The Prevalence of Campylobacter jejuni in Various Sources in Kayseri, Turkey, and Molecular Analysis of Isolated Strains by PCR-RFLP*

Fuat AYDIN1,**, K. Semih GÜMÜŞSOY1, Tuba İÇA1, Bülent SÜMERKAN2, Duygu EŞEL2, Mehmet AKAN3, Ayşe ÖZDEMİR4

1Department of Microbiology, Faculty of Veterinary Medicine, Erciyes University, Kayseri - TURKEY
2Department of Microbiology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY
3Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY
4Kayseri State Hospital, Laboratory of Microbiology, Kayseri - TURKEY

Received: 11.07.2005

Abstract: The objective of this study was to isolate, identify, and genotype Campylobacter jejuni from various sources in the province of Kayseri, Turkey. A total of 6667 samples consisting of 5167 human fecal swabs, 600 dog rectal swabs, 600 cattle gallbladders, and 300 chicken carcasses were examined. The samples were plated onto mCCDA (cefoperazone charcoal desoxycholate agar) agar. In order to identify C. jejuni, phenotypic tests and PCR (polymerase chain reaction) were performed. C. jejuni was isolated in 1.43%, 43.50%, 31.16%, and 56% of the human, dog, cattle, and chicken samples, respectively. Among the 690 C. jejuni strains that were isolated during the study period, 200 C. jejuni strains (50 strains from each species) were randomly selected. The selected strains were typed by using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) fla-typing. DdeI and HinfI restriction enzymes were used for molecular typing. Following the DdeI enzyme application, the strains produced various numbers of bands between 4 and 7, with a total of 20 different band profiles. No similar band profiles were seen among the strains isolated from different sources. It was found that HinfI was not a more discriminative enzyme for fla-typing of C. jejuni isolates.

Key Words: Human, dog, chicken, cattle, Campylobacter jejuni, PCR-RFLP (fla-typing)

Kayseri’de (Türkiye) Çeşitli Kaynaklarda Campylobacter jejuni’nin Prevalansı ve İzole Edilen Suşların PCR-RFLP ile Moleküler Analizi

Özet: Bu çalışma Kayseri’de çeşitli kaynaklardan Campylobacter jejuni’nin izolasyonu,_identifikasyonu ve idente edilen suşların genetik olarak tiplendirilmesi amacıyla yapıldı. Çalışma kapsamına, 5167 insan dışkı swabı, 600 adet köpek rektal swabı, 600 adetissement safrı kesesi ve 300 adet (paket) tavuk eti olmak üzere toplam 6667 adet numune incelendi. Örnekle izolasyon amacıyla mCCDA (cefoperazone charcoal desoxycholate agar) agarı ekildi. C. jejuni’nin identifikasyonunda fenotipik testler ve PCR (Polimeraz Zinciri Reaksiyonu)’den yararlanıldı. İnsan, köpek, dışkı ve tavuk örneklerinden sırasıyla % 1,43, % 43,50, % 31,16 ve % 56 oranlarında C. jejuni izole edildi. Çalışma periyodu boyunca izole edilen 690 adet C. jejuni suşu içerisinde 200 adet seçilen PCR-RFLP, (Polimeraz Zinciri Reaksiyon-Restriction Fragment Length Polymorphism) fla-typing yöntemi ile tiplendirildi. Moleküler tiplendirme amacıyla DdeI ve HinfI restriksiyon enzimleri kullanıldı. DdeI enzimi ile muamelede sonra suşlar 4 ile 7 arasında band oluşturdu ve incelenen tüm suşlar 20 farklı band profili gösterdi. Farklı kaynaklardan izole edilen suşlar arasında benzer band profili veren suşlara rastlanmadı. Bu çalışmada HinfI enziminin suşların tiplendirilmesinde aynı olmay工作总结landı.

Anahtar Sözcükler: İnsan, köpek, tavuk, dışkı, Campylobacter jejuni, PCR-RFLP (fla-typing)

* This work was supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey) (VHAG-1834).
** E-mail: faydin@erciyes.edu.tr
Introduction

Campylobacter is commonly found in the gastrointestinal tracts of domestic and wild animals and is commensal (1,2). Many strains, however, particularly Campylobacter jejuni (C. jejuni), are enteric human pathogens. It is widely assumed that campylobacteriosis is primarily a food-borne disease. Contaminated meat, milk, and water are thought to be the major sources of human infection. Domestic pets, wild birds, and wild animals are also potential sources of C. jejuni infection in humans. Transmission occurs through the consumption of contaminated water and animal products (e.g., meat and milk), direct contact with infected animals, or handling undercooked poultry (3).

Several strain typing methods (e.g., phenotyping and genotyping) have been developed to understand the epidemiology of and to identify the transmission routes of C. jejuni, particularly in regard to humans. Although various phenotyping methods have been described, such as serotyping, biotyping, and phage-typing, these methods require specialist skills and a reagent, and are time consuming. It is also difficult to standardize these methods globally (4). Recently, several new genotyping techniques have been developed, including ribotyping, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), flagellin typing (fla-typing), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) (4,5).

The flagellin gene locus of C. jejuni contains 2 flagellin genes (flaA and flaB). This locus is suitable for PCR-RFLP analysis of PCR products because both genes are highly conserved, and variable regions are present. Thus, it has been reported that the use of a primer specifically designed for the amplification of fla in PCR-RFLP (fla-typing) is a useful, reliable, simple, and valuable subtyping technique for epidemiological studies (4).

The current study was undertaken to determine the prevalence of C. jejuni in various sources in Kayseri, Turkey. A secondary objective was the detection of C. jejuni subtypes using PCR-RFLP (fla-typing).

Materials and Methods

Bacterial strains

Between September 2002 and August 2003, 619 C. jejuni strains were recovered from different sources. The origin and number of these isolates are presented in Table 1. Human samples were taken from diarrheic patients. The dog and cattle samples were obtained from healthy animals. C. jejuni NCTC 11168 was used as the reference strain.

Isolation of enteric campylobacters

Modified CCDA (mCCDA) (LAB M lab 112) and a selective supplement (LAB M, cefoperazone-amphotericin, X112) were used for primary isolation of enteric campylobacters. Incubation was performed under microaerobic conditions for 24 to 48 h at 37 °C. All strains were identified using classical methods (2,6).

Identification of C. jejuni

A. Phenotyping assay

C. jejuni was identified by observing characteristic morphology and motility using phase contrast microscopy and also by using a phenotyping assay, which included growth patterns at various temperatures (25 °C and 42 °C), catalase production, oxidase reaction, hippurate hydrolysis, H₂S production, and susceptibility to nalidixic acid and cephalothin (2,7).

B. Identification of C. jejuni by PCR

CeuE gene-specific primers (JEJ1 5'-CCT GCT ACG GTG AAA GTT TTG C-3' and JEJ2 5'-GAT CTT TTT GTT TGC TGC-3') were used for identification of C. jejuni (8).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), (fla-typing)

Among the 690 C. jejuni strains that were isolated during a 1-year period, 200 C. jejuni strains (50 human, 50 chicken, 50 cattle, and 50 dog) were randomly selected. They were genotyped by a slightly modified PCR-RFLP fla-typing method, which is described elsewhere (9).

Bacterial DNA was prepared using a commercial DNA isolation kit (Genomic DNA Purification Kit, Fermentas, Lithuania). DNA concentrations were measured using a spectrophotometer (A₂₆₀) and diluted with sterile water to approximately 20 ng/µl.
FlaA genes (approximately 1700 bp) were amplified with specific primers (A1: 5’-GGA TTT CGT ATT AAC ACA AAT GGT GC-3’, A2: 5’-CTG TAG TAA TCT TAA AAC ATT TTG-3’) and digested separately using DdeI (Promega, USA) and HinfI (Fermentas, Lithuania). Amplified and digested fragments were visualized using a GeneSnap-Gene Genius Bio Imaging System (Syngene, Cambridge, UK) and analyzed using Gene Tools software from Syngene. Genetic similarity among strains was calculated on a simple matching coefficient (10). The size of digested fragments on the gel was calculated from migration distances using UPGMA (unweighted pair group method with arithmetic mean) algorithms (11).

Results
Isolation of enteric campylobacters and identification of C. jejuni

The number of enteric campylobacters and the isolation rates of C. jejuni are given in Table 1. All presumptive C. jejuni strains identified with phenotyping methods were found to be positive by PCR; 793 bp fragments were observed on agarose gel.

PCR-RFLP fla-typing

PCR products amplified with the flaA gene-specific primer were present in bands of 1700 bp (Figure 1). After the digestion of the amplicon with DdeI and HinfI, bands ranging from 100 to 1100 bp were detected.

DdeI restriction

Analysis of the 200 strains selected randomly from different isolates resulted in 20 different band profiles consisting of 4 to 7 bands each, after digestion with DdeI. The patterns of each band were evaluated as a group. No relationship among the strains of different origins could be detected (Table 2).

Fifty human isolates formed 4 different groups; 28 isolates were detected in the first group, 8 in the second group, and 7 isolates each in the third and fourth groups (Figure 2). Similarity levels among the groups were 57.14% to 66.67%.

Table 1. Number of enteric campylobacters and isolation rates of C. jejuni recovered from different sources.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of samples</th>
<th>No. of enteric campylobacters</th>
<th>C. jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of positive samples</td>
</tr>
<tr>
<td>Humans *</td>
<td>5167</td>
<td>108</td>
<td>74</td>
</tr>
<tr>
<td>Dogs**</td>
<td>600</td>
<td>331</td>
<td>261</td>
</tr>
<tr>
<td>Cattle**</td>
<td>600</td>
<td>272</td>
<td>187</td>
</tr>
<tr>
<td>Chickens</td>
<td>300</td>
<td>230</td>
<td>168</td>
</tr>
<tr>
<td>**TOTAL</td>
<td><strong>6667</strong></td>
<td><strong>941</strong></td>
<td><strong>690</strong></td>
</tr>
</tbody>
</table>

* Fecal samples were taken from diarrheic patients
** Dog rectal swab samples and cattle gallbladders were taken from healthy animals
Fifty dog isolates formed 5 different groups; 7 isolates were detected in the first group, 9 in the second, 10 in the third, 11 in the fourth, and 13 in the fifth (Figure 3). Similarity levels among the groups were 52.63% to 70%.

Fifty cattle isolates formed 5 distinct groups; 5 isolates were detected in the first group, 9 in the second, 10 in the third, 11 in the fourth, and 15 in the fifth (Figure 3). Similarity levels among the groups were 47.06% to 75%.

Fifty poultry isolates formed 6 different groups; 5 isolates were detected in the first group, 6 in the second, 7 in the third, 8 in the fourth, 11 in the fifth, and 13 in the sixth (Figure 3). Similarity levels among the groups were 50% to 77.78%.

HinfI restriction

Analysis of the 200 strains resulted in 2 different banding patterns formed by 2 to 3 bands (Table 3). In all, 190 isolated strains of different origins were detected in the first group and 10 strains were detected in the second group (Figure 4). In the first group, 40 human,
and all dog, cattle, and chicken strains were detected. The rest of the human strains were detected in the second group. The similarity level between the 2 groups was 57.14%.

### Discussion
Various studies have revealed that campylobacters present in the intestinal contents of chickens spread to their carcasses during slaughter, contaminating the carcasses, thus resulting in a public health risk (12). Isolation rates of *C. jejuni* from contaminated carcasses show variability. The *C. jejuni* rates found in chicken carcasses in different countries included 56% by Yıldız and Diker (13), 54% by Kwiatek et al. (14), 61% by Shih (15), 41% by Quinones-Ramirez et al. (16), and 50% by Özer and Ergün (17).

*C. jejuni* is commensally present in the intestinal flora of cattle (18,19) and dogs (20,21). In addition to the above-mentioned chicken carcasses, dogs and cattle also present a risk factor for human campylobacteriosis. Torre and Tello (20) isolated *C. jejuni* from healthy dogs at a...
rate of 14.36% and Sandberg et al. (21) isolated it at a rate of 3%. Diker and Istanbulluoglu (22) found C. jejuni in 62% of healthy cattle and 27% of healthy calves. Diker (6) found C. jejuni at the rate of 14% in both cattle gallbladders and stool samples. Çetin et al. (23) found it in 7% of cattle feces.

In the present investigation, C. jejuni was isolated at a rate of 56% from chicken carcasses, 31.16% from cattle gallbladders, and 43.50% from dog rectal swabs. The C. jejuni rate determined in this study was similar to those found in previous studies of chicken intestines in Turkey and other countries; however, the isolation rate of C. jejuni in dogs and cattle was higher than those found in other studies. Differences in isolation rates of C. jejuni may be attributed to several factors, such as sample size, medium, and isolation and identification procedure.

Compared to other pathogens, campylobacters are the most frequently isolated agents causing gastroenteritis in developed countries (3). In Turkey, C. jejuni isolation rates in humans with enteritis were found to be 7.5% by Işık et al. (24), 8.80% by Yıldırım et al. (25), and 2.25% by Aktaş and Tuncel (26).

In our study, C. jejuni was isolated at the rate of 1.43% in the feces of humans with enteritis. The isolation rate of C. jejuni in humans was lower in our study than in other studies performed in other cities. These differences may be due to variances in consumption rates and cooking procedures for meat, number of examined samples, direct contact with domestic animals, and milk and water hygiene in Kayseri.

Fla-typing methods are applied to understand the epidemiology of campylobacteriosis, and in particular to establish sources of outbreaks and transmission routes. The restriction enzymes used in PCR-RFLP fla-typing methods, such as AluI, DdeI, HinfI, MboI, EcoRI, and PstI, are generated from different PCR product fragments and used in various combinations (4,27).

Lindstedt et al. (28) performed DdeI digestion on 84 C. jejuni strains of different origins and observed 18 different band patterns. In other studies, 19 differently and 19 similarly originated C. jejuni isolates were analyzed with RFLP and digested with DdeI. The differently originated strains revealed 6 band patterns and similarly originated strains displayed 5 band patterns. The patterns ranged from 3 to 7 bands. However, when digestion was performed with HinfI, fewer bands formed with DdeI (29). Ertaş et al. (30) recorded 57 C. jejuni strains isolated from broiler chicken carcasses, which formed into 7 different band patterns after fla-typing.

Nielsen et al. (5) typed 80 C. jejuni strains (isolated from humans, cattle, and chickens) using 6 different genotyping methods, including PCR-RFLP with DdeI and AluI, and detected 40 different band patterns. Harrington et al. (11) reported that DdeI appears to provide the best discrimination level, which can be enhanced by combining DdeI with HinfI patterns.

DdeI and HinfI enzymes were used independently in the present study. After digestion by DdeI, strains formed bands ranging from 4 to 7, resulting in 20 different band profiles. No similarities were detected among the band profiles of isolates with different origins. After the completion of RFLP, human, dog, cattle, and chicken isolates were separated into 4, 5, 5, and 6 groups, respectively, according to their banding patterns. However, when isolates were restricted with HinfI, 2 different banding patterns consisting of 2 to 3 bands each were observed. Results of the present study are in agreement with those of previous studies of PCR-RFLP fla-typing (11,31). Harrington et al. (11) also emphasized that HinfI alone was not very discriminatory.

In conclusion, the PCR-RFLP fla-typing method demonstrated particular usefulness for subtype identification of C. jejuni isolates. It was observed that the use of enzyme combinations in these techniques provides more information of strain genotyping levels and assists in the understanding of the epidemiological surveillance of strains. It should be noted that the prevalence of C. jejuni is high in Kayseri, especially in chicken carcasses, dogs, and cattle.
References


