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Full Length Research Paper

Campylobacter occurrence and antimicrobial resistance in samples from ceca of commercial turkeys and quails in Tehran, Iran

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Infected poultry is considered as the main source for transferring *Campylobacter* spp. to humans. Several studies have been carried out to determine the prevalence of *Campylobacter* infection and antimicrobial resistance rate of the isolates in broiler chickens, but related information regarding other meat producing birds, such as turkeys and quails is lacking in Iran. A total of 125 samples (75 turkeys and 50 quails) were collected from April to September 2010 and examined for *Campylobacter* spp. A total of 52 (41.6%) *Campylobacter* strains consisting of 41 and 11 isolates of turkeys and quails (54.6 and 22% infection rate) were isolated respectively. All of the isolated *Campylobacter* spp. from quails and 19.5% of turkey isolates were *Campylobacter jejuni*, while other isolates of turkeys were identified as *Campylobacter coli*. Susceptibilities of 52 isolates were determined for eight antimicrobial drugs by using the disk diffusion assay. Highest resistance rate was seen against ampicillin (84.6%), followed by resistance to tetracycline (69.2%), ciprofloxacin (50%) and nalidixic acid (34.6%). All of nalidixic acid resistant isolates were identified as *C. jejuni* by hippurate test. Quail *C. jejuni* strains unlike turkey isolates showed resistance to neomycin. None of the isolates were resistant to erythromycin, chloramphenicol and gentamicin.

Key words: *Campylobacter* spp., turkeys, quails, antimicrobial resistance.

INTRODUCTION

Campylobacter spp. is a gram negative, non- spore forming micro-aerophilic organism, associated with diverse diseases in humans and animals. However, it is a commensal bacterium in poultry (Zhang, 2008). Campylobacteriosis is the most commonly found food-borne bacterial disease in both developed and developing countries (Zhang, 2008; Friedman et al., 2000). In addition to acute enteric disease, *Campylobacter* spp. may cause late-onset complications such as Guillan-Barré syndrome which is one of the most remarkable post-infection complications in humans (Hariharan et al., 2009). The most important *Campylobacter* species associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* (Dickins et al., 2002).

Contaminated raw or undercooked poultry products constitute a significant risk for human campylobacteriosis (Zhang, 2008; Rasschaert et al., 2006). Several epidemiological studies demonstrated the high prevalence of *Campylobacter* in chickens, ducks and turkeys (Dickins et al., 2002). During slaughter, intestinal contents can contaminate poultry carcasses and isolates of *Campylobacter* carcasses often come from birds' gastrointestinal tracts (Pearson et al., 2000). It has been shown that contamination with even small amount of cecal contents during processing at slaughterhouse can cause significant increase in the number of *Campylobacter* on eviscerated poultry carcasses. Hygienic efforts at the slaughterhouse to reduce *Campylobacter* contamination may have a limited efficiency in eliminating infection risk in consumers (Perko-Mäkelä et al., 2009; Black et al., 1998; Mead et al., 1995).

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Table 1. *Campylobacter* isolates from ceca of 125 sampled turkeys and quails.

***Campylobacter* isolates**	**Number and percent of positive cases**	
	Turkeys (n= 75)	**Quails (n= 50)**
Positive for *Campylobacter spp.*	41 (54.6%)	11 (22%)
Campylobacter coli	33 (80.5%)	0 (0%)
Campylobacter jejuni	8 (19.5%)	11 (100%)

As *Campylobacter* may be transferred from animals to humans via food, the emergence of antimicrobial resistance in this enteric microorganism is a matter of concern (Luber et al., 2003). Although most human cases of *Campylobacter* enteritis may not need pharmacotherapy, in patients with severe campylobacteriosis, administration of antibiotics such as erythromycin or ciprofloxacin is often recommended (Aarestrup and Engberg, 2001).

Several studies have been carried out to determine the prevalence of *Campylobacter* infection and antimicrobial resistance rate of the isolates in broiler chickens (Taremi et al., 2006; Soltan Dallal et al., 2010; Ansari- Lari et al., 2010), but there is no information regarding isolation and antimicrobial sensitivity of *Campylobacter* spp. in other meat producing birds, such as turkeys and quails in Iran. This study was conducted to determine the occurrence of *Campylobacter* in commercial turkeys and quails at the time of slaughter and to investigate the antimicrobial resistance profile of the isolates.

MATERIALS AND METHODS

Sampling procedure

During the period of April to September 2010, one hundred and twenty five birds including 75 turkeys which were slaughtered at one slaughterhouse and 50 quails at different abattoir were sampled at the evisceration line during the slaughter. The intestines, including ceca of the turkeys and quails were placed in sterile bags and transported to the laboratory in a cool box for processing same day.

Isolation of *Campylobaacter* spp.

At the laboratory, the ceca were aseptically opened and a loopful of content was plated on *Campylobacter* selective agar with *Campylobacter* selective supplements (Merck, Germany). 5% of defibrinated sheep blood was also added. Inoculated plates were incubated under microaerophilic conditions employing Anaerocult C (Merck, Germany) at 42^{o}C for 48 h. Colonies were subcultured onto blood agar plates to confirm typical morphology by gram staining.

Biochemical tests

Identification of the isolates was based on key phenotypic properties as recommended by Nachamkin (Nachamkin, 2003). Presumptive *Campylobacter* isolates were confirmed using standard biochemical procedure including catalase (3% H_2O_2) and oxidase reactions. All isolates were also tested for hydrolysis of sodium hippurate. Hippurate positive isolates were identified as *C. jejuni* (Zhang, 2008; Hariharan et al., 2009).

Antibiotic resistance test

Antibiotic resistance test was conducted according to the method described by the National Committee for Clinical Laboratory Standard (NCCLS, 2000). Mueller Hinton agar medium with 5% defibrinated sheep blood was used. The antimicrobial susceptibility test disks used in this study were: ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), neomycin (30 µg), ciprofloxacin (5 µg) and tetracylin (30 µg). All isolates were also tested for their susceptibility to nalidixic acid (30 µg). Nalidixic acid susceptible, hippurate negative isolates were identified as *C. coli* (Zhang, 2008; Hariharan et al., 2009).

RESULTS

Isolation and identification of *Campylobacter* spp.

Forty one of 75 turkey samples (54.6%) were positive for *Campylobacter*. Of these, 33 of the isolated strains which were negative for hippurate test but susceptible to nalidixic acid were considered as *C. coli;* and 8 isolates which were positive for hippurate test were determined as *C. jejuni*. In quails samples, 11 of 50 (22%) were positive for *Campylobacter* (Table 1). All of the quail isolates were *C. jejuni*. Oxidase and catalase tests were positive for all isolates.

Antibiotic resistance test

Antibiotic resistance against 8 drugs for 41 *Campylobacter* isolates from turkeys showed 80.5%

Table 2. Resistance of 52 *Campylobacter* isolates from turkeys and quails to 5 antibiotics.

Antibiotic*	Number of resistant isolates		Total (n= 52)
	C. coli (n= 33)	*C. jejuni* (n= 19)	
Ampicillin	25 (75.7%)	19 (100%)	44 (84.6%)
Ciprofloxacin	10 (30.3%)	16 (84.2%)	26 (50%)
Neomycin	0	11 (57.8%)	0
Tetracycline	31 (93.9%)	5 (26.3%)	36 (69.2%)
Nalidixic acid	0	18 (94.7%)	18 (34.6%)

*No resistance rate was seen against chloramphenicol, erythromycin and gentamicin.

resistance rate to ampicillin, 78% to tetracycline, 36.6% to ciprofloxacin and 19.5% to nalidixic acid. All 11 quail isolates were resistant against ampicillin, neomycin and ciprofloxacin, 90.9% to nalidixic acid, while 36.3% showed resistant against tetracycline. Table 2 shows resistance rate of the *Campylobacter* isolates in respect to bacterial species. In general, highest resistance rate was seen against ampicillin, since 84.6% of the isolates were resistant to this antibiotic, followed by resistance to tetracycline (69.2%), ciprofloxacin (50%) and nalidixic acid (34.6%). All nalidixic acid resistant isolates were identified as *C. jejuni* (hippurate positive). All of the isolates, despite their origin, showed no resistance to chloramphenicol, gentamicin and erythromycin.

DISCUSSION

Campylobacter is a common contaminant of poultry carcasses in processing plants (Alter et al., 2005). The results of several studies showed that *Campylobacter* spp. was present in all stages of the slaughtering process and this contamination could not be eliminated completely during the process (Rahimi et al., 2010; Alter et al., 2005).

Poultry meat is often contaminated with *Campylobacter* during processing and constitutes a risk to human health. The intestinal tracts of all avian including chicken, turkey and quail which are of higher importance in providing poultry meat than other poultry, is a favorable environment for *Campylobacter* colonization. Unlike in humans, colonization in poultry is often at a high level but with little or no disease (Evans and Sayers, 2000). Since *Campylobacter* infective dose is very low, about 500-1000 CFU, infected poultry is considered as a major risk factor for human infection (Black et al., 1998).

Several studies have been carried out on *Campylobacter* infection and the findings have indicated prevalence ranges from 3 to 98% in poultry (Newell and Wagenaar, 2000; Newell and Fearnley, 2003). Results of the present study indicated that 54.6 and 22% of sampled turkeys and quails were positive for *Campylobacter* spp., by means of bacterial culture respectively. This result is in agreement with documented infection rates of commercially reared poultry (Newell and Wagenaar, 2000). As quails are usually slaughtered at younger ages in comparison with turkeys, lower infection rate for quails might be due to shorter rearing period which could limit the chance of infection.

Very recent study on prevalence of *Campylobacter* infection in chickens in Iran showed that 76% of broiler flocks were positive for *Campylobacter* spp., in which 22% were positive for *C. jejuni*, 32% for *C. coli* and 22% for both species by using molecular method (Ansari- Lari et al., 2010). In another study, the contamination of turkey carcasses by *Campylobacter* spp. during processing at slaughterhouse was evaluated by using PCR method and the results showed 62.1% infection rate in commercial turkeys in Iran (Rahimi et al., 2010).

Traditional *Campylobacter* identification relies on bacteriologic culturing, followed by biochemical assays, such as hippurate hydrolysis. This test is still routinely used to differentiate between *C. jejuni* (hippurate positive) and *C. coli* (hippurate negative). *C. coli* was reported to be the predominant species found in turkeys (Zhang, 2008). Similarly, in the present study, *C. coli was* found in most turkey isolates (80.5%).

Although *Campylobacter* is normally susceptible to various antimicrobials, increasing resistance to several drugs including fluoroquinolones, erythrpmycin and tetracyclines has been documented with *Campylobacter* isolates from animals and humans (Zhang, 2008).

Resistance to quinolones and fluoroquinolones is of particular concern to all. In a study of clinical isolates of *Campylobacter* spp. from diarrheic children in Tehran, Iran, 62% of the isolates were resistant to ciprofloxacin (Feizabadi et al., 2007). High resistance rates to ciprofloxacin were also reported in *Campylobacter* isolates from retail chicken and beef in Tehran, Iran, as 47 and 69.4% of the isolates in different studies were resistant to this antimicrobial (Soltan Dallal et al., 2010; Taremi et al., 2006). Present study alarmingly indicated that 84.2% of the *C. jejuni* and 30.3% of *C.coli* from turkeys and quails were resistant to ciprofloxacin. Over

80% resistance of *C. jejuni* isolates from chicken and human to ciprofloxacin was also observed elsewhere (Senok et al., 2007). Enrofloxacin is closely related to ciprofloxacin and is widely in use in poultry to treat infection with *Escherichia coli*. High resistance rate to ciprofloxacin observed here might be due to common use of enrofloxacin during rearing of birds. As poultry is considered a main source of human *Campylobacter* infections and since quinolone antimicrobials have clinical relevance in human and veterinary medicine, the development of fluoroquinolone resistant *Campylobacter* in poultry is regarded as a threat to public health (Zhang, 2008).

High resistance against tetracycline among our *Campylobacter* spp. was compared with previous studies (Taremi et al., 2006; Hariharan et al., 2009; Han et al., 2007). But our results, unlike previous reports, showed significant difference in rates for tetracycline resistance between *C. coli* and *C. jejuni*.

Resistance to ampicillin (84.6%) was the most common finding among our isolates which was greater than other studies (Soltan Dallal et al., 2010; Han et al., 2007). In Iran poultry is often treated with tetracycline, fluoroquinolone and penicillins. Hence, emerging of antimicrobial resistance against these drugs, especially quinolones such as ciprofloxacin which is considered as the first choice for treatment of acute diarrhea in Iran is important. There was no erythromycin- resistant strain of *Campylobacter* from turkeys or quails in our study. No considerable resistance to erythromycin was also seen in previous reports of chicken or human isolates of Iran and other countries (Soltan Dallal et al., 2010; Taremi et al., 2006; Han et al., 2007; Senok et al., 2007; Hariharan et al., 2009). This result validates the continued use of this agent.

Use of antibiotics as growth promoter or disease control which were implemented in poultry flocks during production period may have positive effect on lowering infection rate of the birds with *Campylobacter,* but this is not recommended for the prevention strategy due to emergence of antimicrobial resistance and public health concerns. The presence of antimicrobial- resistant *Campylobacter* in ceca of slaughtered meat producing poultry which may contaminate the carcasses shows a public health significance especially in developing countries, where widespread and uncontrolled use of antibiotics in growing poultry might occur (Hart and Kariuki, 1998).

Program for the routine surveillance of *Campylobacter* in turkey and quail flocks does not exist in Iran, but positive results from this study clearly stated that control of the infection at the farm level prior to slaughter and also effort to good management practices of critical control points during slaughter are essential.

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