RESEARCH ARTICLE

Molecular Analyses of the Prevalence of *Campylobacter* Detected from the Poultry Meat and its Byproducts

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Abstract

Campylobacter is one of the most common bacteria that is usually transmitted between humans and animals. Among the animal source foods (ASF), the presence of the *Campylobacter* spp. in poultry meat has been extensively reported, while the consumption of undercooked meat is the most important means of transmission of this bacterium to humans. Hence, determining the prevalence of *C.jejuni* and *C.coli* in poultry meat and byproducts through the bacteriological and molecular methods was the pivot of this research. This descriptive cross-sectional study was conducted on poultry carcasses collected from the slaughterhouses of Jahrom-Iran. The presence of *Campylobacter* spp. in the samples was checked through bacteriological and molecular methods. Obtained data were analyzed by SPSS-16 on the descriptive statistics level. In the bacteriological examination, 0.91% and 3.98% of the samples were found to be infected with *C.jejuni* and other *Campylobacter* spp., respectively. In the molecular examination, 9.8%, 1.2%, and 0.9% of the samples were found to be infected with *C.coli*, *C.jejuni*, and other *Campylobacter* spp., respectively. Thus, it was concluded that the prevalence of the *Campylobacter* species in poultry meat, Jahrom is lower than their prevalence in the poultry meat in other cities of Iran and other countries.

Introduction:

Campylobacter is a curved gram-negative, non-spore-forming, and motile bacillus from the *Campylobacteraceae* family (Kaakoush *et al.*, 2015). This bacterium, which has numerous species and hosts, is one of the most threaten and most common bacteria transmitted between humans and animals (Allos & Blaser, 1995; Silva *et al.*, 2011). *Campylobacter jejuni* (*C.jejuni*), *Campylobacter coli* (*C.coli*), and other *Campylobacter* spp. account for 95%, 4%, and 1% of the *Campylobacteriosis* cases in humans, respectively (Adopted 28 September, Coral Gables, Fla. (2007). However, *C.jejuni* is the main source of more than 85% of the bacterial intestinal infections, gastroenteritis (Sahin *et al.*, 2002).

As per the world-wide record, 20-35% of human diarrheas are caused by *C.jejuni* (Henao *et al.*, 2015). Other rare *Campylobacter* infections are septic arthritis and bacteremia (Fitzgerald, 2015). In addition, *Campylobacter* infections can result in chronic

complications and acute paralysis (Vandeplas *et al.*, 2008; Vicente *et al.*, 2008). *Campylobacteriosis* is substantially important in humans, especially those who consume undercooked or raw poultry and cow meat. *Campylobacter* spp. have a greater pathogenic potential in children, old age people, and patients suffering from immuno-deficiency (Grennan *et al.*, 2001). Thus, it is become necessary to study and identify the causes of contamination with *Campylobacter* in slaughterhouses due to increasing pathogenesis of this bacterium. Studying the prevalence of the *Campylobacter* spp. in poultry meat and byproducts in the slaughterhouses of Jahrom-Iran through bacteriological and molecular methods was the overarching goal of this research.

Materials and methods:

In this descriptive cross-sectional study, 328 samples of poultry carcasses were randomly collected from the slaughterhouses in Jahrom- Iran, Jan.2017-Jun.2017. Each sample was washed with 400 ml of 0.1% sterilized peptone

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water, and the solution resulting from this process was filtered using a clean porous sterilized cloth and then was centrifuged for 30 minutes at 6000xg at 42°C. After throwing out the liquid on the surface, the sediment was cultured in 5 ml of the Exeter broth enrichment medium containing; Cefoperazone 1.5 mg/Lit, Amphotericin B 2 mg/Lit, Trimethoprim 10 mg/Lit, Rifampin 5 mg/Lit, and Polymyxin 2500 IU/Lit. Following 48 hr of microaerophilic incubation (5% O₂, 10% CO₂, 85% N₂, 42°C), 0.1 ml of the cultured sample in the enriched medium was isolated and cultured on a selective Skirrow Agar containing vancomycin 10 mg/Lit, polymyxin B 250 mg/Lit, trimethoprim 5 mg/Lit, and 5% of defibrinated horse blood 5%. Following 48 hours of microaerophilic incubation (5% O₂, 10% CO₂, 85% N₂, 42°C), hemolytic, gray, 1-5 mm, circular, and aqueous colonies were selected and examined after gram staining. Gram-negative, spiral rod-shaped bacilli were isolated and were exposed to the oxidase and catalase tests. Bacteria with oxidase and catalase positive tests were transferred to a heart infusion broth containing sodium hippurate for the hippurate hydrolysis test and were incubated for 24-48 hr at 42°C. Thereafter, 0.8 ml of the medium was mixed with 0.2 ml of the FeCl₃ solution and stored for 30 minutes at the room temperature. The samples containing sediments were considered to contain *C.jejuni* (the hippurate in the medium was hydrolyzed and transformed into benzoic acid and glycine) and samples with no sediments were considered to contain other *Campylobacter* spp. (Henao *et al.*, 2015). In this study, the standard *C.jejuni* strain ATCC33560 was used as a positive control. For the molecular assay, the kits produced by the Iranian Nedaye Fan Company (Cat.NO: PR881613) were used to extract the DNA from the poultry carcasses samples. The PCR tests were also carried out using Jenet Bio kits (Cat.No: G-2000) in accordance with the kit instructions. The primers were selected from the relevant articles and were examined in the <https://blast.ncbi.nlm.nih.gov/Blast.cgi>.

Table-1: Primer Sequence used for detection of *Campylobacter* spp.

Amplicon	Primer	Primer sequence(5/-3/)
1062 bp	Universal	F: 5'-GGAGGC AGC AGT AGG GAA TA-3' R: 5'-TGACGGGCGGTGAGTACAAG-3'
840 bp	C.spp (16srRNA)	F: 5'-GGAGGATGACACTTTTCGGAGCG-3' R: 5'-TCGCGGTATTGCGTCTCATTGTATATGC-3'
344 bp	<i>C.jejuni</i> (Hippo)	F: 5'-GACTTCGTGCAGATATGGATGCTT-3' R: 5'-GCTATAACTATCCGAAGAAGCCATCA-3'
500 bp	<i>C.coli</i> (asp)	F: 5'-GGTATGATTCTACAAAGCGAG-3' R: 5'-ATAAAAGACTATCGTCGCGTG-3'

In the molecular process, 4 primer pairs were used (Table 1); i) A 16s Universal primer for internal control (Persson & Olsen, 2005), ii) A primer 16s rRNA for detection of all species of *Campylobacter* (Vanniasinkam *et al.*, 1999), iii) A hippo primer (hippuricase gene) for detection of *C.jejuni*

(Persson & Olsen, 2005), iv) A asp primer (aspartokinase gene) for detection of *C.coli* (Misawa *et al.*, 2002).

The PCR tests were conducted on the DNAs, extracted from the poultry carcasses using the specific *Campylobacter* primer. In the samples when the presence of the *Campylobacter* DNA was confirmed, each of the *C.coli* and *C.jejuni* species was subjected to molecular examinations using their genus-specific primers. The results from the PCR reactions were examined through an agarose gel (1.5%) electrophoresis and ethidium bromide staining under ultraviolet radiation and the data were statistically analyzed in SPSS on the descriptive statistics level.

The presence of the specific DNA of *Campylobacter* in a molecular test was considered as the contamination of that sample with *Campylobacter*. The presence of specific *C.coli* and *C.jejuni* DNA in the molecular study was considered as the contamination of the sample with *C.coli* and *C.jejuni*.

Results:

According to the bacteriological assay results, 201 samples (61.28%) of the 328 samples were infected with *Campylobacter* spp. The prevalence of *C.jejuni* and other *Campylobacter* spp. was 109 (33.23%) and 92 (28.05%), respectively. In the molecular assay (Fig.-1), 67.99% of the samples contained the *Campylobacter* (Table-2).

Figure-1: The result of the electrophoresis of the products of the PCR-based amplification of DNA extracted from the samples.

Table-2: Distribution of various *Campylobacter* spp.in the samples

Contamination	Molecular Frequency Number	Percent
<i>Campylobacter jejuni</i>	116	35.37
<i>Campylobacter coli</i>	65	19.82
Co-Contamination: <i>C.jejuni</i> & <i>C.coli</i>	29	8.84
Other <i>Campylobacter</i> species	13	3.96
All <i>Campylobacter</i> contamination	223	67.99
Non-contamination with <i>Campylobacter</i>	105	32.01
Total	328	100

Discussion:

The results from this study revealed that the frequency of contamination with *Campylobacter* spp. in the poultry carcasses samples collected from the slaughterhouses of Jahrom was 61.28% and 67.99% from the bacteriological

and molecular assays, respectively.

In other studies conducted in different cities of Iran, the frequency of meat contamination with *Campylobacter* was as follows: Isfahan 56.1% (Rahimi & Tajbakhsh, 2008), Tehran 63.2% (Dallal *et al.*, 2010), Mashhad 76%, and Shahr-e Kord 47% (Rahimi & Ameri, 2011). These findings are parallel with the results obtained from the present study. The frequency of meat contamination with *Campylobacter* reported from the studies conducted elsewhere was as follows: Turkey 91.8% (Yildirim *et al.*, 2005), Korea 68.3% (Han *et al.*, 2007), Canada 62.4% (Valdivieso-Garcia *et al.*, 2007), and Japan 40-77% (Sallam, 2007). These findings from mentioned researches are also resembling with our present results.

The molecular frequency of the *C.jejuni* was higher than *C.coli* (35.37% vs. 19.82%) found in our study. These results have a consistency with the results of previous findings (Eyigor *et al.*, 1999; Van Looveren *et al.*, 2001). Therefore, the results from the present study may not comply with the results of the aforementioned studies. This statistical difference could be attributed to the lower prevalence of *Campylobacter* in the present study as compared to the other studies, the difference between the sampling techniques, the difference between the prevalence of *Campylobacter* in different seasons (with the lowest prevalence in spring and winter and the highest prevalence in autumn and summer), and the difference between the geographical regions. For instance, we carried out this research in the hot and arid regions.

In this study, the comparison between the molecular and bacteriological analyses revealed that 201 samples had both positive molecular and bacteriological performances. However, 22 samples had positive PCR results and negative bacteriological results. Their considerable difference between the diagnoses by the two methods is also worth mentioning. Majdani (2016) used the culture and PCR methods to study the prevalence of human diarrhea caused by *C.jejuni* and *C.coli* in Isfahan. He reported that of the 196 diarrhea cases, 18 were infected with *Campylobacter* following the PCR tests from which 18 cases 14 had the *C.jejuni* infection and 4 had an infection with *C.coli*. In the bacteriological examinations, 15 of the 196 diarrhea samples had positive *Campylobacter* results: 12 samples were infected with *C.jejuni* and the other 3 were infected with *C.coli* (Havaei *et al.*, 2006; Majdani, 2016). The findings from present research and Fazli's research confirm the greater potential of the molecular method for diagnosing the contamination of foodstuff with *Campylobacter* spp. and other bacteria. As regards the research constraints, the small sample size was one of the limitations on this research, while sampling was carried out within a short period of time only in the low-prevalence seasons, viz. spring and winter.

Finally, the results from the present study indicate that

C.coli accounts for most of the poultry contamination in Jahrom-Iran. However, its prevalence is extremely lower than the results reported in the previous studies. The low prevalence of *Campylobacter* in the poultry meat in this part of Iran can be attributed to the application of the health principles in the chicken coops and slaughterhouses of this area.

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