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Antimicrobial resistance patterns of *Campylobacter* spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran

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ABSTRACT

This study was conducted to determine the prevalence and antimicrobial resistance pattern of *Campylobacter* spp. isolated from retail raw poultry meats in Iran. From July 2009 to March 2010, a total of 494 raw meat samples from chicken ($n = 200$), turkey ($n = 170$), quail ($n = 86$), partridge ($n = 17$), and ostrich ($n = 21$) were purchased from randomly selected retail outlets in Shahrekord, Iran. Using cultural method, 187 meat samples (37.9%) were contaminated with *Campylobacter*. The highest prevalence of *Campylobacter* spp. was found in chicken meat (47.0%) followed by quail (43.0%), partridge (35.3%), turkey (28.8%), and ostrich (4.8%) meat. The most prevalent *Campylobacter* species was *Campylobacter jejuni* (92.0%). The PCR assay could identify 38 *Campylobacter*-contaminated samples that were negative using the cultural method. Antimicrobial susceptibility test results showed that 98.4% of isolates were resistant to one or more antimicrobial agents. Resistance to tetracycline was the most common findings (70.6%), followed by resistance to nalidixic acid (54.0%), and ciprofloxacin (49.7%). Significantly higher prevalence rates of *Campylobacter* spp. ($P < 0.05$) were found in meat samples taken in summer (51.1%). To our knowledge, the present study is the first report of the isolation of *Campylobacter* spp. from raw partridge meat in Iran.

1. Introduction

Campylobacter spp. represent as one of the major causes of foodborne diseases in humans beings worldwide and are recognized as one of the most prevalent causes of human foodborne diarrhea illness in children and young adults in developing countries (Franchin, Ogliari, & Batista, 2007; Kemp, Aldrich, Guera, & Scheider, 2001; Oberhelman & Taylor, 2000). *Campylobacter* spp. are gram-negative bacteria in the family Campylobacteriacae with microaerobic growth requirement. Infection with *Campylobacter* in humans is widely held to be the result of handing and consuming of raw poultry and cross-contamination of uncooked products (Corry & Atabay, 2001). The most important *Campylobacter* species associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* (Wesley et al., 2000).

Studies have demonstrated high levels of contamination with *Campylobacter* spp. on poultry from farm (Stern, Clavero, Bailey, Cox, & Robach, 1995) and retail poultry meat with contamination rates ranging from 40% to 100% (Dickins et al., 2002; Taremi et al., 2006; Hussain, Mahmood, Akhtar, & Khan, 2007; Suzuki & Yamamoto, 2009). Poultry carcasses are commonly contaminated with *Campylobacter* in poultry processing plants (Corry & Atabay, 2001; Franchin et al., 2007). Contamination during processing occurs directly via intestinal contents or indirectly from bird to bird, via equipment and water (Corry & Atabay, 2001).

The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter* (Aarestrup & Engberg, 2001), which has potentially serious impact on food safety in both veterinary and human health (Van Looveren et al., 2001). Although *Campylobacter* with resistance to antimicrobial agents has been reported worldwide (Han, Jang, Choo, Heu, & Ryu, 2007; Praakle-Amin, Roasto, Korkeala, & Hänninen, 2007; Van Looveren et al., 2001), the situation seems to deteriorate more rapidly in developing countries, where there is widespread and uncontrolled use of antibiotics (Hart & Kariuki, 1998; Taremi et al., 2006).

Campylobacter is a difficult organism to culture and maintain in the laboratory (Solomon & Hoover, 1999). Currently, *Campylobacter* is identified using selective culture media and a confirmatory series of biochemical tests. These methods are expensive and time-consuming (Todor, 2004). In recent years, PCR assay has increasingly been applied in the detection and identification of *Campylobacter*. Several studies have shown PCR assay as an accurate and rapid

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method with high diagnostic sensitivity (Englen & Fedorka-Cray, 2002).

Currently, there is limited information regarding the prevalence and antimicrobial resistance of *Campylobacter* in poultry products in Iran. Therefore, this study was conducted to determine the prevalence and antimicrobial resistance patterns of *Campylobacter* spp. isolated from raw chicken, turkey, quail, partridge, and ostrich meat in Iran.

2. Materials and methods

2.1. Samples

From July 2009 to March 2010, 494 poultry meat samples including chicken ($n = 200$), turkey ($n = 170$), quail ($n = 86$), partridge ($n = 17$), and ostrich ($n = 21$) were randomly purchased from 12 retail outlets in Shahrekord, Iran. Shahrekord is the capital of Chaharmahal va Bakhtiari province which is located in the central and southern part of Iran with about 850,000 inhabitants. Samples collected in this study included leg (chicken, turkey and ostrich) and leg and breast (quail and partridge). All samples were taken by using sterilized utensils, placed in separate sterile plastic bags to prevent spilling and cross contamination, and were immediately transported to the laboratory in a cooler with ice packs.

2.2. Isolation and identification Campylobacter

The samples were processed immediately upon arrival using aseptic techniques. Of each meat sample, 25 g from each was homogenized and transferred to 225 mL of Preston enrichment broth base (HiMedia Laboratories, Mumbai, India, M899) containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India, FD042) and 5% (v/v) defibrinated sheep blood. After incubation at 42 °C for 24 h in a microaerophilic condition (85% N2, 10% CO2, 5% O2), 0.1 mL of the enrichment was then streaked onto *Campylobacter* selective agar base (HiMedia Laboratories, Mumbai, India, M994) containing an antibiotic supplement for the selective isolation of *Campylobacter* species (HiMedia Laboratories, Mumbai, India, FD006) and 5% (v/v) defibrinated sheep blood and incubated for 48 h at 42 °C under the same condition. For the chiller tank samples, 50 mL of water samples were added to 50 mL double-strength *Campylobacter* enrichment broth (Preston enrichment broth base, HiMedia Laboratories, M899) and incubated as described above. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and identification was performed using standard microbiological and biochemical procedures including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, and susceptibility to cephalotin (Bolton, Wareing, Skirrow, & Hutchinson, 1992; Whyte et al., 2004).

2.3. DNA extraction and PCR conditions

DNA from 494 samples was extracted from Preston broth after the enrichment step using a Genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. The PCR procedures used in this study have been described previously (Denis et al., 1999). Three genes selected for the identification of the *Campylobacter* spp., *C. jejuni*, and *C. coli* were the *16S rRNA* gene (Linton, Lawson, Owen, & Stanley, 1997), the *mapA* gene (Stucki, Joachim, Nicolet, & Burnens, 1995), and the *ceuE* gene (Gonzalez, Grant, Richardson, Park, & Collins, 1997), respectively. The sequences of the three sets of primers used for gene amplification are presented in Table 1. Amplification reactions were performed in a 30 μL mixture containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 μmol 1^{-1} of each dNTP, 0.11 μmol 1^{-1} of MD16S1 and MD16S2 primers, and 0.42 μmol 1^{-1} of MDmapAl, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradiant, Eppendrof, Germany) with the following program: one cycle of 10 min at 95 °C, 35 cycles each consisting of 30 s at 95 °C, 1 min and 30 s at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The amplification generated 857 bp, 589 bp, and 462 bp DNA fragments corresponding to the *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively. *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) were used as the positive controls and DNase free water was used as the negative control. The PCR products were stained with 1% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose (Figs. 1–3).

Table 1
Primers for polymerase chain reaction (PCR) amplification of campylobacterial DNA for identification DNA.

Organism	Primer	PCR product (bp)	Sequence
Campylobacter spp.	*16SrRNA*	857	5′ ATC TAA TGG CTT AAC CAT TAA AC 3′ 5′ GGA CGG TAA CTA GTT TAG TAT T 3′
Campylobacter jejuni	*mapA*	589	5′ CTA TTT TAT TTT TGA GTG CTT GTG 3′ 5′ GCT TTA TTT GCC ATT TGT TTT ATT A 3′
Campylobacter coli	*ceuE*	462	5′ AAT TGA AAA TTG CTC CAA CTA TG 3′ 5′ TGA TTT TAT TAT TTG TAG CAG CG 3′

2.4. Antimicrobial susceptibility testing

One strain from each *Campylobacter*-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing for 172 *C. jejuni* and 15 *C. coli* isolated strains was performed by the Kirby–Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2006). The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 μg), ciprofloxacin (15 μg), erythromycin (15 μg), tetracycline (15 μg), streptomycin (30 μg), gentamicin (10 μg), amoxicillin (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), and enrofloxacin (10 μg). After incubation at 42 °C for 48 h in a microaerophilic atmosphere, the susceptibility of the *Campylobacter* spp. to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). *Staphylococcus aureus* and

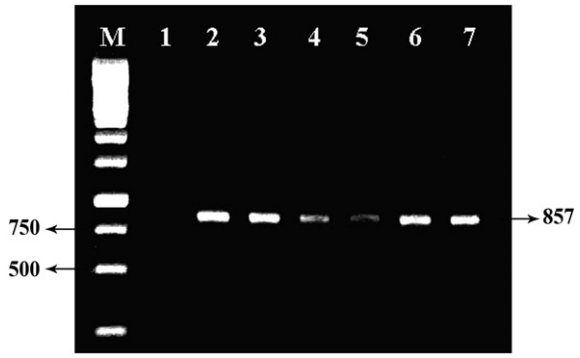

Fig. 1. Ethidium bromide-stained agarose, polymerase chain reaction (PCR) gels. M: 1 kb DNA ladder; lane 1: negative control, distilled water substituted for DNA template; lanes 2–7: positive amplification of *Campylobacter* DNA from culture.