

## Prevalence and characteristic of *Campylobacter* species isolated from raw duck and goose meat in Iran

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**Abstract.** Poultry meat is frequently contaminated with *Campylobacter* strains and is thought to be the major source of organisms causing human *Campylobacter* enteritis. This study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail duck and goose raw meats in Iran. From August 2009 to August 2010, a total of 169 raw duck and goose meat samples were purchased from randomly selected retail outlets in Gilan province, Iran and were evaluated for the presence of *Campylobacter*. Using conventional bacteriological methods, 39 of 110 duck meat samples (35.5%) and 13 of 49 goose meat samples (26.5%) were contaminated with *Campylobacter*, respectively. The most prevalence *Campylobacter* species isolated from samples was *Campylobacter jejuni* (88.5%); the remaining isolates were *Campylobacter coli* (11.5%). All of *Campylobacter* identified as *Campylobacter jejuni* and *Campylobacter coli* by using conventional bacteriological method were also positive Using the PCR assay. Susceptibilities of 52 *Campylobacter* isolates were determined for ten antimicrobial drugs using the disk diffusion assay. Resistance to ciprofloxacin was the most common finding (40.4%), followed by resistance to tetracycline (32.7%) and nalidixic acid (30.8%). Significantly higher prevalence rates of *Campylobacter* spp. ( $P < 0.05$ ) were found in samples taken in spring and summer. To our knowledge, the present study is the first report of the isolation of *Campylobacter* spp. from raw duck and goose meat in Iran.

**Keywords:** *Campylobacter*, raw poultry meat, duck; goose, antimicrobial resistance

### 1. Introduction

*Campylobacter* species are one the most common cause of acute bacterial gastroenteritis in human beings. The most important *Campylobacter* species associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* [1]. Animal-derived foods considered as significant sources of infection. Consumption and handling of poultry and poultry products are the major sources of human *Campylobacter*-enteritis [2]. Analysis of human and poultry strains by serotyping and with different genotyping methods demonstrated an extensive congruence of strains colonizing poultry and infecting humans despite the broad variability in genotypes isolated from poultry and humans [3, 4]. Several epidemiological studies demonstrated high prevalences in chickens, ducks and turkeys, ranging from 40% to 100% [4, 5].

Most patients with *Campylobacter* infections have a self-limited illness and do not require antimicrobial drugs except in cases with sever or prolonged symptoms, or in immunocompromised patients [6]. The use of antimicrobial agents in food animals has resulted in the emergence and dissemination of antimicrobial-resistant bacteria, including antimicrobial-resistant *Campylobacter* [7], which has potentially serious impact on food safety in both veterinary and human health [8]. Although *Campylobacter* with resistance to antimicrobial agents has been reported worldwide [8, 9], the situation seems to deteriorate more rapidly in

developing countries, where there is widespread and uncontrolled use of antibiotics [10]. The present study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from retail raw duck and goose meat in Gilan, Iran.

## 2. Materials and methods

### 2.1. Sample collection

From August 2009 to August 2010, a total of 169 raw duck and goose meat samples were purchased from randomly selected retail outlets in Gilan province, Iran. All samples were 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. Microbiological analysis

The samples were processed immediately upon arrival using aseptic techniques. Of each meat sample, 10 g was homogenized and transferred to 90 mL of Preston enrichment broth base containing *Campylobacter* selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. After inoculation at 42°C for 24 h in a microaerophilic condition (85% N<sub>2</sub>, 10% CO<sub>2</sub>, 5% O<sub>2</sub>), 0.1 mL of the enrichment was then streaked onto *Campylobacter* selective agar base (HiMedia Laboratories, Mumbai, India) supplemented with an antibiotic supplement for the selective isolation of *Campylobacter* species (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood and incubated at 42 °C for 48 h under the same condition. One presumptive *Campylobacter* colony from each selective agar plate was subculture and tested by standard microbiological and biochemical procedure including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxylacetate hydrolysis and susceptibility to cephalotin [11].

### 2.3. DNA Extraction and PCR Condition

Only *Campylobacter* spp. isolates identified by bacteriological methods were tested by PCR. The PCR procedures used in this study have been described previously [12]. Briefly, 1 mL of pure culture of *Campylobacter* was centrifuged at 13000 g for 5 min at room temperature. The DNA was then extracted using a genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. Three genes selected for the identification of the *Campylobacter* spp., *Campylobacter jejuni* and *Campylobacter coli* were the *16S rRNA* gene [13], the *mapA* gene [14], and the *ceuE* gene [15], 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 0.6U Taq polymerase (Fermentas, GmbH, Germany), 100 µmol l<sup>-1</sup> of each dNTP, 0.11 µmol l<sup>-1</sup> of MD16S1 and MD16S2 primers, and 0.42 µmol l<sup>-1</sup> of MDmapA1, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradient, Eppendorf, 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, *Campylobacter jejuni* and *Campylobacter coli*, respectively. *Campylobacter coli* (ATCC 33559) and *Campylobacter 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.

### 2.4. Antimicrobial susceptibility testing

One strain from each *Campylobacter*-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing 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 [16]. The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 µg), ciprofloxacin (15µg), erythromycin (15 µg), tetracycline (15µg), 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 *Escherichia coli* were used as quality control organisms in antimicrobial susceptibility determination.

## 2.5. Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), chi-square test and fisher's exact two-tailed test analysis were performed and differences were considered significant at values of  $P < 0.05$ .

## 3. Results and Discussion

In this study, 30.8% (52 of 169) of all meat samples were contaminated with *Campylobacter* spp. *Campylobacter* were detected in 35.5% and 26.5% duck and goose meat samples respectively. All of *Campylobacter* identified as *Campylobacter* by using conventional bacteriological method were also positive Using the PCR assay. There were not significant differences between duck meat and goose meat samples. The present data showed duck to be prominent reservoirs of *Campylobacter*. These findings are comparable with those reported from Thailand [17] however, are higher than the prevalence reported from Philippines [18]. Boonmar *et al.* (2007) showed that 31% of duck meat samples by PCR were positive for *Campylobacter* species. In the Philippines, Magrtrado *et al.* (2001) found only 6% prevalence of *Campylobacter* isolated from a total 145 duck and chicken samples using both standard culture method and PCR. Noga and Muhairwa (2010) showed that isolation rates of *Campylobacter* species in free range domestic duck in Morogoro municipality, Tanzania 80% [19]. In similar study Abrahams *et al.* (1990) reported a prevalence of 43.5% [20]. No previous report could be found on the occurrence of *Campylobacter* spp. on the goose meat. *Campylobacter* spp. are frequently found in the intestinal tract of poultry where colonization leads to contamination of carcasses during processing, especially at the defeathering, evisceration, and chilling stages. Variations in isolation rates may be due to several reasons. Such as differences in sampling techniques, seasonal effects, laboratory methodologies employed local prevalence of *Campylobacter* in the specific region and management of duck where the study was conducted.

The most prevalence *Campylobacter* species isolated from samples was *Campylobacter jejuni* (88.5%); the remaining isolates were *Campylobacter coli* (11.5%). *Campylobacter jejuni* has been reported to be the most frequent species recovered from food of animal origin specially poultry meat, whereas *Campylobacter coli* is dominant in pigs [21, 22]. Our results on the prevalence of *Campylobacter jejuni* in raw duck and goose meat are in agreement with data from other countries [11, 14, 17, 21, 22].

The highest incidence of *Campylobacter* spp. (48.6%) occurred in summer, with high isolation rate in spring (41.7%), and lower isolation rate in winter (19.5%) and in fall (15.6%). In the current study, no apparent pattern in the seasonality of *Campylobacter* prevalence was observed in retail duck meat samples.

The resistance pattern of *Campylobacter* isolates to 10 antimicrobial agents tested in this study is shown in Table 1. Overall, 43 of 52 *Campylobacter* isolates (82.7%) were resistant to one or more antimicrobial agent. Resistance to ciprofloxacin was the most common finding (40.44%), followed by resistance to tetracycline (32.7%), and nalidixic acid (30.8%). All *Campylobacter* isolates were susceptible to amoxicillin, chloramphenicol, erythromycin, and gentamicin. These results are comparable to those reported by other investigators [8, 23, 24]. The results of antibiotic resistance found in this study are correlated to antibiotic drugs that are being used to treat infection in food animals in Iran. For example, enrofloxacin which is closely related to ciprofloxacin is widely used in animal to treat infection with *Escherichia coli*. We recommend that in vitro susceptibility testing of *Campylobacter* be performed and appropriate treatment be instituted for specific cases with food borne campylobacteriosis, especially in children, the elderly and immunocompromised patients.

TABLE 1. ANTIMICROBIAL RESISTANCE PROFILES OF CAMPYLOBACTER STRAINES ISOLATED FROM DUCK MEAT IN GILAN.

Antimicrobial agent	<i>Campylobacter</i> spp. (N = 52)	<i>C. jejuni</i> (N = 46)	<i>C. coli</i> (N = 6)
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Amoxicillin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ampicillin	5 (9.6%)	5 (10.9%)	0 (0.0%)
Chloramphenicol	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin	21 (40.4%)	19 (41.3%)	2 (33.3%)
Enrofloxacin	7 (13.5%)	7 (15.2%)	0 (0.0%)
Erythromycin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gentamicin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Nalidixic acid	16 (30.8%)	14 (30.4%)	2 (33.3%)
Streptomycin	1 (1.9%)	1 (2.2%)	0 (0.0%)
Tetracycline	17 (32.7%)	14 (30.4%)	3 (50.0%)

The present results showed that duck meat and goose meat carcasses from retail shops proved to be reservoirs of *Campylobacter*. Consequently, implementation of good cooking techniques and good kitchen and personal hygiene during preparation are necessary. Moreover there is a strong need to train and educate food handlers in microbial risks associated with poultry meat and how to control them.

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