Prevalence and antimicrobial resistance of *Campylobacter* species isolated from chicken carcasses during processing in Iran

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ABSTRACT The objective of this study was to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from chicken carcasses during different stages of broiler processing in a major commercial poultry processing plant in southwestern Iran. Overall, 84 chicken carcasses were sampled from 4 sites along the processing line during a total of 7 visits. In addition, 14 water samples from the chiller tank were taken. Using the cultural method, 186 of 336 (55.4%) carcasses were positive for *Campylobacter*. *Campylobacter jejuni* was more frequently isolated (89.4%) than *Campylobacter coli* (10.6%). The frequency of *Campylobacter* spp. on carcasses was 54.8% after defeathering, 51.2% after evisceration, 69.0% 20 min after the chilling period started, and 46.4% 24 h after the chilling period completed. *Campylobacter* was positive in 85.7% of the samples taken from the chilling water. The frequency of *Campylobacter* spp.-positive carcasses was reduced in complete chilled chickens but not during the slaughtering process. Susceptibilities of *Campylobacter* isolates were determined for 10 antimicrobial drugs using the disk diffusion method. Of the 198 *Campylobacter* isolates tested, 92.9% were resistant to one or more antimicrobial agents. Resistance to tetracycline was the most common finding (78.3%), followed by resistance to ciprofloxacin (62.1%), nalidixic acid (58.6%), and enrofloxacin (44.4%).

Key words: *Campylobacter*, chicken, *Campylobacter jejuni*, *Campylobacter coli*, antimicrobial resistance

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INTRODUCTION

Campylobacter is the most common cause of acute bacterial gastroenteritis in human beings (Mead et al., 1999). The most important *Campylobacter* spp. associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* (Wesley et al., 2000). Chicken carcasses are commonly contaminated with *Campylobacter* in poultry processing plants (Atabay and Corry, 1997; Franchin et al., 2007). Studies have demonstrated high levels of *Campylobacter* on broiler chickens from farm (Stern et al., 1995) and retail chickens (Rahimi and Tajbakhsh, 2008), ranging from 40 to 100% (Dickins et al., 2002).

Contamination during processing occurs directly via intestinal contents or indirectly from bird to bird, via equipment and water (Corry and Atabay, 2001). Bacteria adhere to the skin surface and will subsequently form a biofilm that is difficult to remove. These microorganisms encounter certain stressful events during slaughter and processing (Newell et al., 2001) but are largely protected from biocidal activities within that biofilm (Buswell et al., 1998). Several investigators have reported a high prevalence of *Campylobacter* spp. through broiler chicken processing, such as on carcasses collected after defeathering (Cason et al., 1997; Franchin et al., 2007), after evisceration and after water chilling (Cason et al., 1997; Franchin et al., 2007), as well as in the final chilled product (Line, 2001). Consequently, undercooked and raw poultry meats are common vehicles for the transmission of human campylobacteriosis (Corry and Atabay, 2001).

Currently, there is limited information regarding the prevalence and antimicrobial susceptibility patterns of *Campylobacter* in poultry products in Iran. Furthermore, little is known about how processing procedures in poultry processing plants may affect the prevalence of *Campylobacter* in Iran. Therefore, the present study was conducted to determine the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated during

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different stages of broiler processing in a major commercial poultry processing plant in southwestern Iran.

MATERIALS AND METHODS

Samples

Chicken carcasses were collected during 7 visits to a commercial poultry processing plant in Ahvaz from November 2007 to December 2008. Ahvaz, located in southwestern Iran, is the capital of the Khuzestan province, which has more than 4 million inhabitants. This processing plant is the primary poultry plant, with an average of 140,000 chickens being processed per day. Carcasses were randomly chosen and collected by hand using new latex gloves for each carcass from 4 sites along the processing line including after defeathering, after evisceration, 20 min after the chilling period started, and 24 h after the chilling period completed. Overall, 84 chicken carcasses were sampled at each technological step through the processing line. The samples were placed in separate sterile plastic bags to prevent spilling and cross-contamination. All carcasses were subjected to a whole-carcass rinse. Carcasses collected were shaken with 100 mL of sterile distilled water for 60 s. The rinses were poured into 50-mL sterile specimen cups and refrigerated at 4°C. In addition, 14 water samples from the chiller tank were taken during the slaughter process and collected in sterile 50-mL plastic syringes. The samples were immediately transported to the laboratory in a cooler with ice packs and processed within 2 h.

Isolation and Identification of Campylobacter

Twenty-five milliliters of sample was transferred to 225 mL of Preston enrichment broth base (M899, HiMedia Laboratories, Mumbai, India) containing *Campylobacter* selective supplement IV (FD042, HiMedia Laboratories) and 5% (vol/vol) defibrinated sheep blood. After incubation at 42°C for 24 h in a microaerophilic condition (85% N_2, 10% CO_2, 5% O_2), 0.1 mL of the enrichment was streaked onto *Campylobacter* selective agar base (M994, HiMedia Laboratories) containing an antibiotic supplement for the selective isolation of *Campylobacter* spp. (FD006, HiMedia Laboratories) and 5% (vol/vol) defibrinated sheep blood and incubated for 48 h at 42°C under the same condition. For the chiller tank sample, 50 mL of water samples was added to 50 mL of double-strength *Campylobacter* enrichment broth (Preston enrichment broth base, M899, HiMedia Laboratories) and incubated as described above. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and tested by standard microbiological and biochemical procedures (Bolton et al., 1992).

DNA Extraction and PCR Conditions

Deoxyribonucleic acid from all 336 samples (4 × 84) was extracted from Preston broth after the enrichment step using a Genomic DNA Purification Kit (K0512, Fermentas GmbH, St. Leon-Rot, Germany; Sambrook and Russell, 2001) 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 et al., 1997), the *mapA* gene (Stucki et al., 1995), and the *ceuE* gene (Gonzalez et al., 1997), respectively. The sequences of the 3 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 of Taq polymerase (Fermentas GmbH), 100 $\mu mol \cdot l^{-1}$ of each deoxynucleoside triphosphate, 0.11 $\mu mol \cdot l^{-1}$ of MD16S1 and MD16S2 primers, and 0.42 $\mu mol \cdot l^{-1}$ of MDmapAl, MDmapA2, COL3, and MDCOL2 primers in the Fermentas buffer (Fermentas GmbH). Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradiant, Eppendorf, Hamburg, Germany) with the following program: 1 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-, 589-, and 462-bp DNA fragments corresponding to the *Campylobacter* genus, *C. jejuni*, and *C. coli*, respectively. *Campylobacter 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 a 1% solution of ethidium bromide and were visualized under UV light after gel electrophoresis on 1.5% agarose (Figure 1).

Table 1. Primers for PCR amplification of campylobacterial DNA for identification

Organism	Primer	PCR product (bp)	Sequence[1]
Campylobacter spp.	16S rRNA	857	F: 5′-ATC TAA TGG CTT AAC CAT TAA AC-3′ R: 5′-GGA CGG TAA CTA GTT TAG TAT T-3′
Campylobacter jejuni	*mapA*	589	F: 5′-CTA TTT TAT TTT TGA GTG CTT GTG-3′ R: 5′-GCT TTA TTT GCC ATT TGT TTT ATT A-3′
Campylobacter coli	*ceuE*	462	F: 5′-AAT TGA AAA TTG CTC CAA CTA TG-3′ R: 5′-TGA TTT TAT TAT TTG TAG CAG CG-3′

[1]F = forward; R = reverse.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (MV1084, HiMedia Laboratories) supplemented with 5% defibrinated sheep blood, according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003) guidelines. The antimicrobial agents tested and their corresponding concentrations were as follows: 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 incubating the inoculated plate aerobically 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 NCCLS (2003). *Staphylococcus aureus* and *Escherichia coli* were used as quality control organisms in antimicrobial susceptibility determination.

Statistical Analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL), a χ^2 test and Fisher's exact 2-tailed test analysis were performed and differences were considered significant at values of $P < 0.05$.

RESULTS

Using cultural techniques, 186 of 336 carcasses (55.4%) were positive for *Campylobacter* spp. (Table 2). No significant differences ($P > 0.05$) were present between the number of *Campylobacter* spp. isolated after defeathering and after evisceration stages. However, the number of positive samples increased significantly 20 min after the chilling period started and decreased significantly after the chilling period completed ($P < 0.05$). The number of *C. jejuni* isolated from carcasses in each processing line stage and in the chiller tank water was significantly higher than *C. coli* ($P < 0.05$).

Significantly more chicken carcass samples were found to contain *Campylobacter* spp. by the PCR assay than the cultural method ($P < 0.05$; Table 2). Overall, 186 carcasses samples were positive for *Campylobacter* spp. using both the cultural method and the PCR assay. The PCR assay could identify 27 *Campylobacter*-contaminated carcass samples that were negative using the cultural method.

Table 3 summarizes the resistance pattern of *Campylobacter* isolates to 10 antimicrobial agents tested in this study. Of the 198 *Campylobacter* isolates tested, 92.9% (n = 178) were resistant to one or more antimicrobial agents. A high proportion of the *Campylobacter* isolates was resistant to tetracycline, ciprofloxacin, and nalidixic acid and to a lesser extent to enrofloxacin. All *Campylobacter* isolates were susceptible to gentamicin. *Campylobacter coli* isolates were susceptible to erythromycin, amoxicillin, and chloramphenicol.

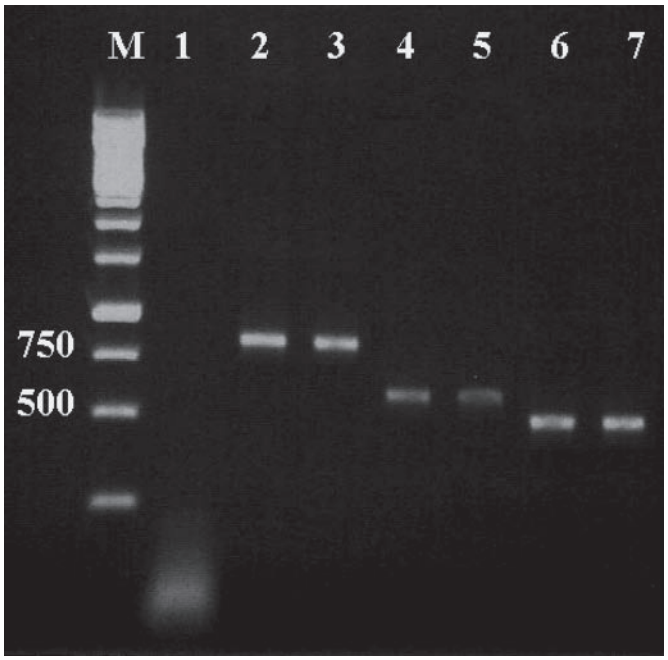

Figure 1. Ethidium bromide-stained agarose PCR gel. M = 1-kb DNA ladder; lane 1 = negative control, distilled water substituted for DNA template; lanes 2 and 3 = positive amplification of *Campylobacter* DNA from culture; lane 4 = positive control, DNA from *Campylobacter jejuni* (ATCC 33560); lane 5 = positive amplification of *C. jejuni* DNA from culture; lane 6 = positive control, DNA from *Campylobacter coli* (ATCC 33559); lane 7 = positive amplification of *C. coli* DNA from culture.

DISCUSSION

In this study, *Campylobacter*-contaminated carcasses were detected in all 4 sites along the processing line in a commercial poultry processing plant in southwestern Iran. The frequencies of contaminated carcasses with *Campylobacter* spp. after defeathering and after evisceration found in this study (54.8 and 51.2%, respectively) are in agreement with those reported by Franchin et al. (2007; 68 and 69.4%, respectively); however, they are lower than those observed by Vashin and Stoyanchev (2004; 92 and 100%, respectively). As previous studies (Vashin and Stoyanchev, 2004; Franchin et al., 2007; Son et al., 2007) and our study showed, no significant differences were found between the frequency of contaminated carcasses with *Campylobacter* spp. after defeathering and after evisceration. This finding raises the possibility that evisceration is not the main factor responsible for cross-contamination. However, because rupture of the viscera with extravasations of intestinal content is always possible during the evisceration stage, the processes preceding evisceration such as defeather-

Table 2. Prevalence of *Campylobacter* spp. in chicken carcasses during the processing line and from the water of the chiller tank using cultural technique and PCR assay

Source of samples	No. of samples	Number (%) of samples positive using cultural technique			No. of samples	Number (%) of samples positive using PCR assay		
		Campylobacter spp.	*Campylobacter jejuni*	*Campylobacter coli*		*Campylobacter* spp.	*Campylobacter jejuni*	*Campylobacter coli*
After defeathering	84	46 (54.8)	41 (89.1)	5 (10.9)	84	53 (63.1)	47 (88.7)	6 (11.3)
After evisceration	84	43 (51.2)	40 (93.0)	3 (7.0)	84	57 (67.9)	51 (89.5)	6 (10.5)
20 min after chilling stage started	84	58 (69.0)	51 (87.9)	7 (12.1)	84	59 (70.2)	52 (88.1)	7 (11.9)
24 h after chilling stage completed	84	39 (46.4)	35 (89.7)	4 (10.3)	84	44 (52.4)	40 (90.9)	4 (9.1)
Chiller water	14	12 (85.7)	10 (83.3)	2 (16.7)	0	—	—	—
Total	350	198 (56.7)	177 (89.4)	21 (10.6)	336	213 (63.4)	190 (89.2)	23 (10.8)

ing and scalding should be controlled to reduce *Campylobacter* contamination.

A significant increase in the number of *Campylobacter* spp. isolates was noted in the carcass samples obtained 20 min after the chilling stage started. This finding suggests that the chiller is probably the main source of the cross-contamination. This was further supported by the high number of *Campylobacter* isolates detected in the chiller tank water. Our results showed that the number of contaminated carcasses was significantly decreased 24 h after the chilling stage completed; however, the complete elimination of the agent was not achieved. It has been shown that the number of contaminated carcasses can be reduced by stressors such as drying of the skin surface or chilling (Newell et al., 2001; Alter et al., 2005; Son et al., 2007). The ability to survive under such environmental stressors is dependent on the presence and expression of several stress response genes (Newell et al., 2001).

In our study *C. jejuni* was far more common than *C. coli*. *Campylobacter jejuni* has been reported to be the most frequent species recovered from poultry (Jørgensen et al., 2002) and poultry carcasses (Son et al., 2007; Rahimi and Tajbakhsh, 2008).

Antibiotic susceptibility test results of this study indicated that there is an overall increase in the resistance of *Campylobacter* to tetracycline, ciprofloxacin, and nalidixic acid. Results of this study are comparable to the results reported by others (Ge et al., 2003; Yildirim et al., 2005; Andersen et al., 2006; Taremi et al., 2006). The widespread use of antibiotics as supplements for prophylaxis and growth promotion has promoted the selection of antimicrobial-resistant bacterial strains at the farm level during poultry production. High antimicrobial resistance rates to ciprofloxacin and tetracycline observed in this study might be due to the fact that during the past decade enrofloxacin, which is closely related to ciprofloxacin, and tetracycline have been commonly used in veterinary medicine in Iran. Because campylobacteriosis is transmitted primarily through food, particularly poultry meat, the presence of antimicrobial-resistant *Campylobacter* in raw meat products has important public health implications, especially in developing countries where there is widespread and uncontrolled use of antibiotics (Hart and Kariuki, 1998). Therefore, the use of in vitro susceptibility testing of *Campylobacter* may take on greater importance in ensuring rapid and appropriate management of patients with foodborne campylobacteriosis.

In this study, *Campylobacter* was better detected by the PCR assays than the cultural method. This could be due to the higher analytical and diagnostic sensitivities of the PCR assays. However, care must be taken to avoid false-positive results arising from DNA contamination, as well as false-negative results caused by inhibitory substances in foods or enrichment broths. Because the possibilities for amplicon contamination were minimized by separation of the preparation and amplification-detection laboratories and the blank con-

Table 3. Antibiotic resistance profiles of *Campylobacter* strains isolated from chicken carcasses during the processing line and from the water of the chiller tank

Antimicrobial agent	*Campylobacter* spp. (n = 198)	*Campylobacter jejuni* (n = 177)	*Campylobacter coli* (n = 21)
Nalidixic acid	116 (58.6%)	105 (59.3%)	11 (52.4%)
Ciprofloxacin	123 (62.1%)	119 (67.2%)	4 (19.0%)
Erythromycin	3 (1.5%)	3 (1.7%)	0 (0.0%)
Tetracycline	155 (78.3%)	141 (79.7%)	14 (66.7%)
Streptomycin	12 (6.1%)	11 (6.2%)	1 (4.8%)
Amoxicillin	14 (7.1%)	14 (7.9%)	0 (0.0%)
Ampicillin	37 (18.7%)	33 (18.6%)	4 (19.0%)
Gentamicin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Chloramphenicol	1 (0.5%)	1 (0.6%)	0 (0.0%)
Enrofloxacin	88 (44.4%)	85 (48.0%)	3 (14.3%)

trols were negative, false-positive results due to amplicants are unlikely to have occurred.

In conclusion, the results of this study showed a high prevalence of *Campylobacter* spp. in poultry carcasses in a major poultry processing plant in Ahvaz, Iran. *Campylobacter* spp. was isolated from the initial processing steps to the final products. Most of the isolates were antimicrobial-resistant strains. Contamination of poultry carcasses with *Campylobacter* spp. during processing constitutes a risk for consumers, especially from consumption of undercooked or postcooking contaminated chicken products. Therefore, the incidence of *Campylobacter* spp. in chicken products should be reduced by increasing biosafety measures during rearing on the chicken farm, transportation, preslaughter operations, and during the processing of the poultry carcasses.

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