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Incidence and Antibiotic Resistance Properties of Campylobacter Species Isolated From Poultry Meat

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Abstract

Background: F *Campylobacter* species are imperative foodborne bacteria because of the contaminated poultry meat consumption.

Objectives: This study was conducted to recognize the incidence and antimicrobial resistance profile of *Campylobacter* species recovered from raw poultry meat samples.

Materials and Methods: A total of 695 poultry meat samples were collected and assessed by culture technique. Bacterial species were identified by polymerase chain reaction (PCR). Antimicrobial resistance was assessed by disk diffusion method (DDM).

Results: The contamination rate of samples with *Campylobacter* spp. was 44.75% with higher contamination rate of wild duck (84%), wild goose (83.33%), coot (78.26%), chicken (67.78%), and wild pheasant (66.66%), respectively. *Campylobacter jejuni* and *C. coli* bacteria were found in 84.24% and 15.76% of *Campylobacter* spp., respectively. The highest incidence of *C. jejuni* was obtained in partridge (95.45%), quail (95%), pheasant (92.31%), and wild duck (90.48%) meat samples, respectively. The highest incidence of *C. coli* was found in turkey (52.63%) and wild pheasant (22.22%) meat samples, respectively. Moreover, *C. jejuni* had the highest resistance to tetracycline (76.34%), nalidixic acid (65.65%), ciprofloxacin (58.78%), enrofloxacin (39.69%), and ampicillin (38.55%), respectively. *C. coli* had the highest resistance to nalidixic acid (48.99%), ciprofloxacin (40.82%), and enrofloxacin (38.78%), respectively.

Conclusion: Poultry meat, particularly partridge, quail, pheasant, turkey, and wild avian are the main sources of *Campylobacter* transmission. Furthermore, higher incidence and antibiotic resistance of *C. jejuni* was found. Proper cooking of poultry meat and monitoring the antibiotic prescription can lessen the occurrence of antibiotic-resistant *Campylobacter* spp. in poultry meat.

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Background

Poultry meat is an excellent source of numerous vitamins and minerals.[1] It is a prevalent diet among people all around the world.[1] Nevertheless, the poultry meat inspection and their purchase by humans augmented the foodborne diseases.[1-5]

Campylobacter species are gram-negative and microaerophile bacteria measured as the most common cause of acute gastroenteritis. *Campylobacter jejuni* and *C. coli* are the most significant species of this family accountable for the occurrence of human disorders.[6,7] Campylobacteriosis is acknowledged with abdominal cramping, fever, and diarrhea.[6,7] Plain cases are mostly faced with severe diarrhea associated with blood, and occasionally may develop complicated syndromes such as Guillain Barré branded by ataxia, areflexia, immune-mediated neuropathies, ophthalmoplegia, and death.[6,7]

Campylobacter spp. are principally resistant to numerous kinds of antimicrobial agents including penicillins, quinolones, macrolides, cephalosporins, and tetracyclines.[8] Thus, higher loads of cost for a longer period of time should be performed to treat campylobacteriosis cases.[8]

Due to the high risk of transmission of *Campylobacter* spp. through poultry products, particularly meat,[9,10] and absence of epidemiological surveys in this field in Iran, the current study was carried out to signify the incidence and antibiotic resistance properties of *Campylobacter* spp., *C. jejuni*, and *C. coli* isolated from different kinds of poultry meat samples.

Materials and Methods

Samples

A total of 695 poultry meat samples including turkey (n=90), chicken (n=90), quail (n=90), duck (n=80), partridge (n=80), goose (n=60), pheasant (n=50), ostrich (n=50), wild duck (*Anas crecca*) (n= 25), wild pheasant (*Phasianus colchicus*) (n= 27), wild goose (*Anser anser*) (n= 30), and coot (*Fulica atra*) (n= 23) were purchased from the retail centers of Mazandaran province, Iran in the period of January 2018 to January 2019. Samples (100 g from the femur muscle) were aseptically collected using separate plastic bags, then transferred to the Department of Poultry Diseases, Veterinary Organization of Mazandaran, Iran.

Isolation of Campylobacter spp.

To isolate *Campylobacter* spp., 10 g of the macerated shells was added to 100 mL of Bolton broth Base supplemented with 25 mL of defibrinated horse blood along with the following antibiotic combination: 20 mg/L of cefoperazone, 20 mg/L of vancomycin, 20 g/L of trimethoprim, 10 mg/L of amphotericin B. Media were incubated at 42C for 24 hours in microaerophilic conditions.[11] The identification test was performed immediately to confirm the characteristics of *Campylobacter* colonies. Identification of the isolates was conducted based on method described by Nachamkin.[12] One colony from each suspected medium was subjected to standard Biochemical tests including Gram-staining, oxidase and hydrolysis of hippurate, production of catalase (3% H2O2), hydrolysis of indoxyl acetate, urease activity, and resistance pattern toward cephalothin.[12]

Polymerase Chain Reaction Detection of Campylobacter spp.

Campylobacter isolates were cultured on nutrient broth (Merck, Germany) and further incubated at 42°C for 24 hours. Principles of producing factory of DNA extraction kit (Cinnagen, Iran) were applied for DNA extraction. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280). Polymerase Chain Reaction (PCR) was conducted rendering beforehand documents (Table 1).[13] Thermo-cycler device (Flexrcycler^2, Germany) was used to detect *Campylobacter* spp., *C. jejuni*, and *C. coli*. Next, 15 µL of the PCR products was electrophoresed using 1.5% agarose gel. Runs comprised a negative control (PCR grade water) and two positive controls (*C. jejuni* ATCC 33291 and *C. coli* ATCC 33559).

Antibiotic Resistance Test

Phenotypic profile of antibiotic resistance of *Campylobacter* spp. isolates were examined by disk diffusion method (DDM). To achieve this aim, Mueller–Hinton agar media (Merck, Germany) with 5% sheep blood were applied following the protocols of the Clinical and Laboratory Standards Institute (CLSI).[14] Diverse antibiotic disks (Oxoid, UK) including ampicillin (10 µg/disk), amoxicillin (30 µg/disk), cephalothin (30 µg/disk), colistin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg/disk), ciprofloxacin (5 µg/disk), enrofloxacin (5 µg/disk), erythromycin (15 µg/disk), neomycin (30 µg/disk), streptomycin (30 µg/disk), gentamicin (10 µg/disk), and tetracycline (30 µg/disk) were applied for this goal. Plates containing bacteria and also antibiotic agents were incubated for 48 hours at 42°C in microaerophilic conditions.

Statistical Examination

Data collected from the experimentations were classified in the Excel software. SPSS/21.0 was used for numerical examination. Chi-square and Fisher exact two-tailed tests were applied to measure any noteworthy association. Statistical signification was determined at a *P* value < 0.05.

Results

Incidence of Campylobacter spp.

Table 2 signifies the incidence of *Campylobacter* spp. in different kinds of poultry meat samples. Out of 695 (44.75%) poultry meat samples, 311 cases were contaminated with *Campylobacter* spp. Moreover, wild duck (84%), wild goose (83.33%), coot (78.26%), chicken (67.78%), and wild pheasant (66.66%) were the most commonly contaminated samples. From 311 *Campylobacter* spp. contaminated samples, 262 (84.24%) and 49 (15.76%) isolates were identified as *C. jejuni* (84.24%) and *C. coli* (15.76%), respectively. All *Campylobacter* strains isolated from wild goose and ostrich were identified as *C. jejuni*. Samples from partridge (95.45%), quail (95%), pheasant (92.31%), and wild duck (90.48%) had the highest incidence of *C. jejuni*. Meanwhile, samples from turkey (52.63%) and wild pheasant (22.22%) had the highest incidence of *C. coli*. There was a statistically significant difference between different kinds of poultry meat samples and incidence of *Campylobacter* spp. ($P < 0.05$). Furthermore, there was a statistically significant difference between the incidence of *C. jejuni* and *C. coli* bacteria ($P < 0.05$).

Antibiotic Resistance Pattern of *Campylobacter jejuni* and *Campylobacter coli*

Table 1. PCR Circumstances Applied for Identification of *Campylobacter* spp., *Campylobacter jejuni*, and *Campylobacter coli*

Target Gene	Primer Sequence (5'-3')	PCR Product (bp)	PCR Volume (50 µL)	PCR Programs
16SrRNA (*Campylobacter* genus)	F: ATCTAATGGCTTAACCATTAAAC R: GGACGGTAACTAGTTTAGTAT T	857	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template	1 cycle: 94°C ----------- 1 min. 35 cycle: 94 °C ----------- 30 s 60°C ----------- 30 s 72°C ----------- 40 s 1 cycle: 72°C ----------- 3 min
MapA (*C. jejuni*)	F: CTATTTTATTTTTGAGTGCTTGTG R: GCTTTATTTGCCATTTGTTTTATTA	589		
CeuE (*C. coli*)	F: AATTGAAAATTGCTCCAACTATG R: TGATTTTATTATTTGTAGCAGCG	462		

Table 2. Incidence of *Campylobacter* spp. in Different Poultry Meat Samples.

Poultry Meat Samples	N. Samples Collected	N. samples Positive for Bacteria (%)		
		Campylobacter spp.	*C. jejuni*	*C. coli*
Chicken	90	61 (67.78)	51 (83.61)	10 (16.39)
Turkey	90	38 (42.22)	18 (47.37)	20 (52.63)
Quail	90	40 (44.44)	38 (95)	2 (5)
Partridge	80	22 (27.50)	21 (95.45)	1 (4.54)
Duck	80	34 (42.50)	30 (88.24)	4 (11.76)
Goose	60	19 (31.67)	16 (84.21)	3 (15.79)
Pheasant	50	13 (26)	12 (92.31)	1 (7.69)
Ostrich	50	2 (4)	2 (100)	-
Wild duck	25	21 (84)	19 (90.48)	2 (9.52)
Wild pheasant	27	18 (66.66)	14 (77.77)	4 (22.22)
Wild goose	30	25 (83.33)	25 (100)	-
Coot	23	18 (78.26)	16 (88.88)	2 (11.11)
Total	695	311 (44.75)	262 (84.24)	49 (15.76)

Table 3 signifies the antibiotic resistance pattern of *C. jejuni* and *C. coli* recovered from different kinds of poultry meat samples. *C. jejuni* showed the highest resistance to tetracycline (76.34%), nalidixic acid (65.65%), ciprofloxacin (58.78%), enrofloxacin (39.69%), and ampicillin (38.55%) antibiotic agents, respectively. Furthermore, *C. coli* showed the highest resistance to nalidixic acid (48.99%), ciprofloxacin (40.82%), and enrofloxacin (38.78%) antibiotic agents, respectively. *C. jejuni* showed a higher resistance to the examined antibiotic agents than *C. coli* ($P < 0.05$).

Discussion

Campylobacteriosis is a common disease with a high incidence rate in both developing and developed countries.[15] Human infection with *Campylobacter* spp. can occur by direct contact with infected animals or by consumption of their contaminated products. Domestic and wild poultry have been identified as the main sources of contamination with *Campylobacter* spp.[16]

Table 3. Antibiotic Resistance Properties of *Campylobacter* spp. Isolated from Different Poultry Meat Samples

Antimicrobial Agent	Antibiotic Resistance (%)	
	C. jejuni (262)	*C. coli* (49)
Amoxicillin	46 (17.56)	-
Ampicillin	101 (38.55)	9 (18.37)
Nalidixic acid	172 (65.65)	24 (48.99)
Ciprofloxacin	154 (58.78)	20 (40.82)
Enrofloxacin	104 (39.69)	19 (38.78)
Streptomycin	15 (5.73)	3 (6.12)
Gentamycin	3 (1.15)	-
Neomycin	52 (19.85)	1 (2.04)
Erythromycin	50 (19.08)	-
Chloramphenicol	11 (4.20)	-
Tetracycline	200 (76.34)	18 (36.73)
Colistin	39 (14.89)	9 (18.37)

The current study was carried out to evaluate the incidence rate and antibiotic resistance of *Campylobacter* spp. recovered from raw turkey, quail, chicken, duck, partridge, goose, pheasant, ostrich, wild duck, wild pheasant, wild goose, and coot meat samples. Overall, 44.75% of the examined samples were contaminated with *Campylobacter* spp. in which *C. jejuni* and *C. coli* were identified in 84.24% and 15.76% of isolates, respectively. While raw partridge, quail, pheasant, and wild duck had the highest incidence of *C. jejuni*, turkey and wild pheasant had the highest incidence of *C. coli*.

Campylobacter spp., particularly *C. jejuni* and *C. coli*, are well adapted to growth and survival in poultry meat. It is possibly due to the higher body temperature of poultry, which facilitates growth and survival of *Campylobacter* spp. and diminishes the growth and survival of other bacteria. There were some probable reasons for the high incidence of *Campylobacter* spp.in poultry meat samples. The likelihood of cross-contamination occurrence in the aviculture and also *Campylobacter* transmission from contaminated environment to meat are the most important factors. Furthermore, cross-contamination through different stages of the slaughter, transmission of bacteria from infected staff of abattoirs and retail centers to poultry carcasses, and bacterial transmission due to contaminated water used for washing poultry carcasses are other main risk factors. Moreover, direct contact of wild poultry with the contaminated environment and also infected birds might be a probable reason for the high incidence of *Campylobacter* spp. Living in damp and sludgy environment and different feeding patterns of duck, goose, and wild poultry might be the probable reasons for the high incidence of *Campylobacter* in the examined samples.

Different epidemiological surveys have been conducted in the field of campylobacteriosis in food samples with animal origins. The total incidence rates of *Campylobacter* spp. in poultry meat samples in Austria,[17] Denmark,[17] Finland,[17] France,[18] Germany,[17] The Netherlands,[17] Hungary,[17] Poland,[19] Slovakia,[17] Slovenia,[17] Spain,[17] and Turkey[20] were 71%, 12%, 11%, 76%, 38%, 32%, 24%, 50%, 41%, 36%, 54% and 70%, respectively. Dabiri et al[21] stated that the incidence of *Campylobacter* spp. in chicken meat samples recovered from Iran shopping centers was 44% in which *C. jejuni* and *C. coli* were identified in 79% and 21% of isolates, respectively. Di Giannatale et al[22] reported that *Campylobacter* spp. was identified in 219 (17.38%) poultry meat samples in which *C. jejuni* and *C. coli* were identified in 58.45% and 41.55% of isolates, respectively. Szosland-Fałtyn et al[23] reported that the incidence of *Campylobacter* spp. among the raw chicken, turkey, duck, and goose meat samples was 49.70%, 18.38%, 43.80%, and 6.60%, respectively. Moreover, in their study, the incidence rates for *C. jejuni* and *C. coli* were 36.31% and 13.11% among the examined raw chicken, 12.10% and 6.50% among turkey, 27.23% and 16.14% among duck, and 4.30% and 2.20% among goose meat samples. A higher incidence of *C. jejuni* than *C. coli* in poultry meat samples was also reported in some recent surveys.[11,24-28]

The contamination rate of poultry products with *Campylobacter* spp. varies in different studies. This might be due to different factors, including sampling time and location, method of sampling, types of samples, and even different laboratory techniques. Moreover, different hygienic levels of poultry flocks may affect the incidence of bacteria in different studies.

The current study revealed that resistance of bacteria to tetracycline, nalidixic acid, ciprofloxacin, enrofloxacin, and ampicillin antibiotic agents was high. Similarly, a high resistance to tetracycline, nalidixic acid, ciprofloxacin, enrofloxacin, and ampicillin antibiotic agents was reported from Iran,[29] Tunisia,[24] Italy,[30] Algeria,[31] and Pakistan.[32] This might be due to the unlawful prescription and unauthorized sale of antibiotic agents and additionally excessive use of antibiotics in poultry farms. Adzitey et al[33] revealed that the *C. jejuni* isolated from poultry products in Malaysia showed a higher resistance to antibiotics than *C. coli*, which was consistent with our findings. They exhibited that the resistance of *C. jejuni* isolates to ampicillin, cefotaxime, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, norfloxacin, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline was 81%, 20%, 51%, 99%, 7%, 76%, 1%, 5%, 84%, 80%, 50%, 96%, and 96%, respectively. Similar resistance rates for *Campylobacter* spp. were also reported in surveys conducted in China,[34] Poland,[35] Iran,[36] Malaysia,[37] and Latvia.[33]

We found that the incidence of *Campylobacter* spp. among the examined samples was 44.75%. Higher incidence of *Campylobacter* spp. in poultry meat samples was reported from Ireland (80%-100%) [38] and Japan (80%-100%),[39] while lower incidence was reported from Italy (17.38%),[22] Denmark (12%),[17] and Finland (11%).[17] A lower incidence of contamination of ruminant meat with *Campylobacter* spp. has also been reported in previous studies.[40,41] The lower levels of *Campylobacter* in pork and beef may be due to a lower incidence of these organisms in swine and cattle populations than in poultry, as well as the sensitivity of *Campylobacter* to atmospheric oxygen and other environmental stresses during transport, processing, and storage of the products tested.

Conclusion

The current study was conducted to assess the incidence rate and antibiotic resistance of *Campylobacter* spp. isolated from raw turkey, quail, chicken, duck, partridge, goose, pheasant, ostrich, wild duck, wild pheasant, wild goose, and coot meat samples. The findings revealed that the incidence of *Campylobacter* spp. was 44.75% among the examined poultry meat samples with higher incidence of bacteria in wild goose (83.33%) and coot (78.26%), respectively. Furthermore, a higher incidence of *C. jejuni* than *C. coli* was observed. The current research is one of the most comprehensive studies to evaluate the incidence of antibiotic-resistant *Campylobacter* spp., particularly *C. jejuni* and *C. coli* bacteria isolated from poultry meat samples in Iran. A higher resistance of *C. jejuni* than *C. coli* to antibiotic agents was obtained. Moreover, our findings showed that the poultry meat samples, particularly partridge, quail, pheasant, wild duck, and turkey meat samples were the reservoirs of resistant-*Campylobacter* spp. Additionally, because of the high resistance of isolated bacteria to tetracycline, nalidixic acid, ciprofloxacin, enrofloxacin, and ampicillin antibiotic agents, they are not recommended for treating *Campylobacter* food poisoning cases. Furthermore, our results showed that consuming raw or undercooked poultry meat is a major public health hazard. Proper cooking of poultry meat and monitoring the prescription of antibiotics can reduce the risk of antibiotic-resistant *Campylobacter* transmission from poultry meat to humans.

Authors' Contributions

Authors equally contributed particularly to the concept, laboratory experiments, and analysis of the data.

Ethical Approval

The ethical codes of the current study were approved by Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Conflict of Interest Disclosures

Authors declare that they have no conflict of interests.

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