Archives of Hygiene Sciences
Qom University of Medical Sciences

https://jhygiene.muq.ac.ir/
10.34172/AHS.12.2.1.393
Vol. 12, No. 2, 2023, 93-98

Original Article

Prevalence of *Campylobacter* spp. Among Broiler Carcasses at Industrial Slaughterhouses in Hamedan, Iran

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Abstract

Background & Aims: Today, food-borne diseases are known as one of the most important public health concerns in many countries. *Campylobacter* is one of the most prevalent food-borne pathogens. Raw chicken meat has been reported as the main source of human campylobacteriosis. The present study was conducted to investigate the prevalence of *Campylobacter* species among broiler carcasses at industrial slaughterhouses in Hamedan province, west of Iran.

Materials and Methods: Totally, 100 samples were collected using sterile swabs from chicken skin at the post-scalding stage. The samples were enriched in Brucella broth containing *Campylobacter* selective supplement and incubated at 42°C for 48-72 hours under microaerophilic conditions. The molecular detection and identification of *Campylobacter* species were performed by the polymerase chain reaction (PCR) using cadF and Hip primers for detecting *Campylobacter* species and *Campylobacter jejuni*, respectively.

Results: The results of this study revealed that 81% and 31% of broiler carcasses were positive for the presence of *Campylobacter* species and *C. jejuni*, respectively.

Conclusion: Due to the high contamination rate of chicken meat with this pathogen, precise hygienic control of poultry meat and an increase in consumer awareness seem necessary to decrease human campylobacteriosis.

Keywords: Foodborne diseases, *Campylobacter*, Public health, Chickens, Abattoirs

Received: April 5, 2022, **Accepted:** October 3, 2022, **ePublished:** May 31, 2023

1. Introduction

Human campylobacteriosis is the most common food-borne disease in the United States, accounting for 1.9 million cases per year [1,2]. Campylobacteriosis is one of the most important diseases transmitted through the gastrointestinal, especially in children younger than 5 years. According to the latest reports by the European Center for Disease Control and Prevention, campylobacteriosis was reported as the most prevalent zoonotic disease in 2012 [3]. Commonly, in developing countries, there is no national surveillance program for campylobacteriosis, thus the exact amount of its incidence in these countries is unavailable [4]. This disease is associated with self-limiting dysentery, abdominal cramps, and fever; however immunodeficient people may need antibiotic therapy [2,5].

Thermophilic campylobacters, especially *Campylobacter jejuni*, and *Escherichia coli* have been recognized as the most important causes of intestinal infections in humans in many countries [6-8]. In addition to intestinal disease, *C. jejuni* is known as one of the important causes of Guillain-Barre syndrome [9,10].

Campylobacter is a Gram-negative and spiral bacterium, which is sensitive to oxygen and grows in microaerophilic conditions, including 3-5% oxygen and 3-10% carbon dioxide with a temperature range of 40-42 °C [8,11]. Further, water activity (aw) < 0.987, NaCl > 2%, and pH < 4.9 inhibit bacterial growth [12]. The main mechanisms for *Campylobacter* pathogenesis are adhesion, invasion, and toxin production [11-15].

Contaminated poultry meat has been reported as the major source of human campylobacteriosis in the food supply [16,17]. Overall, 50-80% of human cases of Campylobacteriosis are related to the consumption of poultry meat [18]. The *Campylobacter* contamination rate in poultry carcasses at slaughterhouses has been found to be between 34.9% and 100% [19]. Weak hygiene practices and subsequently carcass contamination with feces and intestinal contents during slaughter processing lead to an increase in the contamination rate by *campylobacter* [20]. Inappropriate handling, preparation, and consumption of raw or semi-cooked poultry meat increase the risk of campylobacteriosis in humans. Poultries such as chicken, turkey, duck, goose, and wild birds are infected with campylobacters and mainly with *C. jejuni* and *E. coli* [21].

Campylobacters are fastidious bacteria [22], and

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the isolation and identification of campylobacters via conventional microbiological culture methods require enrichment culture, subculture to selective media, optimization of growth condition, and antibiotic support to reduce the co-cultured bacteria [23,24]. In addition, these methods are time-consuming and do not provide an accurate measure of the frequency and diversity of *Campylobacter* species associated with food [25,26]. Furthermore, rapid, specific, and sensitive molecular techniques such as the polymerase chain reaction (PCR) have become increasingly routine for the detection of foodborne pathogens in food supply and feces [26-28]. The numbers of PCR assay methods and target genes have been described previously for the detection of campylobacters in foods, water, feces, and environmental samples [29-31].

Considering the importance of the poultry industry in Iran, the significant role of *Campylobacter* in human infections, and the limited information about the contamination status of poultry herds in Hamedan, this study sought to investigate the prevalence of *Campylobacter* species among poultry carcasses at slaughterhouses in Hamedan province, west of Iran using the PCR method.

2. Materials and Methods

2.1. Sample Collections and Culture Conditions

This study was performed on 100 specimens collected from broiler chickens at two slaughterhouses in Hamedan province, west of Iran from February to June 2018.

After the scalding stage, the samples were collected from all areas of chicken skin using a sterile swab. Then, they were enriched by inoculation in Brucella broth media (Condalab, Spain) supplemented with vancomycin (1.0 mg/mL), trimethoprim (5.0 mg/mL), and polymyxin B (25 000 IU/mL) at 42 ˚C in microaerophilic conditions for 48-72 hours.

2.2. DNA Extraction

Genomic DNA was extracted from bacterial strains using FavorPrep™ Genomic DNA Extraction Kit (Favorgen Biotech, Taiwan). For this purpose, all enriched bacterial suspensions were centrifuged at 13 000 rpm for 2 minutes. Moreover, the supernatant was discarded, and the DNA of the pellet was extracted according to the manufacturer's instructions. The quality and quantity of genomic DNA were checked by NanoDrop 2000™ (Thermo Scientific, Wilmington, USA) and gel electrophoresis in a 1% (W/V) agarose.

2.3. PCR Assay and Sequencing

Campylobacter isolates were detected and identified by the PCR using primers described in Table 1. cadF.F and cadF.R primers flanking the outer membrane protein-encoding gene (*cadF*), a conserved gene in *Campylobacter* species, were used for the detection of *Campylobacter* species. The HIP400F and HIP1134R primers targeting the hippuricase (*hip*) gene, which is absent from campylobacters other than *C. jejuni*, were used to amplify the DNA of *C. jejuni* [32].

PCR assay for molecular detection was performed in a 25 µL reaction mixture containing 12.5 µL of Taq DNA Polymerase 2X Master Mix® (Ampliqon, Odense, Denmark), 0.25 µL of each primer pair (10 pmol–Bioneer, Daejeon, South Korea), 5 µL template DNA, and 7 µL distilled deionized water. PCR amplification was performed in a SimpliAmp® thermal cycler (Applied Biosystem, USA) with conditions including initial denaturation at 95˚C for 5 minutes, followed by 30 cycles of denaturation at 95˚C for 45 seconds, 60 seconds at the annealing temperature specific for each primer (Table 1), extension at 72˚C for 1 minute, and a final extension at 72˚C for 5 minutes.

The amplified products were analyzed by agarose gel electrophoresis in a 1.5% (W/V) agarose gel stained with ethidium bromide (0.5 µg/mL, SinaClon, Iran) at 110V for 55 minutes. The gels were viewed under UV light and photographed using UV Imager (Transluminator, France). Further, a 100 bp molecular weight marker (SinaClon, Iran) was used as the size standard. The *Campylobacter* strain, kindly provided by Staji [33], and distilled deionized water were employed as positive and negative controls, respectively.

After the amplification of the target genes, the PCR product of three samples amplified by the *cadF* gene was sequenced, and all DNA sequencing of the PCR product was performed by Takapouzist Company (DynaBio™, Iran) in an applied Biosystems 3500 (ABI) genetic analyzer (Applied Biosystems Inc, USA). The obtained nucleotide sequences were compared with the other sequences of *Campylobacter* species, deposited in GenBank by using the BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

Out of 100 DNA extracts, 81 isolates were determined to be positive for *Campylobacter* spp. using genus-specific primers (Figure 1), and 31 strains were identified as *C. jejuni* using *C. jejuni*-specific primers (Figure 2).

The obtained nucleotide sequence revealed > 98% identity to various *Campylobacter* species deposited in NCBI. The nucleotide sequences of the *cadF* gene were deposited in GenBank® (accession number: MN603165).

4. Discussion

Nowadays, the vital role of poultry in providing animal protein for humans is noticeable in different communities. This role is mainly due to low production costs, short breeding periods, high food efficiency, and white meat nutritional superiority. Continuous monitoring to ensure

Table 1. Oligonucleotide primers used for the detection and identification of *Campylobacter* species and *Campylobacter jejuni*

Primer	Oligonucleotide sequence (5′→3′)	Target gene	Annealing temperature (°C)	Amplicon size(bp)	Reference
cadF.F	TTGAAGGTAATTTAGATATG	cadF	44	400	(32)
cadF.R	CTAATACCTAAAGTTGAAAC				
HIP400F	GAAGAGGGTTTGGGT GGTG	hip	66	735	(32)
HIP1134R	AGCTAGCTTCGCATAATAACTTG				

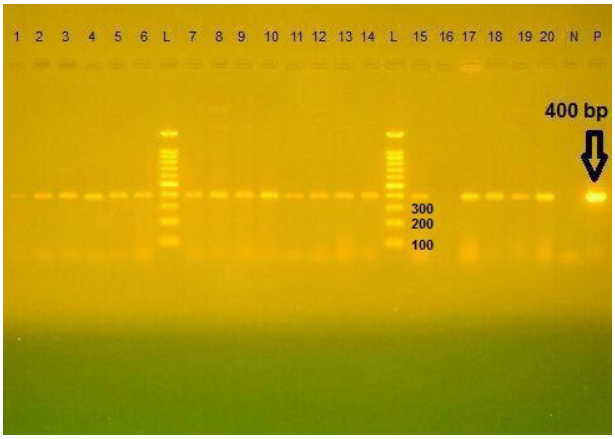

Figure 1. PCR identification of *Campylobacter* species isolated from broiler carcasses. *Note*. PCR: Polymerase chain reaction; Lanes 1-20: *Campylobacter* species isolated from broiler carcasses; Lane P: Positive control; Lane N: Negative control (distilled water); Lane L: Molecular weight ladder.

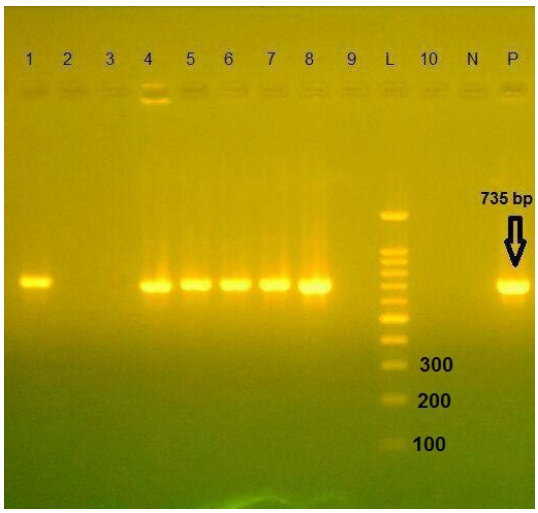

Figure 2. PCR identification of *Campylobacter jejuni* isolated from broiler carcasses. *Note*. PCR: Polymerase chain reaction; Lanes 1 and 4-8: *C. jejuni* isolated from broiler carcasses; Lane P: Positive control; Lane N: Negative control (distilled water); Lane L: Molecular weight ladder

that poultry meat is free of pathogens seems important and necessary due to the valuable role of poultry in the food supply. *Campylobacter* is one of the most important zoonotic pathogens that has a potential risk for poultry meat consumers. Extensive studies have been conducted on the contamination sources of campylobacteriosis in humans in many countries, and identifying and controlling these sources could play an important role in campylobacteriosis control and prevention [34].

Campylobacter species are the intestinal microflora in many domestic animals, thus they are considered a potential risk to food safety due to the contamination of carcasses in slaughterhouses. *Campylobacter* contamination can occur at various stages throughout the food chain including production, processing, mishandling distribution, transportation, and preparation [12].

In Iran and other countries, various investigations have focused on the contamination of industrial poultries with *Campylobacter*, indicating different contamination rates in feces and chicken meat [35-37]. In this study, out of 100 samples collected from broiler carcasses at Hamedan slaughterhouses, 81 and 31 *Campylobacter* species and *C. jejuni* were isolated and identified, respectively. These results are in accordance with those of other studies conducted in different regions of Iran.

The high prevalence of *Campylobacter* in feces, meat, and other poultry-related products has been reported worldwide. The prevalence of *Campylobacter* species was reported at 68.8% (40.2% of *E. coli* and 28.5% of *C. jejuni*) in chicken carcasses at the slaughterhouse in the south of Spain [38]. The results of the study by Panzenhagen et al showed that the prevalence of *campylobacter* infections was 45% (53.66% of *C. jejuni* and 46.34% of *E. coli*) in the poultry carcasses at the six slaughterhouses in Rio de Janeiro, Brazil [39]. Han et al reported that the *campylobacter* infection rate was 56.1%, 31.0%, and 17.0% in caecal samples, carcasses, and carcass parts, respectively, at slaughter in China [40]. Gonsalves et al demonstrated that infection rates for *C. jejuni* and *E. coli* were 72% and 38%, respectively [41]. In the study conducted in India, the contamination rate of *campylobacter* in both whole and sliced chicken was 13.2% and 30%, respectively [42]. Huang et al compared the prevalence of *Campylobacter* in chickens slaughtered by traditional and industrial methods in China. The results of this study represented that the highest rate of infection was related to conventional slaughter, while the overall prevalence of *Campylobacter* was 51.3%. The results of this study indicated that *Campylobacter* infection is present in live chickens; therefore, weak hygiene practices during slaughter processes will increase the spread of the disease [43]. In the study performed on 200 samples collected from chicken meat from retailers in Lahore, Pakistan, the infection rate of *Campylobacter* species was 29% using the conventional culture method and the PCR technique [44]. In a study conducted in Accra, Ghana

to compare the level of the contamination of chicken meat with *C. jejuni* in wet markets and supermarkets, the chicken meat offered in wet markets showed a higher level of *C. jejuni* contamination, confirming the necessity of keeping chicken meat in proper storage conditions after transferring the chicken to the slaughterhouse [45].

In Iran, several studies have been conducted on the prevalence rate of *C. jejuni* in chicken carcasses. Sari et al reported a contamination rate of 28% in poultry carcasses by *C. Jejuni* in Mashhad Industrial Slaughterhouse [46]. Babaie Najad Basiri et al found that out of 150 fecal swab samples collected from healthy broilers, 98 (65.3%) samples were infected with the thermophilic strains of *Campylobacter*. Out of 98 *Campylobacter* isolates, 79.59% and 20.4% belonged to *C. jejuni* and *E. coli*, respectively [47]. The prevalence of *Campylobacter* in chicken skins at a slaughterhouse in Urmia was reported at 58.75% using bacterial culture methods [48]. In the study by Zendehbad et al, the prevalence of *Campylobacter* in broiler poultry in Mashhad was 63%, and the highest infection rate belonged to *jejuni* species and occurred in the summer season (78.9%). The prevalence of contamination reported in this study was higher than in the current research [49]. In Shiraz, Ansari-Lari et al investigated the infection rate of broiler carcasses with *C. jejuni* and *E. coli*. Based on this study, 33.31% to 35% and 36.7% to 40% of broiler carcasses were infected with *C. jejuni* and *E. coli*, respectively [35]. Nouri Gharajalar et al concluded that the prevalence of *C. jejuni* and *E. coli* in the chicken liver was 72% and 28%, respectively [36]. Rahimi et al found that improving the poultry diet by adding probiotics and prebiotics to the diet is effective in the maturation of the intestinal immune system of poults, which, in turn, prevents the contamination of the poultry intestine with pathogenic microorganisms such as salmonella and *Campylobacter* [37].

The conflict between the results of the mentioned studies with the present study could be attributed to temporal and spatial differences between the studies, including differences in the method of slaughter and the application of hygienic practices during the slaughter process.

5. Conclusion

The results of this study revealed that 81% of broiler carcass samples taken from chicken slaughterhouses in Hamedan province were positive for the presence of *Campylobacter* species. Further, the results of this study showed that PCR is a fast, sensitive, and accurate molecular method for the identification of *Campylobacter*. Therefore, to determine the definitive identification of *campylobacter*, in addition to culture, the simultaneous use of other methods such as PCR can be extremely helpful. In conclusion, it is necessary to make good hygiene practices during different slaughter stages and distribution chains to prevent the occurrence of human campylobacteriosis.

Acknowledgments

The author would like to thank Ms. Sakineh Azami and Dr. Pejman Mahmoodi for their technical help.

Authors' Contributions

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Writing–review & editing: Abbas Ali Sari, Ali Goudarztalejerdi.

Competing Interests

The authors declared no conflict of interests.

Ethical Approval

Samples were collected with the permission of the Ethics Committee of Bu-Ali Sina University, Hamedan, Iran (code: IR.BASU.REC.406903).

Funding

This work was supported by a grant from the Bu-Ali Sina University, Hamedan, Iran (Grant No. 98-64 to Abbas Ali Sari).

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