International Journal of Food Microbiology 178 (2014) 29–38

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Genetic diversity and antibiotic resistance profiles of *Campylobacter jejuni* isolates from poultry and humans in Turkey

Secil Abay [a,*], Tuba Kayman [b], Baris Otlu [c], Harun Hizlisoy [a], Fuat Aydin [a], Nurhan Ertas [d]

[a] Erciyes University, Faculty of Veterinary Medicine, Department of Microbiology, Kayseri, Turkey
[b] Kayseri Training and Research Hospital, Microbiology and Clinical Microbiology Laboratory, Kayseri, Turkey
[c] Inonu University, Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey
[d] Erciyes University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kayseri, Turkey

ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form 26 February 2014
Accepted 3 March 2014
Available online 13 March 2014

Keywords:
Antibiogram
Campylobacter jejuni
Pulsed-Field Gel Electrophoresis
Repetitive extragenic palindromic-polymerase chain reaction

ABSTRACT

In this study, the investigation of clonal relations between human and poultry *Campylobacter jejuni* isolates and the determination of susceptibilities of isolates to various antibiotics were aimed. A total of 200 *C. jejuni* isolates concurrently obtained from 100 chicken carcasses and 100 humans were genotyped by the Pulsed-Field Gel Electrophoresis (PFGE) and automated Repetitive Extragenic Palindromic PCR (Rep-PCR, DiversiLab system) methods and were tested for their susceptibility to six antibiotics with disk diffusion method. The minimum inhibitory concentration (MIC) values of ciprofloxacin (CI), enrofloxacin (EF) and erythromycin (EM) were evaluated by E-test. By using PFGE 174 of (87.0%) the isolates were able to be typed. The clonally related strains were placed in 35 different clusters and 115 different genotypes were obtained. All of the two hundred isolates could be typed by using Rep-PCR and were divided into 133 different genotypes. One hundred and fourteen clonally related isolates (57.0%) were included in 47 clusters. In disk diffusion test, while the susceptibility rates of AMC and S to human and chicken derived *C. jejuni* isolates were 84.0%–96.0% and 96.0%–98.0%, respectively, all isolates were susceptible to gentamicin. The resistance rates of human isolates to AMP, NA and TE were detected as 44.0%, 84.0% and 38.0% of the resistances of chicken isolates to these antibiotics were 34.0%, 95.0% and 56.0%, respectively. The MIC values of human and chicken isolates to CI, EF and EM were detected as 81.0–93.0%, 85.0–88.0% and 6.0–7.0%, respectively. The clonal proximity rates were detected between human and poultry origin *C. jejuni* isolates. The discriminatory power of PFGE and Rep-PCR was similar, with Simpson's diversity indexes of 0.993 and 0.995, respectively. Concordance of the two methods as determined by Adjusted Rand coefficient was 0.198 which showed the low congruence between Rep-PCR and PFGE. High rates of quinolone resistance were detected in *C. jejuni* isolates.

This study demonstrated that chicken meat played an important role for infections caused by *C. jejuni* in Turkey and erythromycin, amoxicillin clavulanic acid and gentamicin are recommended for the treatment of Campylobacteriosis in humans.

1. Introduction

Campylobacteriosis is a foodborne infection and a dominant species *Campylobacter jejuni* is an important cause of acute gastroenteritis in humans. Acute symptoms of *C. jejuni* in humans are diarrhea, fever and abdominal pain. Furthermore, it causes colitis, reactive arthritis and neurological complications including Miller-Fisher and Guillain-Barré syndromes (Butzler, 2004). The most important sources of this infection in humans are contaminated meat, milk and water. In particular, poultry meat (Gormley et al., 2008) is known as an important source. In addition, pet animals (cats, dogs), wild birds and other animals are sources of infection (Broman et al., 2004; Peterson, 2003; Wolfs et al., 2001). In order to clarify the epidemiology of campylobacteriosis in humans and animals, molecular methods such as Ribotyping (Ge et al., 2006), Pulsed Field Gel Electrophoresis (PFGE) (Eyles et al., 2006), Flagellin Typing (fla typing) (Aydin et al., 2007; Broman et al., 2004), Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) (Wardak and Jagielski, 2009), Amplified Fragment Length Polymorphism (AFLP) (Lévesque et al., 2012) and Repetitive Extragenic Palindromic PCR (Rep-PCR) (Behringer et al., 2011) are widely used. Comparative studies are of the utmost importance because poultry and mammals are attributed as sources of infection for human campylobacteriosis (Magnússon et al., 2011).

Pulsed Field Gel Electrophoresis (PFGE) is used for the typing of campylobacters and many other bacteria (Lehner et al., 2000), and is based upon the restriction fragment length polymorphism technique, which is a highly discriminative, reproducible and effective molecular typing method. Among all molecular typing methods, it is considered

* Corresponding author.
E-mail addresses: ranaabay@hotmail.com, sabay@erciyes.edu.tr (S. Abay).

http://dx.doi.org/10.1016/j.ijfoodmicro.2014.03.003
0168-1605/

as "gold standard" (Barbuddhe et al., 2009). The Rep-PCR-based DiversiLab system, used to determine the genetic proximity of various infectious agents, is an easy to use, rapid and standardized molecular method (Healy et al., 2005). In particular, in nosocomial infections and epidemic outbreaks, DiversiLab is useful for investigating the sources as soon as possible (Fluit et al., 2010).

In recent years, especially in campylobacters, the increasing rates of resistance to fluoroquinolones both worldwide and in Turkey, are of great importance. In some studies, the relationships between the antibiotic resistance of campylobacters isolated from human infections and environmental and food origin campylobacters are emphasized (Cokal et al., 2009; Nelson et al., 2007; Ongen et al., 2007; Praakle-Amin et al., 2007; Savasan et al., 2004; Tadesse et al., 2011; Unicomb et al., 2006). In particular in the European Union, this situation is controlled and the monitoring of antimicrobial resistance profiles of zoonotic pathogens which threaten public health is obligatory (Directive, 2003/99/EC of The European Parliament and of the Council).

In Turkey, no studies report the antimicrobial profile of human and chicken *C. jejuni* strains and simultaneously used PFGE and Rep-PCR for the subsequent strain typing. Therefore, in this study, we aimed to determine the molecular typing of *C. jejuni* isolates obtained from chicken meat (carcass) and human gastroenteritis cases by using PFGE and the Rep-PCR based DiversiLab system and to determine the susceptibilities of these isolates to various antibiotics.

2. Materials and methods

2.1. Human C. jejuni isolates

One hundred human isolates were randomly selected from 152 *C. jejuni* strains isolated from stool samples of patients with diarrhea which were sent to the Kayseri Training and Research Hospital, Microbiology Laboratory, in Kayseri, Turkey (Kayman et al., 2013).

2.2. Chicken C. jejuni isolates

One hundred chicken isolates were randomly selected from 150 *C. jejuni* strains isolated from chicken carcasses belonging to various firms, which were purchased from supermarkets in Kayseri city center. For the isolation of *C. jejuni*, the chicken carcasses were washed in stomacher bag with buffered peptone water (Oxoid, CM0509, UK). The rinses were then plated onto mCCD agar by using swab and the inoculated plates were incubated at 42 °C under microaerobic atmosphere for 72–96 h (Aydin et al., 2007).

All the strains mentioned above were isolated between March 2010 and March 2011. The *C. jejuni* isolates were identified by phenotypic (Aydin et al., 2001; Quinn et al., 1998) and molecular methods (Wang et al., 2002).

2.3. Reference strain

Campylobacter jejuni NCTC 11168 was used as a reference strain at all stages of the study.

2.4. Pulsed-Field Gel Electrophoresis (PFGE)

For the typing of *C. jejuni* isolates by PFGE, the standardized protocol, used in the PulseNet program by the Centers for Disease Control and Prevention (CDC), was applied with minor changes (Ribot et al., 2001). Pure culture *C. jejuni* colonies were collected with a plastic loop and were suspended in 2 ml of cell suspension buffer (CSB; 100 mM Tris, 100 mM EDTA, pH 8). Bacterial density was adjusted by using a spectrophotometer (UV/Vis. Spectrophotometer, Boeco, Germany) with a 610 nm wavelength and about 0.8 absorbance. Low melting point agarose (LMP) (2%) (Gibco, UK) was prepared in TE buffer (10 mM Tiris, 1 MM EDTA, pH 8) and mixed with an equal volume of cell suspension. Then 25 μl of Proteinase K (20 mg/ml stock solution) (Sigma-Aldrich, Dorset, UK) was added. The cell suspension-LMP-Proteinase K mixture was distributed into plug molds (Bio-Rad, Hercules, CA) and allowed to cool for 15 min at 4–8 °C. The solidified plugs were transferred in 5 ml of cell lysis buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% sarcosine, 1.5 mg/ml Proteinase K) and incubated in a shaking water bath for 2 h at 55 °C. After lysis, the plugs were washed four times with 4 ml of sterile ultra pure water at 50 °C for 15 min then followed by washing four times with 4 ml of TE (Tris–EDTA) buffer at 50 °C for 15 min at 200 rpm within the water bath. Genomic DNA in the plugs was restricted by 20 U of *Sma*I (Fermentas Corporation, USA) and DNA fragments were separated on 1% agarose gel run in 0.5× TBE buffer by using a CHEF-DR II system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14 °C at 6 V/cm^2 (≅165 mA) for 18 h. The initial and final switch times were 6.75 s and 38.35 s, respectively. The gel was stained with ethidium bromide (5 μg/ml) and photographed under UV light. The DNA band profiles were analyzed with the GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). The clonal relationships and UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrogram of the strains were performed using the Dice similarity coefficient with 1% tolerance and position tolerance of 1% for comparisons of bands. Isolates with >80% similarity according to the dendrogram were clustered. The PFGE protocol was repeated three times for the untyped isolates.

2.5. Repetitive extragenic palindromic-PCR (REP-PCR)

2.5.1. Culture of C. jejuni and DNA extraction

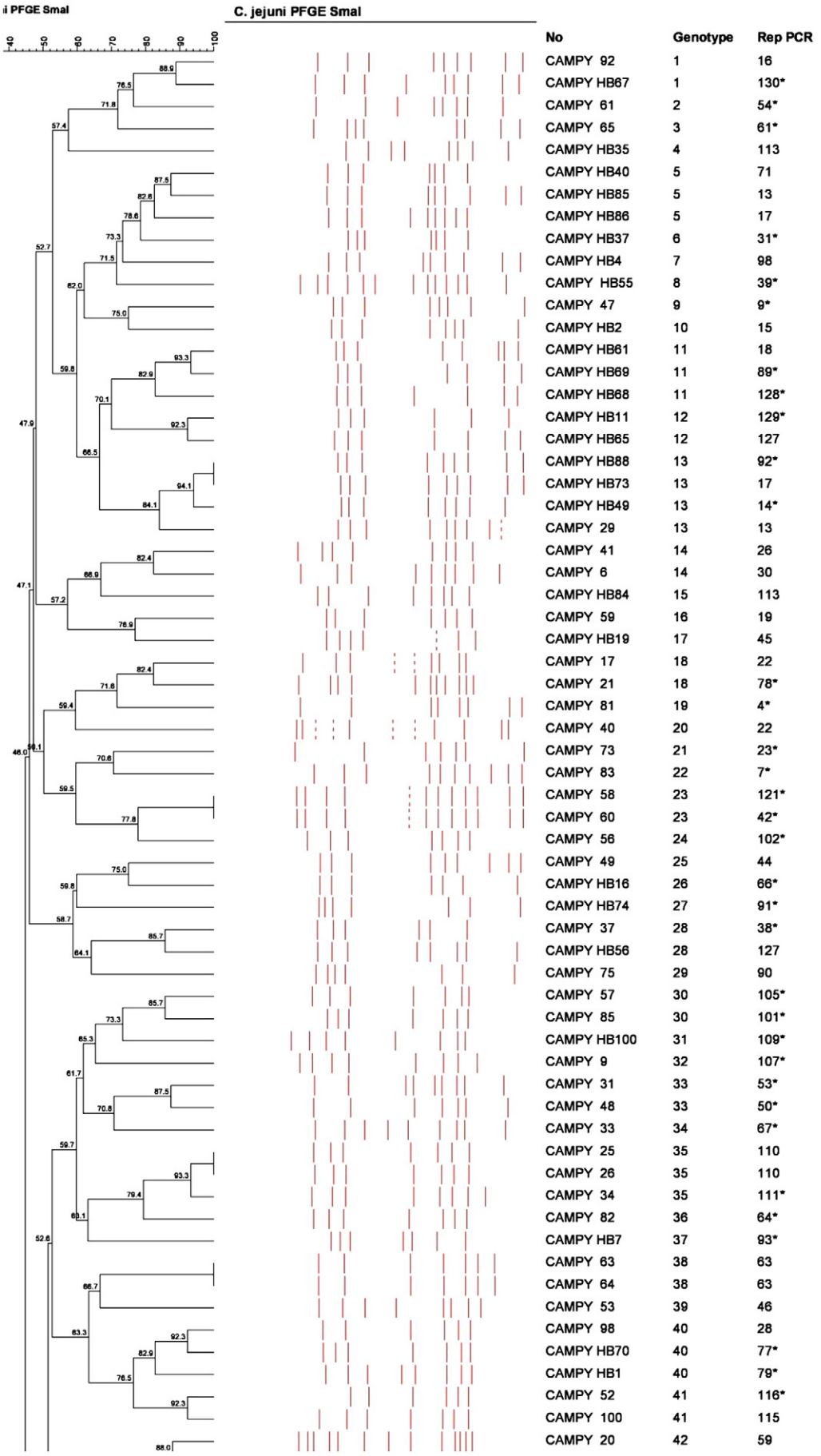
C. jejuni isolates were cultured on 5% sheep blood agar for 48 h at 42 °C. DNA from each isolate was extracted using the Ultra Clean microbial DNA isolation kit (Mo Bio Laboratories, 12224-250, Carlsbad, CA.) following the manufacturer's instructions. DNA concentration was adjusted to approximately 25 ng/μl for each sample and the presence of DNA was confirmed by 1.5% agarose gel electrophoresis with the comparison of 10–40 ng/μl positive DNA controls.

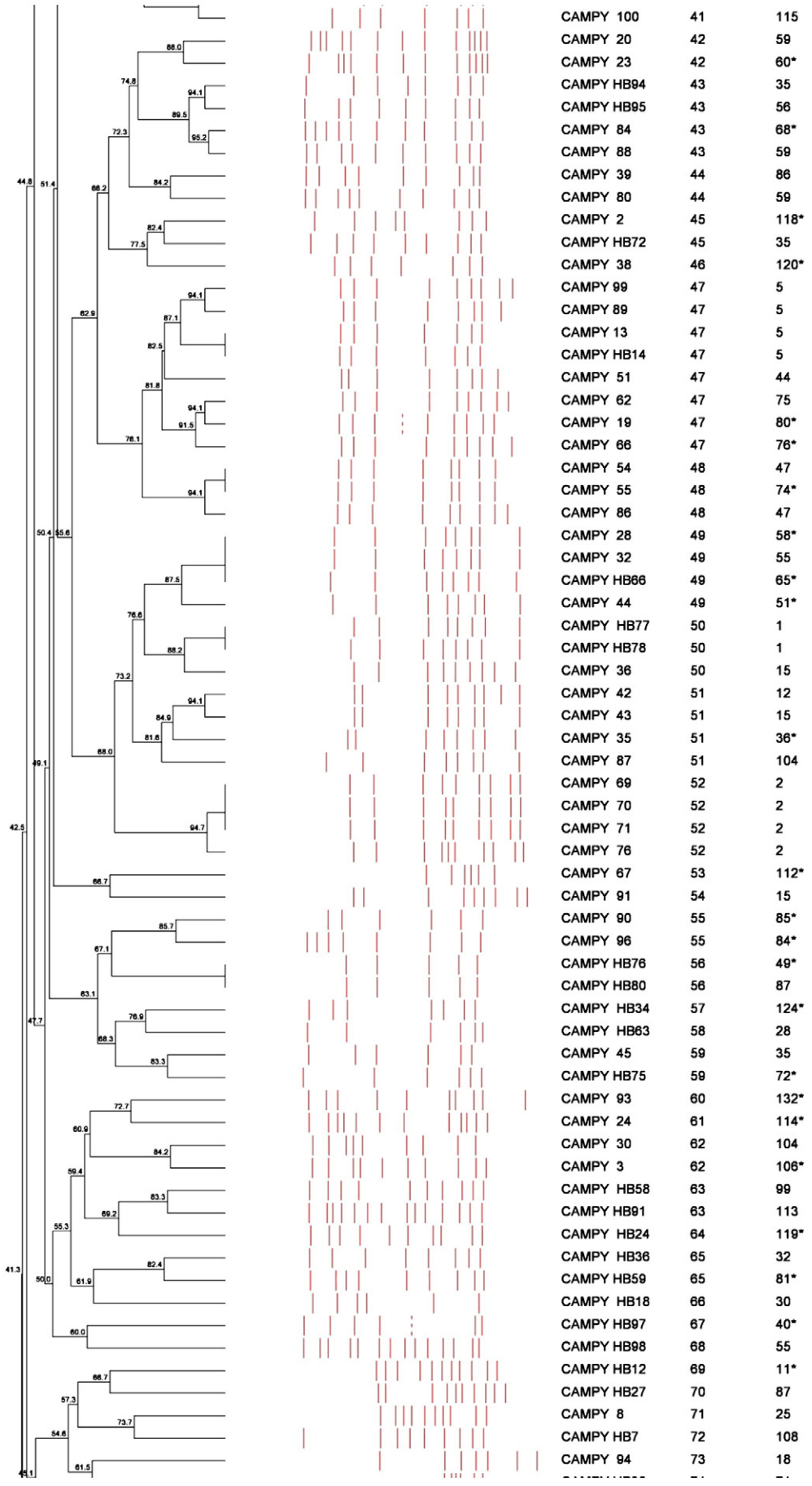
2.5.2. Rep-PCR DNA fingerprinting

All DNA samples were amplified using the DiversiLab System (BioMérieux) Campylobacter kit (Bacterial Barcodes, 270607) standardized with positive and negative controls for DNA fingerprinting following the manufacturer's instructions. Briefly, 25–50 ng/μl, 2 μl of genomic DNA, 0.5 μl of AmpliTaq polymerase, 2.5 μl of 10× PCR buffer (Applied Biosystems) and 2 μl of primer mix were added to the 18 μl Rep-PCR master mix in a total volume of 25 μl per reaction. The Rep-PCR amplification was performed with an initial denaturation of 94 °C for 2 min, followed by 35 cycles of each consisting of 94 °C for 30 s, 50 °C for 30 s and 70 °C for 90 s, and final extension at 70 °C for 3 min (Touchgene Gradient, Techne, UK). Rep-PCR profiles were obtained using microfluidic DNA chips (Bacterial barcodes) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, California). DNA fingerprint patterns were evaluated with electropherograms and the results of the dendrogram with a similarity matrix and a virtual gel image of the fingerprint for each DNA sample. Rep-PCR fingerprinting profiles were compared by DiversiLab® (version 3.4) software using the Pearson correlation coefficient (Bacterial Barcodes).

2.6. Statistical analyses of PFGE and REP-PCR results

The discriminatory power of each method by determining the Simpson's index of diversity (SID) was calculated and the concordance between Rep-PCR type and PFGE types was determined by calculating the adjusted Rand and Wallace coefficients using the online tool for Quantitative Assessment of Classification Agreement (http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Tool). Approximately 95% Confidence Interval (CI) was calculated for SID, adjusted Rand and Wallace coefficients, and the statistical differences for typing


i PFGE SmaI
C. jejuni PFGE SmaI
40
50
60
70
80
90
100
No
Genotype
Rep PCR
CAMPY 92 1 16
CAMPY HB67 1 130*
CAMPY 61 2 54*
CAMPY 65 3 61*
CAMPY HB35 4 113
CAMPY HB40 5 71
CAMPY HB85 5 13
CAMPY HB86 5 17
CAMPY HB37 6 31*
CAMPY HB4 7 98
CAMPY HB55 8 39*
CAMPY 47 9 9*
CAMPY HB2 10 15
CAMPY HB61 11 18
CAMPY HB69 11 89*
CAMPY HB68 11 128*
CAMPY HB11 12 129*
CAMPY HB65 12 127
CAMPY HB88 13 92*
CAMPY HB73 13 17
CAMPY HB49 13 14*
CAMPY 29 13 13
CAMPY 41 14 26
CAMPY 6 14 30
CAMPY HB84 15 113
CAMPY 59 16 19
CAMPY HB19 17 45
CAMPY 17 18 22
CAMPY 21 18 78*
CAMPY 81 19 4*
CAMPY 40 20 22
CAMPY 73 21 23*
CAMPY 83 22 7*
CAMPY 58 23 121*
CAMPY 60 23 42*
CAMPY 56 24 102*
CAMPY 49 25 44
CAMPY HB16 26 66*
CAMPY HB74 27 91*
CAMPY 37 28 38*
CAMPY HB56 28 127
CAMPY 75 29 90
CAMPY 57 30 105*
CAMPY 85 30 101*
CAMPY HB100 31 109*
CAMPY 9 32 107*
CAMPY 31 33 53*
CAMPY 48 33 50*
CAMPY 33 34 67*
CAMPY 25 35 110
CAMPY 26 35 110
CAMPY 34 35 111*
CAMPY 82 36 64*
CAMPY HB7 37 93*
CAMPY 63 38 63
CAMPY 64 38 63
CAMPY 53 39 46
CAMPY 98 40 28
CAMPY HB70 40 77*
CAMPY HB1 40 79*
CAMPY 52 41 116*
CAMPY 100 41 115
CAMPY 20 42 59


CAMPY 100 41 115
CAMPY 20 42 59
CAMPY 23 42 60*
CAMPY HB94 43 35
CAMPY HB95 43 56
CAMPY 84 43 68*
CAMPY 88 43 59
CAMPY 39 44 86
CAMPY 80 44 59
CAMPY 2 45 118*
CAMPY HB72 45 35
CAMPY 38 46 120*
CAMPY 99 47 5
CAMPY 89 47 5
CAMPY 13 47 5
CAMPY HB14 47 5
CAMPY 51 47 44
CAMPY 62 47 75
CAMPY 19 47 80*
CAMPY 66 47 76*
CAMPY 54 48 47
CAMPY 55 48 74*
CAMPY 86 48 47
CAMPY 28 49 58*
CAMPY 32 49 55
CAMPY HB66 49 65*
CAMPY 44 49 51*
CAMPY HB77 50 1
CAMPY HB78 50 1
CAMPY 36 50 15
CAMPY 42 51 12
CAMPY 43 51 15
CAMPY 35 51 36*
CAMPY 87 51 104
CAMPY 69 52 2
CAMPY 70 52 2
CAMPY 71 52 2
CAMPY 76 52 2
CAMPY 67 53 112*
CAMPY 91 54 15
CAMPY 90 55 85*
CAMPY 96 55 84*
CAMPY HB76 56 49*
CAMPY HB80 56 87
CAMPY HB34 57 124*
CAMPY HB63 58 28
CAMPY 45 59 35
CAMPY HB75 59 72*
CAMPY 93 60 132*
CAMPY 24 61 114*
CAMPY 30 62 104
CAMPY 3 62 106*
CAMPY HB58 63 99
CAMPY HB91 63 113
CAMPY HB24 64 119*
CAMPY HB36 65 32
CAMPY HB59 65 81*
CAMPY HB18 66 30
CAMPY HB97 67 40*
CAMPY HB98 68 55
CAMPY HB12 69 11*
CAMPY HB27 70 87
CAMPY 8 71 25
CAMPY HB7 72 108
CAMPY 94 73 18

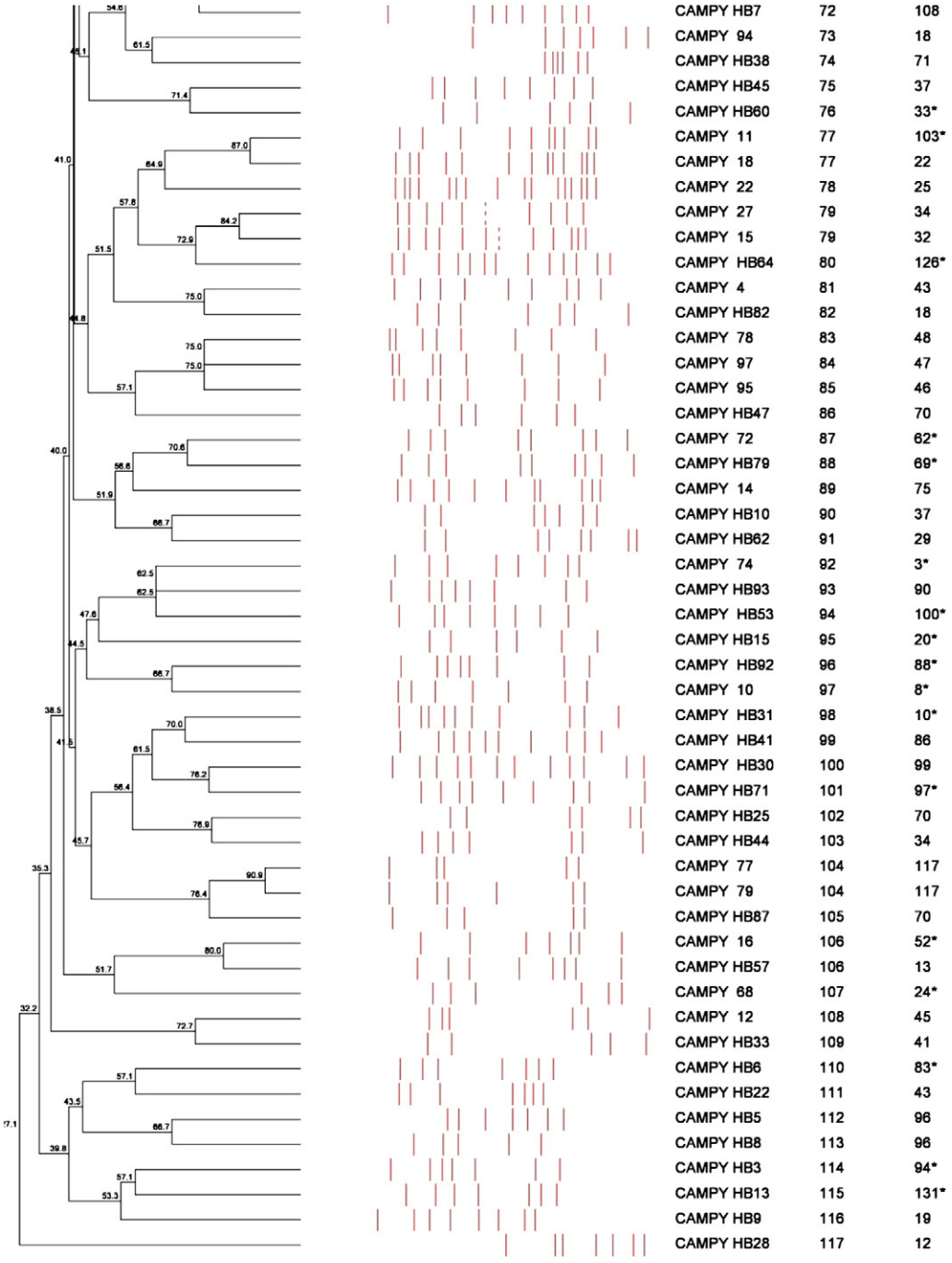


Fig. 1. Dendrogram of *Sma*I digestion fingerprints and Rep-PCR profile for 174 *C. jejuni* isolates. Genetic similarity values between fingerprints were calculated based on Dice coefficient. CAMPY HB and CAMPY represented as human and chicken *C. jejuni* isolates, respectively. *: means that unique band patterns of Rep-PCR.

methods based on the categorization of data by the adjusted Rand and Wallace coefficients (Carrico et al., 2006; Pinto et al., 2008). The Simpson's index of diversity (SID) demonstrates the discriminatory ability of typing techniques. This index is given by the following equation:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} a_j$$

where a_j is the number of strains in the population which are indistinguishable from the jth strain, and N is the number of strains in the population (Hunter, 1990). Adjusted Rand index, provides an overall measure of the congruence between two typing methods while the Wallace's coefficient is more informative and offers a clear interpretation since it represents the probability that a pair of strains which are assigned to the same type by one method are also classified in the same type by the other method (Carrico et al., 2006; Pinto et al., 2008).

2.7. Antibacterial susceptibility testing

The antibiotic susceptibilities of *C. jejuni* isolates to amoxicillin clavulanic acid (AMC, 30 μg), ampicillin (AMP, 10 μg), gentamicin (CN, 10 μg), nalidixic acid (NA, 30 μg), streptomycin (S, 10 μg), and tetracycline (TE, 30 μg) were determined by using the disk diffusion test (Bauer et al., 1966). Erythromycin (EM), enrofloxacin (EF) and ciprofloxacin (CI) were evaluated by E-test. The antimicrobial disks and E-test strips were purchased from Oxoid, UK and BioMérieux,

France, respectively. The disk diffusion test results were interpreted using the criteria published by Clinical and Laboratory Standards Institute (CLSI, 2010a). For MIC values, the limitations of antibiotic susceptibilities and resistances of isolates were evaluated according to the recommended criteria (CLSI, 2008, 2010b).

3. Results

3.1. PFGE

In total 174 *C. jejuni* isolates (87.0%), 78 human (78/100) and 96 chicken (96/100), were typed by using PFGE (Fig. 1). Twenty-two human and 4 chicken isolates could not be typed. One hundred and fifteen different PFGE profiles were obtained from the 174 isolates typed. Ninety three (53.4%) of the isolates were clonally related. Forty-eight human and 33 poultry *C. jejuni* isolates exhibited unique band pattern. Sixty one chicken and 32 human isolates were found in 35 different clusters. The SID value of PFGE typing of 174 *C. jejuni* isolates was 0.993.

3.2. Rep-PCR fingerprint analysis

All the 200 *C. jejuni* isolates were (100%) identified by Rep-PCR, 133 different band profiles were obtained and 114 isolates were clonally related. Forty-four chicken and 42 human isolates exhibited unique band pattern (Fig. 2). Fifty-six chicken and 58 human isolates were found in 47 clusters. The number of strains found in each cluster varied from two to four. Twenty-two of these clusters included isolates of both origins with a similar band pattern. The dendrogram and similarity matrix of human and poultry *C. jejuni* isolates are shown in Fig. 3. The SID value of Rep-PCR typing of 200 *C. jejuni* isolates was 0.995. It was also found that SID value of Rep-PCR typing of 174 *C. jejuni* isolates was the same value.

3.3. Statistical evaluation of PFGE and REP-PCR

Comparison of PFGE and Rep-PCR results are shown in Tables 1 and 2. Typeability of *C. jejuni* for Rep-PCR (100%) was higher than that of PFGE (87.0%). The discriminatory power of PFGE and Rep-PCR was similar, with Simpson's diversity indexes of 0.993 and 0.995, respectively. Also p-value between SIDs of two methods was $p > 0.05$ and this was not significant. The concordance of two methods as determined by Adjusted Rand coefficient was 0.198 which showed the low congruence between Rep-PCR and PFGE. Adjusted Wallace's coefficient was 0.241 when comparing Rep-PCR with PFGE (indicating that if the isolates were characterized to be the same rep-PCR type, those isolates had 24.1% chances to be identified as the same PFGE type) and the Wallace coefficient of PFGE to Rep-PCR was 0.168 (indicating that if the isolates were characterized to be the same PFGE type, those isolates had 16.8% chances to be identified as the same rep-PCR type) (Table 2).

3.4. Antibacterial susceptibility testing

The antibacterial susceptibility testing results of 200 *C. jejuni* isolates against 6 different antibacterial agents are exhibited in Table 3. In disk diffusion test, while the susceptibilities of human and poultry *C. jejuni* isolates to AMC and S were found as 84.0%–96.0% and 96.0%–98.0%, respectively, it was detected that all isolates were susceptible to gentamicin. While the resistance rates of human isolates to AMP, NA and TE were found as 44.0%, 84.0% and 38.0%, the resistance rates of chicken isolates to these antibiotics were detected as 34.0%, 95.0% and 56.0%, respectively.

The MIC values of human and chicken isolates to ciprofloxacin, enrofloxacin and erythromycin were detected as 81.0–93.0%, 85.0–88.0% and 6.0–7.0%, respectively (Table 3). Human and chicken *C. jejuni* isolates were found to be resistant to one or more antibiotics. It was

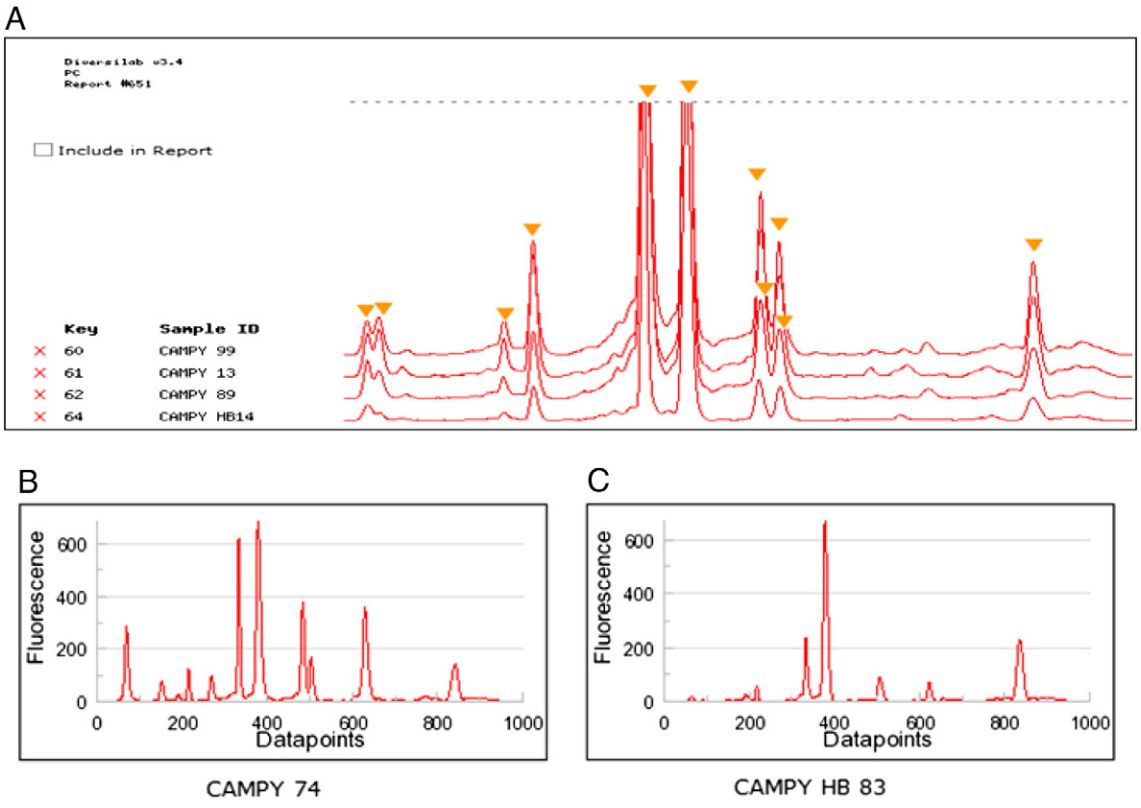


Fig. 2. Electropherograms of clonally related human (CAMPY HB) and chicken (CAMPY) *C. jejuni* isolates (A) Unique electrophoretic patterns of chicken (B) and human (C) *C. jejuni* isolates.

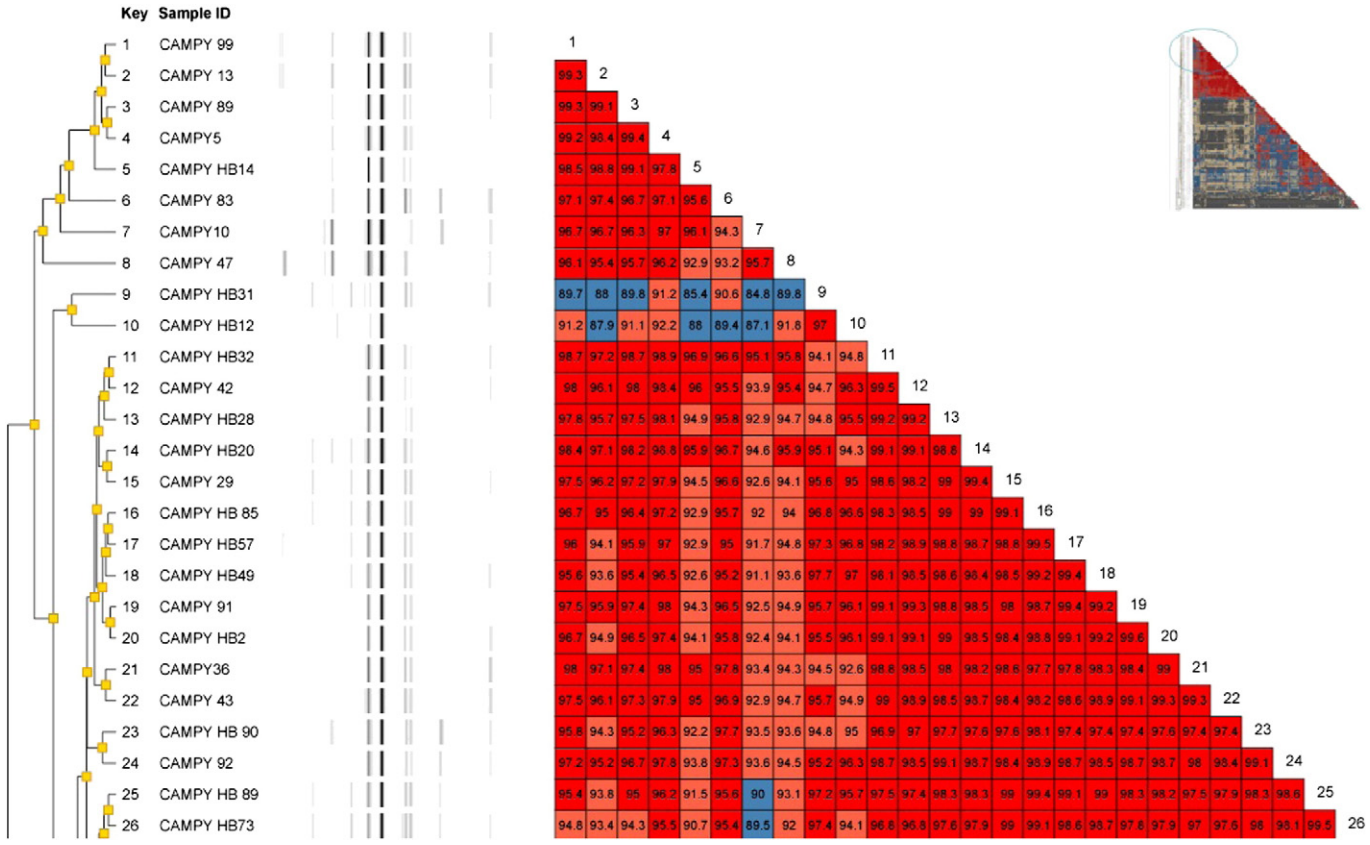


Fig. 3. The dendrogram and similarity matrix of 200 *C. jejuni* isolates (Enlarged scheme of indicated area).

found that the resistance rates of human and chicken *C. jejuni* isolates to at least one antibacterial agent were 92.0% 92/100–96.0% 96/100 and to at least two antibacterial agents were 85.0% 85/100–94.0% 94/100, respectively The data about multidrug resistance of human and chicken *C. jejuni* isolates are presented in Table 4.

4. Discussion

PFGE is a highly discriminatory method used for the genotyping of *Campylobacter* spp. In addition, the discriminatory power of PFGE depends on typed microorganisms and restriction enzymes (*Sal*I, *Kpn*I, *Sac*II, SmaI and *BamH*I) used in the method (Ge et al., 2006; On et al., 1998). PFGE is often used for the purpose of epidemiological studies including molecular characterization of *C. jejuni* isolates recovered from human (Eyles et al., 2006; Magnusson et al., 2011; Ono et al., 2003) and animal clinical specimens (Acke et al., 2010; Sahin et al., 2012), carriage (Acke et al., 2010; Damborg et al., 2004; Saito et al., 2005), contamination (Ono et al., 2003; Saito et al., 2005) and environmental samples (Denis et al., 2011; Eyles et al., 2006).

In this study, the typed 174 isolates showed 115 different PFGE profiles. Ninety three of the 174 isolates (53.4%) were found to be clonally related. In different countries, some authors (Cardinale et al., 2006; Rönner et al., 2005; Zhang et al., 2010) reported that human and poultry *C. jejuni* isolates were clonally related in PFGE analysis. However, Ragimbeau et al. (2008) found that human and poultry isolates had more genotypic patterns than those cattle isolates in PFGE analysis with *Sma*I enzyme. In addition Denis et al. (2009) reported that in PFGE analysis of 151 human and 182 poultry *C. jejuni* isolates, 47 clusters, including poultry and human isolates, were obtained in France. This identical situation in poultry and human *C. jejuni* isolates emphasized the important role of poultry meat in human Campylobacteriosis. Similarly in our study, the clonal relationship detected between human and chicken *C. jejuni* isolates supports the view that chicken meat has a significant role in *Campylobacter* infections in Turkey. Hänninen et al. (1998) found that in the PFGE analysis of 176 *C. jejuni* strains isolated from humans in Finland, 69 PFGE patterns were obtained and also one-third of the isolates had unique band patterns. Forty-eight unique band patterns were determined from 78 human *C. jejuni* isolates in the present study.

In contrast, in a study conducted by Ono et al. (2003) in Japan, PFGE was used for the genotyping of 154 human and chicken *C. jejuni* and 14 *C. coli* isolates. The researchers reported that isolates used in the study

Table 1
Comparison of PFGE and Rep-PCR results.

	Rep-PCR				PFGE			
	Typeability	No. of isolates with unique pattern	No. of clusters	No. of common clusters[a]	Typeability	No. of isolates with unique pattern	No. of clusters	No. of common clusters[a]
Chicken *C. jejuni* isolates (n = 100)	100%	44	13	22	96%	33	18	10
Human *C. jejuni* isolates (n = 100)	100%	42	12		78%	48	7	

[a] The numbers of clusters including human and chicken *C. jejuni* isolates found together with similar band pattern.

Table 2
Comparison of Rep-PCR and PFGE using Simpson's index of diversity (SID), Adjusted Rand and Wallace coefficients and confidence intervals (CI) 95%.

Typing methods	No. of isolates	SID (95% CI)	Adjusted Rand coefficient (95% CI)		Wallace coefficient (95% CI)	
			Rep-PCR	PFGE	Rep-PCR	PFGE
PFGE	174[a]	0.993 (0.991–0.996)		1.000 (1.000–1.000)	0.168 (0.053–0.283)	1.000 (1.000–1.000)
Rep-PCR	174[a]	0.995 (0.994–0.997)	1.000 (1.000–1.000)	0.198 (0.025–0.383)	1.000 (1.000–1.000)	0.241 (0.139–0.344)

[a] 96 chicken and 78 human isolates.

were very different from each other but one human and one chicken isolate were similar. In Poland 117 PFGE profiles were obtained from 128 human *C. jejuni* clinical isolates by Wardak and Jagielski (2009), the researchers found that *C. jejuni* strains isolated from clinical cases in different parts of the country were indistinguishable.

In this study, *Sma*I enzyme was used in PFGE analysis. However, 22% of human and 4% of chicken isolates were not able to be typed. It was assumed that the reason for this situation could be the use of the enzyme *Sma*I for macrorestriction. Similarly, it has been reported that five chicken *Campylobacter* spp. isolates (Praakle-Amin et al., 2007) and 45 *C. jejuni* isolates (Magnússon et al., 2011) could not be restricted by *Sma*I enzyme in PFGE analysis.

There are only limited studies related to the typing of *Campylobacter* spp. by automated Rep-PCR (Behringer et al., 2011; Hernandez and Caldwell, 2010) in literature screening. Behringer et al. (2011) used four molecular typing methods including *fla*A-RFLP, automated Rep-PCR, PFGE and the MLST method for the molecular analysis of 100 *Campylobacter* spp. of broiler origin. The researchers reported that all methods had similar results and stated that 96% and 100% typeability were detected in PFGE using *Sma*I enzyme and Rep-PCR, respectively. Also they found high SID values for each method and the low congruence among the two methods. These results were compatible with our findings. Moreover, Hernandez and Caldwell (2010) reported that a combination of PFGE and DiversiLab Rep-PCR methods for the genotyping of *C. jejuni* isolates obtained from broilers provided highly discriminatory molecular typing results.

The antibacterial susceptibility rates of our isolates are shown in Tables 3–4. Similar findings were also noted by the following researchers. Han et al. (2007) found that all isolates were resistant to at least one antibacterial agent, while most of the isolates were resistant to tetracycline, nalidixic acid and ciprofloxacin. Also, most of them were susceptible to erythromycin, chloramphenicol and gentamicin. Zhang et al. (2010) reported that 44 *C. jejuni* isolates were all susceptible to erythromycin, gentamicin and streptomycin, but resistant to nalidixic acid, levofloxacin and ciprofloxacin. High rates of resistance to quinolones for poultry isolates were detected by Cardinale et al. (2006). In a study conducted in the Czech Republic by Steinhauserová and Mikulicova (2005), as a result of antibacterial susceptibility testing poultry and human *C. jejuni* isolates were found to be resistant to ciprofloxacin at rates of 59% and 69% and the two groups of isolates were resistant to nalidixic acid at a ratio of 30%. Rahimi et al. (2010) found the resistance rates of 177 *C. jejuni* strains isolated from poultry carcasses to tetracycline, ciprofloxacin, nalidixic acid and enrofloxacin to be 79.7%, 67.2%, 59.3% and 48%, respectively. In a study conducted by Salihu et al. (2012) in Nigeria, 70 broiler *C. jejuni* isolates were resistant to ampicillin, ciprofloxacin and tetracycline at rates of 38.6%, 21.4% and 18.6%. Moreover, the researchers reported that 14.3% of the isolates were resistant to a single antibiotic, 24.3% of the isolates were resistant to two antibiotics and 40% of the isolates were resistant to more than two antibiotics. A high resistance rate (80%) was found in 117 human and 33 chicken *C. jejuni* isolates to ciprofloxacin by Senok et al. (2007) in Sudan. In addition all isolates were susceptible to erythromycin except for two human isolates. Kos et al. (2006) stated that 104 poultry *C. jejuni* isolates were resistant to tetracycline, nalidixic acid and ciprofloxacin at rates of 69%, 11% and 8%, respectively, and also reported that all isolates were susceptible to gentamicin and erythromycin. Miflin et al. (2007) reported that 125 broiler *C. jejuni* isolates were found to be resistant to tetracycline and ampicillin at rates of 18.4% and 17.6%, respectively, by disk diffusion test and all isolates were susceptible to erythromycin. Wieczorek et al. (2012) reported that in Poland, 122 *C. jejuni* strains which were isolated from chicken meat were resistant to ciprofloxacin, nalidixic acid and tetracycline at rates of 91%, 89.3% and 49.1%, respectively, and all isolates were susceptible to erythromycin and gentamicin.

Table 3
Antibacterial resistance profiles and MIC distributions of chicken and human *C. jejuni* isolates.

Antibiotics	Chicken (n = 100)			Human (n = 100)		
	S	I	R	S	I	R
AMC	96	–	4	84	4	12
AMP	55	11	34	51	5	44
CN	100	–	–	100	–	–
NA	1	4	95	15	1	84
S	98	–	2	96	1	3
TE	40	4	56	62	–	38

Antibiotics	MIC range			Number of *C. jejuni* isolates according to MIC														
	S	I	R	Origin	0.002	0.094	0.125	0.25	0.5	1	2	4	6	8	12	16	32	256
EF	<0.25	0.5–1	≥2	C	2	2	3		3	2		3	9	3	3		70	
				H	2	3	3		3	4				6	9	8	62	
CI	<1	2	≥4	C		1	1	3	2				3	6		6	78	
				H		2	3	6	5		3				5	6	70	
EM	≤8	16	≥32	C		27	40	21							2	3	3	4
				H	21	11	50									12	3	3

S: susceptible, I: intermediate, R: resistant.
MIC: minimum inhibitory concentration, C: chicken (n = 100), H: human (n = 100).

Table 4
The distributions of multidrug resistance of *C. jejuni* isolates.

Numbers of resistances	Resistance patterns	Origins of the resistant isolates	
		Chicken	Human
2	AMP, EF	1	1
2	AMP, NA	1	1
2	CI, NA	2	–
3	CI, EF, NA	26	24
3	CI, NA, TE	1	–
3	EF, EM, NA	–	1
4	AMC, AMP, EF, TE	–	1
4	AMP, CI, NA, TE.	1	–
4	AMP, CI, EF, NA	6	16
4	CI, EF, NA, TE	30	17
5	AMC, AMP, CI, EF, NA	1	4
5	AMP, CI, EF, EM, NA	1	–
5	AMP, CI, EF, NA, TE	15	9
5	CI, EF, EM, NA, TE	1	–
5	CI, EF, NA, S, TE	-	2
6	AMC, AMP, CI, EF, EM, NA	–	1
6	AMC, AMP, CI, EF, NA, TE	1	3
6	AMP, CI, EF, EM, NA, TE	5	3
7	AMC, AMP, CI, EF, EM, NA, TE	–	1
7	AMC, AMP, CI, EF, NA, S, TE	2	1

In our study, depending on a high rate of multiple antibiotic resistances, there was a low level of compliance in the results of genotyping and antibiotic resistance was detected. Similarly, Han et al. (2007) reported that the relationship between the genotypic characterization and antibiotic resistance was quite low. However Cardinale et al. (2006) and Rönner et al. (2005) reported that there was no correlation between the antibiotic resistance profiles and the PFGE profiles of the isolates.

To our knowledge, this is the first study to use and compare automated Rep-PCR and PFGE for campylobacters in Turkey. Although typeability of *C. jejuni* for Rep-PCR (100%) was higher than that of PFGE (87%), the discriminatory power of PFGE and Rep-PCR was similar. These methods used for genotyping of human and chicken *C. jejuni* isolates were both available and suitable. A dominant epidemic strain was not determined. However, the clonal proximity rates detected in human and chicken *C. jejuni* isolates supported the view that chicken meat plays an important role in infections caused by *C. jejuni* in Turkey. Considering the high rates of quinolone resistance detected in chicken and human *C. jejuni* isolates, erythromycin, amoxicillin clavulanic acid and gentamicin should be considered for the treatment of *C. jejuni* infections.

Acknowledgements

This research was supported by the Scientific Research Council of Erciyes University, Kayseri, Turkey (Project No. TSA-11-3414).

References

Acke, E., McGill, K., Lawlor, A., Jones, B.R., Fanning, S., Whyte, P., 2010. Genetic diversity among *Campylobacter jejuni* isolates from pets in Ireland. Vet. Rec. 166, 102–106.
Aydin, F., Atabay, H.I., Akan, M., 2001. The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). J. Appl. Microbiol. 90, 637–642.
Aydin, F., Gumussoy, S.K., Ica, T., Sumerkan, B., Eşel, D., Akan, M., Özdemir, A., 2007. The prevalence of *Campylobacter jejuni* in various sources in Kayseri, Turkey, and molecular analysis of isolated strains by PCR-RFLP. Turk. J. Vet. Anim. Sci. 31, 13–19.
Barbuddhe, S., Hain, T., Chakraborty, T., 2009. The genus Listeria. In: Goldman, E., Green, L.H. (Eds.), Practical Handbook of Microbiology. CRC Press, USA, p. 549.
Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turek, M., 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45, 493–496.
Behringer, M., Miller, W.G., Oyarbazal, O.A., 2011. Typing of *Campylobacter jejuni* and *Campylobacter coli* isolated from live broilers and retail broiler meat by flaA-RFLP, MLST, PFGE and REP-PCR. J. Microbiol. Methods 84, 194–201.
Broman, T., Waldenstrom, J., Dahlgren, D., Carlsson, I., Eliasson, I., Olsen, B., 2004. Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. J. Appl. Microbiol. 96, 834–843.
Butzler, P.J., 2004. *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect. 10, 868–876.
Cardinale, E., Rose, V., Perrier Gros-Claude, J.D., Tall, F., Rivoal, K., Mead, G., Salvat, G., 2006. Genetic characterization and antibiotic resistance of *Campylobacter* spp. isolated from poultry and humans in Senegal. J. Appl. Microbiol. 100, 209–217.
Carrico, J.A., Silva-Costa, C., Melo-Cristino, J., Pinto, F.R., de Lencastre, H., Almeida, J.S., Ramirez, M., 2006. Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. J. Clin. Microbiol. 44 (7), 2524–2532.
Clinical and Laboratory Standards Institute (CLSI), 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-Third Edition M31-A3 Vol. 28 no. 8 replaces M31-A2 Vol. 22 no. 6. (Wayne, PA) .
Clinical and Laboratory Standards Institute (CLSI), 2010a. Performance Standards for Antimicrobial Susceptibility Testing, Twentieth Informational Supplement M100-S20 Vol. 30, No. 1 Replaces M100-S19 vol. 29 no. 3. (Wayne, PA) .
Clinical and Laboratory Standards Institute (CLSI), 2010b. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline-Second Edition M45-A2 Vol. 30 No. 18 replaces M45-A Vol. 26 No. 19. (Wayne, PA) .
Cokal, Y., Caner, V., Sen, A., Cetin, C., Karagenc, N., 2009. *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: an epidemiological survey study in Turkey. Zoonoses Public Health 56 (3), 105–110.
Damborg, P., Olsen, K.E.P., Nielsen, E.M., Guardabassi, L., 2004. Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J. Clin. Microbiol. 42, 1363–1364.
Denis, M., Chidaine, B., Laisney, M.J., Kempf, I., Rivoal, K., Mégraud, F., Fravalo, P., 2009. Comparison of genetic profiles of *Campylobacter* strains isolated from poultry, pig and *Campylobacter* human infections in Brittany, France. Pathol. Biol. (Paris) 57, 23–29.
Denis, M., Tanguy, M., Chidaine, B., Laisney, M.J., Mégraud, F., Fravalo, P., 2011. Description and sources of contamination by *Campylobacter* spp. of river water destined for human consumption in Brittany, France. Pathol. Biol. 59, 256–263.
Directive 2003/99/EC Of The European Parliament and of The Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF.
Eyles, R.F., Brooks, H.J.L., Townsend, C.R., Burtenshaw, G.A., Heng, N.C.K., Jack, R.W., Weinstein, P., 2006. Comparison of *Campylobacter jejuni* PFGE and Penner subtypes in human infections and in water samples from the Taieri River catchment of New Zealand. J. Appl. Microbiol. 101, 18–25.
Fluit, A.A., Terlingen, A.M., Andriessen, L., Ikawaty, R., Van Mansfeld, R., Top, J., Cohen Stuart, J.W., Leverstein-van Hall, M.A., Boel, C.H., 2010. Evaluation of the DiversiLab System for detection of hospital outbreaks of infections by different bacterial species. J. Clin. Microbiol. 48, 3979–3989.
Ge, B., Girard, W., Zhao, S., Friedman, S., Gaines, S.A., Meng, J., 2006. Genotyping of *Campylobacter* spp. from retail meats by pulsed-field gel electrophoresis and ribotyping. J. Appl. Microbiol. 100, 175–184.
Gormley, F.J., MacRae, M., Forbes, K.J., Ogden, I.D., Dallas, J.F., Strachan, N.J.C., 2008. Has retail chicken played a role in the decline of human Campylobacteriosis? Appl. Environ. Microbiol. 74, 383–390.
Han, K., Jang, S.S., Choo, E., Heu, S., Ryu, S., 2007. Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. Int. J. Food Microbiol. 114, 50–59.
Hänninen, M.L., Pajarre, S., Klossner, M.L., Rautelin, H., 1998. Typing of human *Campylobacter jejuni* isolates in Finland by pulsed-field gel electrophoresis. J. Clin. Microbiol. 36, 1787–1789.
Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R., Woods, C., Versalovic, J., James, R., Lupski, J.R., 2005. Microbial DNA typing by automated repetitive-sequence-based PCR. J. Clin. Microbiol. 43, 199–207.
Hernandez, C.A., Caldwell, D., 2010. Molecular Typing and Antimicrobial Resistance of *Campylobacter* Isolated During Commercial Broiler Production. (Master of science dissertation) Texas A&M University (December).
Hunter, P.R., 1990. Reproducibility and indices of discriminatory power of microbial typing methods. J. Clin. Microbiol. 28, 1903–1905.
Kayman, T., Abay, S., Hizlisoy, H., 2013. Identification of *Campylobacter* spp. isolates with phenotypic methods and multiplex polymerase chain reaction and their antibiotic susceptibilities. Mikrobiyol. Bul. 47, 230–239.
Kos, V.N., Keelan, M., Taylor, D.E., 2006. Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from poultry from Alberta, Canada. Antimicrob. Agents Chemother. 50, 778–780.
Lehner, A., Schneck, C., Feierl, G., Pless, P., Deutz, A., Brandl, E., Wagner, M., 2000. Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre. Epidemiol. Infect. 125, 13–16.
Lévesque, S., St-Pierre, K., Frost, E., Arbeit, R.D., Michaud, S., 2012. Use of amplified-fragment length polymorphism to study the ecology of *Campylobacter jejuni* in environmental water and to predict multilocus sequence typing clonal complexes. Appl. Environ. Microbiol. 78, 2470–2473.
Magnússon, S.H., Guðmundsdóttir, S., Reynisson, E., Rúnarsson, A.R., Harðardóttir, H., Gunnarson, E., Georgsson, F., Reiersen, J., Marteinsson, V.T., 2011. Comparison of *Campylobacter jejuni* isolates from human, food, veterinary and environmental sources in Iceland using PFGE, MLST and fla-SVR sequencing. J. Appl. Microbiol. 111, 971–981.
Miflin, J.K., Templeton, J.M., Blackall, P.J., 2007. Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the South-East Queensland region. J. Antimicrob. Chemother. 59, 775–778.
Nelson, J.M., Chiller, T.M., Powers, J.H., Angulo, F.J., 2007. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. Clin. Infect. Dis. 44, 977–980.

On, S.L.W., Nielsen, E.M., Engberg, J., Madsen, M., 1998. Validity of *Sma*I-defined genotypes of *Campylobacter jejuni* examined by *Sal*I, *Kpn*I, and *BamH*I polymorphisms: evidence of identical clones infecting humans, poultry and cattle. Epidemiol. Infect. 120, 231–237.

Ongen, B., Nazik, H., Kaya, I., 2007. Identification and antibiotic susceptibilities of Campylobacter strains isolated from routine stool culture: evaluation of five year results. ANKEM Derg. 21, 37–41.

Ono, K., Kurazono, T., Niwa, H., Itoh, K., 2003. Comparison of three methods for epidemiological typing of *Campylobacter jejuni* and *C. coli*. Curr. Microbiol. 47, 364–371.

Peterson, M., 2003. *Campylobacter jejuni* enteritis associated with consumption of raw milk. J. Environ. Health 65, 20–21.

Pinto, F.R., Melo-Cristino, J., Ramirez, M., 2008. A confidence interval for the Wallace coefficient of concordance and its application to microbial typing methods. PLoS ONE 3 (11), e3696. http://dx.doi.org/10.1371/journal.pone.0003696.

Praakle-Amin, K., Roasto, M., Korkeala, H., Hannien, M.L., 2007. PFGE genotyping and antimicrobial susceptibility of *Campylobacter* in retail poultry meat in Estonia. Int. J. Food Microbiol. 114, 105–112.

Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R., 1998. *Campylobacter* species. In: Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R. (Eds.), Clinical Veterinary Microbiology. Mosby, London, pp. 268–272.

Ragimbeau, C., Schneider, F., Losch, S., Even, J., Mossong, J., 2008. Multilocus sequence typing, pulsed-field gel electrophoresis, and fla short variable region typing of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry origins in Luxembourg. Appl. Environ. Microbiol. 74, 7715–7722.

Rahimi, E., Momtaz, H., Ameri, M., Ghasemian-Safaei, H., Ali-Kasemi, M., 2010. Prevalence and antimicrobial resistance of *Campylobacter* species isolated from chicken carcasses during processing in Iran. Poult. Sci. 89, 1015–1020.

Ribot, E.M., Fitzgerald, C., Kubota, K., Swaminathan, B., Baret, T.J., 2001. Rapid pulsed field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J. Clin. Microbiol. 39, 1889–1894.

Rönner, A.C., Borch, E., Kaijser, B., 2005. Genetic profiling of *Campylobacter jejuni* strains from humans infected in Sweden or in Thailand, and from healthy Swedish chickens, studied by pulsed-field gel electrophoresis (PFGE). Scand. J. Infect. Dis. 37, 579–584.

Sahin, O., Fitzgerald, C., Stroika, S., Zhao, S., Sippy, R.J., Kwan, P., Plummer, P.J., Han, J., Yaeger, M.J., Zhang, Q., 2012. Molecular evidence for zoonotic transmission of an emergent, highly pathogenic *Campylobacter jejuni* clone in the United States. J. Clin. Microbiol. 50, 680–687.

Saito, S., Yatsuyanagi, J., Harata, S., Ito, Y., Shinagawa, K., Suzuki, N., Amano, K., Enomoto, K., 2005. *Campylobacter jejuni* isolated from retail poultry meat, bovine feces and bile and human diarrheal samples in Japan: comparison of serotypes and genotypes. FEMS Immunol. Med. Microbiol. 45, 311–319.

Salihu, M.D., Junaidu, A.U., Magaji, A.A., Yakubu, Y., 2012. Prevalence and antimicrobial resistance of Thermophilic *Campylobacter* isolates from commercial broiler flocks in Sokoto, Nigeria. Res. J. Vet. Sci. 5, 51–58.

Savasan, S., Ciftci, A., Diker, K.S., 2004. Emergence of quinolone resistance among chicken isolates of *Campylobacter* in Turkey. Turk. J. Vet. Anim. Sci. 28, 391–397.

Senok, A., Yousif, A., Mazi, W., Sharaf, E., Bindayna, K., Elnima, el-A., Botta, G., 2007. Pattern of antibiotic susceptibility in *Campylobacter jejuni* isolates of human and poultry origin. Jpn. J. Infect. Dis. 60, 1–4.

Steinhauserová, I., Mikulicova, M., 2005. The evaluation of antimicrobial resistance of *Campylobacter jejuni* strains of poultry and human origin. Proceedings of the XVII European Symposium on the Quality of Poultry Meat and XI European Symposium on the Quality of Eggs and Egg Products, Golden Tulip Park Hotel Doorwerth, Doorwerth, Netherlands, 23-26 May, pp. 200–204.

Tadesse, D.A., Bahnson, P.B., Funk, J.A., Thakur, S., Morrow, W.E., Wittum, T., DeGraves, F., Rajala-Schultz, P., Gebreyes, W.A., 2011. Prevalence and antimicrobial resistance profile of *Campylobacter* spp. isolated from conventional and antimicrobial-free swine production systems from different U.S. regions. Foodborne Pathog. Dis. 8, 367–374.

Unicomb, L.E., Ferguson, J., Stafford, R.J., Ashbolt, R., Kirk, M.D., Becker, N.G., Patel, M. S., Gilbert, G.L., Valcanis, M., Mickan, L., 2006. Low-level fluoroquinolone resistance among *Campylobacter jejuni* isolates in Australia. Clin. Infect. Dis. 42, 1368–1374.

Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward, D.L., Rodgers, F.G., 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J. Clin. Microbiol. 40, 4744–4747.

Wardak, S., Jagielski, M., 2009. Evaluation of genotypic and phenotypic methods for the differentiation of *Campylobacter jejuni* and *Campylobacter coli* clinical isolates from Poland. II. PFGE, ERIC-PCR, PCR-flaA-RFLP and MLST. Med. Dosw. Mikrobiol. 61, 63–77.

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

Wolfs, T.F.W., Duim, B., Geelen, S.P.M., Rigter, A., Thomson-Carter, F., Fleer, A., Wagenaar, J.A., 2001. Neonatal sepsis by *Campylobacter jejuni*: genetically proven transmission from a household puppy. Clin. Infect. Dis. 32, 97–99.

Zhang, M., Gu, Y., He, L., Ran, L., Xia, S., Han, X., Li, H., Zhou, H., Cui, Z., Zhang, J., 2010. Molecular typing and antimicrobial susceptibility profiles of *Campylobacter jejuni* isolates from north China. J. Med. Microbiol. 59, 1171–1177.