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Isolation and Identification of *Campylobacter jejuni* and *Campylobacter coli* From Various Animal Source Foods by Conventional Methods and PCR*

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Abstract: In this study, 300 samples consisted of chicken meats, ground beef, and gallbladder of cattle and sheep were collected from various markets, butchers and abattoirs in the Eastern Anatolia region in Turkey. The samples were evaluated for the presence of *Campylobacter jejuni* and *Campylobacter coli. Campylobacter spp.* was isolated from 16 (5.3%) of the samples by conventional methods. The isolates were identified by biochemical analyses and the polymerase chain reaction technique, which revealed that 12 (75%) of samples were *Campylobacter jejuni* while 4 (25%) of them were *Campylobacter coli.* As a result, it was considered that *Campylobacter* species, an important category of microorganisms causing acute bacterial gastroenteritis in humans, are commonly transmitted through foods in animal origin.

Key words: *Campylobacter coli*, *Campylobacter jejuni,* Cattle meat, Chicken meat, Sheep meat.

Farklı Hayvansal Gıdalarda *Campylobacter jejuni* ve *Campylobacter coli'*nin Konvansiyonel Kültürel Metod ve PCR ile İzolasyonu ve İdentifikasyonu

Özet: Bu araştırmada Kuzeydoğu Anadolu Bölgesinde bulunan farklı market, kasap ve kesimhanelerden alınan tavuk eti, koyun eti (kıyma), sığır eti (kıyma) ve safra kesesi olmak üzere toplam 300 örnek incelendi. Tüm örnekler *Campylobacter jejuni* ve *Campylobacter coli* yönünden incelendi. Konvansiyonel metodlar ile örneklerin 16'sında (%5.3) *Campylobacter* spp. pozitif bulundu. İzolatlar biyokimyasal analizler ve PCR tekniği ile tanımlandı. İzolatların 12'sinde (%75) *Campylobacter jejuni* ve 4'ünde (%25) *Campylobacter coli* identifiye edildi. Sonuç olarak, *Campylobacter* türlerinin, genellikle insanlara hayvansal gıdalarla bulaşan akut bakteriyel gastroenteritise sebep olan önemli zoonozlardan olduğu düşünülmektedir.

Anahtar kelimeler: *Campylobacter coli, Campylobacter jejuni,* Koyun eti, Sığır eti, Tavuk eti.

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INTRODUCTION

Currently, although numerous microorganism groups lead to enteritis and diarrhoea in humans, *Campylobacter coli* (*C. coli*) and *Campylobacter jejuni* (*C. jejuni*) species of the genus *Campylobacter* are commonly isolated in diarrhoea patients (Friedman et al., 2000). These species also cause abortion and serious economic losses in farm animals (Ertaş et al., 2002). These pathogenic bacteria have a zoonotic character, display a commensalist existence in the intestinal lumen of different animals, and induce various intestinal and extra-intestinal diseases in humans via contaminated food and water (Örmeci, 2007). *Campylobacter* species generally grow at 37 ^{o}C, while *C. jejuni* and *C. coli* species are thermophilic and grow at 42 ^{o}C. Since these two species share many identical phenotypic characters, some difficulties are encountered in their microbiological differentiation. In numerous studies, both DNA-based molecular methods, such as polymerase chain reaction (PCR), in addition to conventional methods were used to differentiate the species. Determination of new target genes specific to the *Campylobacter* species has facilitated the identification of these bacteria (Oyofo et al., 1992; Açık, 2006). In this study, we aimed to identify *C. jejuni* and *C. coli* in samples obtained from animal sources by using conventional methods and PCR technique.

MATERIALS and METHODS

Sample Collection

In this study, 300 samples were collected. The samples consisted of chicken meats (100), ground beef (50) of cattle and sheep (50), gallbladder of cattle (50) and sheep (50). The samples were collected randomly at butchery, abattoirs, and markets in the Eastern Anatolia Region, Turkey. All collected samples were stored in clean bags and freighted to laboratory for preparation. The samples were carried to the laboratory within 1 h and analysed in the same day.

Culture and Isolation

Following the acquisition of chicken meat, ground beef, and gallbladder bile samples under aseptic conditions, pre-enrichment procedure was carried out in c*ampylobacter* selective broth (OXOID CM0067B) at 42 ^{o}C for 48 hours in a microaerophilic environment. After the enrichment, the samples were subcultured into *campylobacter* selective agar (OXOID CM0739B), and the bacterial growths on plates were then evaluated for their colony formation and microscopic characteristics (Oyofo et al., 1992; Ertaş et al., 2002).

Biochemical Tests

The pre-diagnosis was achieved on colony suspected of *Campylobacter spp.*, by using conventional methods and a Vitek II COMPAQ system (Biomerieux). Gram staining, motility, catalase, nitrate reduction, H_2S, hippurate hydrolysis, urea, arginine arylamidase, gamma-glutamyl transferase, and other biochemical tests (Leucyne Arylamidase, Ornithine decarboxylase etc.) were applied for this purpose.

Genotypic Confirmation of Isolates

The DNA extraction from the isolates was performed using a commercial extraction kit (Wizard Genomic DNA Purification Kit, Promega) according to the instructions of the manufacturer. The DNA pellets were dissolved in 50 µl sterile distilled water and stored at -20 ^{o}C until the analysis. During the PCR, primers specific to the *ceu*E gene and capable of amplifying a region of 100 bp length in *C. jejuni* and 130 bp length in *C. coli*, were used (Aydın et al., 2005).

While Cc-F1 Forward (5’-CATATTGTAAAACCAAAGCTTATCG-3’) and Cc-R1 Reverse (5’-AGTCCAGCAATGTGTGCAATG-3’) were used for *C. coli*, Cj-F1 Forward (5’-TGCTAGTGAGGTTGCAAAAGAATT-3’) and Cj-R1 Reverse (5’-TCATTTCGCAAAAAA ATC CAA A-3’)

primers (Tıbmol Biol, Germany) were employed for *C. jejuni*. The reaction mixture consisted of 3.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM dNTP, 0.5 U Taq DNA polymerase enzymes (Fermentas, Lithuania), and 1 µM from each primer in the final concentration of 25 µl. A 2.5 µl target DNA was added into the reaction mixture (22.5 µl) to achieve a total volume of 25 µl. The reaction was carried out in a thermal cycler (Thermo Hybaid, UK) at 95 ^{o}C for 10 minutes in order to reach 40 cycles, at 95 ^{o}C for 20 sec, and at 60 ^{o}C for 1 min (Lagier et al., 2004). Following the amplification, PCR products were run on agarose gel of 2 % at electrophoresis. After that, gel was stained with ethidium bromide and visualized under an ultraviolet (UV) transilluminator.

Reference Strains

Campylobacter jejuni (ATCC33291) and *Campylobacter coli* (ATCC 33559) were used as reference strains for DNA extraction and PCR procedures.

RESULTS

C. jejuni was isolated and identified in 12 (4%) out of 300 samples examined, whereas *C. coli* was isolated and identified in 4 (1.3%) of samples. While *C. coli* was isolated and identified in 2 chicken meat samples (2%) and 2 ground mutton (4%), *C. jejuni* was isolated and identified from 2 chicken meat samples (2%), 9 ground mutton (18%) and only 1 sheep gallbladder (2%). No *Campylobacter spp.* was isolated from the cattle ground meat and gallbladder samples collected (Table 1). The genomic DNAs extracted from those isolates were subjected to PCR using Cc-F1/R1 and Cj-F1/R1 primer pairs. Positive bands were found for *C. jejuni* (100 bp) and *C .coli* (130 bp) in 2% agarose gel (Figures 1 and 2).

Table 1. The distribution of *C. coli* and *C. jejuni* isolates in samples collected.
Tablo 1. Örneklerden izole edilen *C. coli* and *C. jejuni'* nin dağılımı.

Animal sources, (n)	*C. coli* n (%)	*C. jejuni* n (%)
Chicken meat, (100)	2 (2)	2 (2)
Cattle gallbladder, (50)	0	0
Cattle ground beef, (50)	0	0
Sheep gallbladder, (50)	0	1 (2)
Sheep ground beef, (50)	2 (4)	9 (18)

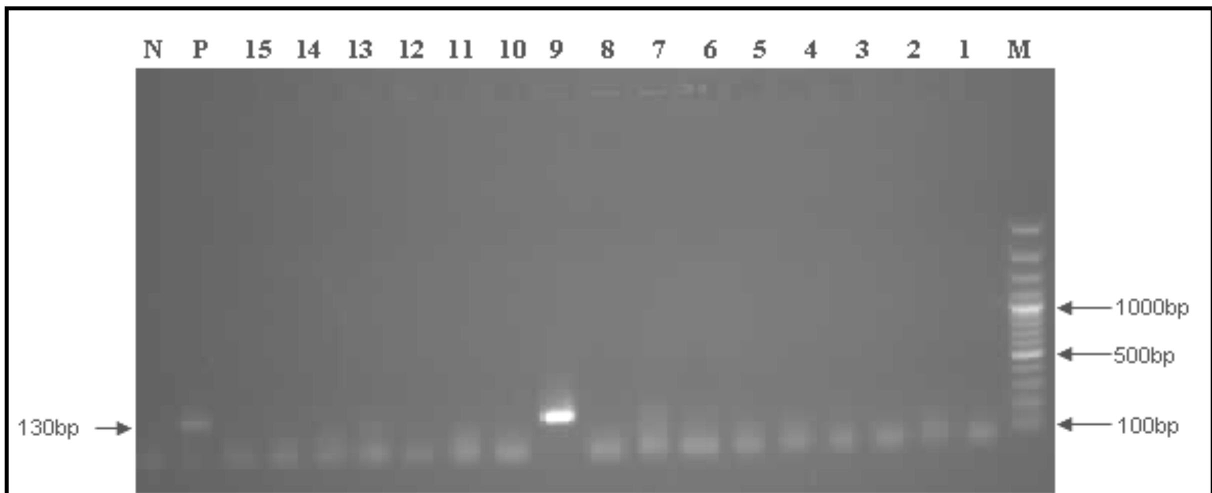

Figure 1. PCR products of *Campylobacter coli* isolate on agarose gel (2%) stained with ethidium bromide. M: Marker, 9: Isolate, P: Positive control, N: negative control (distilled water).
Şekil 1. Ethidium bromid ile boyanan %2'lik agaroz jeldeki *Campylobacter coli* izolatlarına ait PCR ürünleri M: Marker, 9: İzolat, P: Positif kontrol, N: Negatif kontrol (distile su).

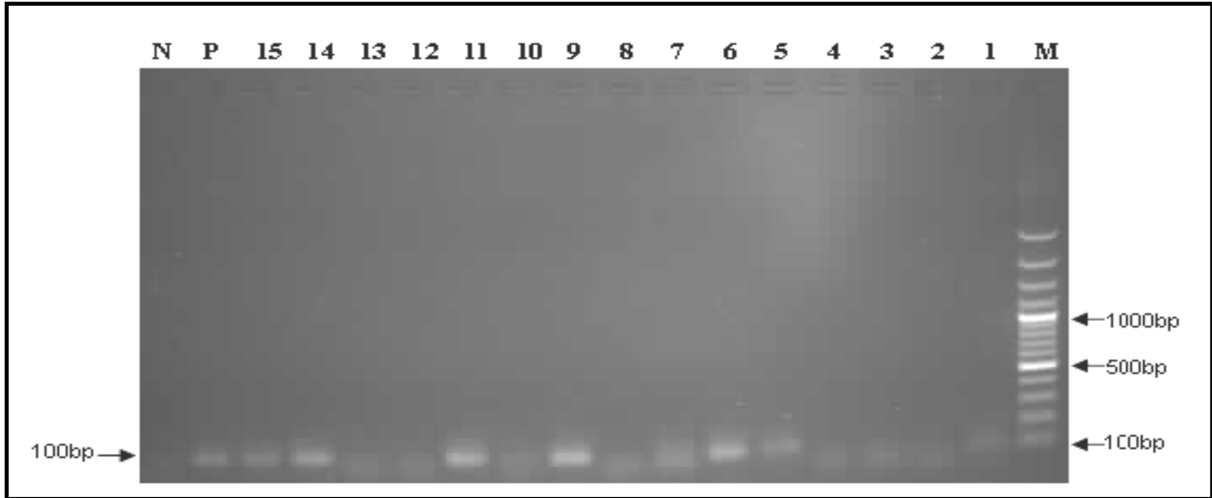

Figure 2. PCR products of *Campylobacter jejuni* isolate on agarose gel (2%) stained with ethidium bromide. M: Marker, 1-15: Isolate, P: Positive control, N: negative control (distilled water).
Şekil 2. Ethidium bromid ile boyanan %2'lik agaroz jeldeki Campylobacter jejuni izolatlarına ait PCR ürünleri M: Marker, 1-15: İzolat, P: Positif kontrol, N: Negatif kontrol (distile su).

DISCUSSION and CONCLUSION

The *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* species of *Campylobacter genus* are thermophilic bacteria that can grow at 42 °C. Among these, *C. jejuni* and *C. coli* are particularly recognized as the two of the most important food-borne zoonotic pathogens for both human and animal health. In humans, acute bacterial gastroenteritis is associated with *C. jejuni* in 90–95 % and *C. coli* in 5–10 % of reported cases (Friedman et al., 2000, Vandamme, 2000). Recently, the prevalence of *Campylobacter*-related food infections has been noted as surpassing the prevalence of *Salmonella*-related infections; thus, the former is predicted to become one of the most frequently isolated food-borne pathogens (Açık, 2006). Etiologic diagnosis has a crucial role to combat with the infections. Although classical (conventional) methods are successful up to the isolation stage, they may lead to inaccurate results in differentiating *C. coli* and *C. jejuni* (Açık, 2006). The reliability and validity of these methods are suspicious since numerous authors have achieved different results from the same isolates, and there is not yet standardization for identification using biochemical tests (On, 1996). Therefore, investigators generally recommend molecular techniques using genomic DNA, such as PCR, for a definitive diagnosis and distinction between the species (Sails et al., 1998; Oyofo et al., 1992; Comi et al., 1995). In this study, the identification after isolation was performed using the automatized Vitek II COMPAQ system (based on a system similar to that used in classical biochemical tests), followed by confirmation by PCR.

Various studies have employed the enrichment method to isolate pathogens from samples studied for the presence of *Campylobacter spp.* (Mehlman and Romero, 1982; Baylis et al., 2000). In the present study, the inoculation and culture technique was used after enrichment in order to improve the isolation rate. Proper collection and transportation of microbiologic samples is critical with regard to accurate diagnosis and isolation because *Campylobacter spp.* is influenced adversely or favourably by transportation factors such as delivery time (duration from the sample collection to submission to the laboratory). Even if the transportation is fully complied with the cold chain and sterility rules, the isolation rate is reduced in samples that are not examined shortly after

collection (Gülmez, 1999). Açık (2006) observed a reduced isolation rate when samples collected from the intestinal contents of animals were transported to the laboratory within approximately 12 hours. In the present study, the delivery times of the gallbladder bile samples collected from the abattoir (6–8 hours) were longer than that of meat samples collected from markets (1–2 hours). Trials involving the isolation of *Campylobacter* species from cattle have produced variable (1-90 %) results in both Turkey and abroad (Rosef et al., 1983, Diker, 1987; Stanley et al., 1998). In one study, the isolation rate of *Campylobacter spp.* from 1.154 rectal swaps and liver samples collected in the Eastern region of Turkey was 26.1% (Açık, 2006). In our study, no thermophilic *Campylobacter* species was isolated from the cattle ground meat and bile samples, whereas the samples collected from sheep demonstrated an isolation rate of 12%. As food-borne pathogens, these species generally cause infections by contaminating poultry meat (Stern and Meinersmann, 1989; Gülmez, 1999). Studies in our country and across the world have shown high isolation rates of *C. coli* and *C. jejuni* in samples of poultry meat (Berry et al., 1988; Gülmez, 1999; Deckert et al., 2010).

By using genotyping techniques in Campylobacter infections, definitive diagnoses, differentiation between species, and various epidemiologic characteristics, such as infection source and routes of transmission, can be determined. Thus, data on isolates can be accurately assessed to develop not only the protection from infections but also new control strategies.

In conclusion, in this study, we were able to isolate and identify *Campylobacter spp.*, particularly in samples obtained from chicken meat and sheep ground beef. Therefore, we believe that the role of animal sources should not be overlooked with regard to public health, and animal products and consumer markets should be frequently inspected to minimize the risk of infection.

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