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

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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

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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