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Original article

Monitoring the Virulence Genes in *Campylobacter coli* Strains Isolated from Chicken Meat in Tehran, Iran

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Background: *Campylobacter* species are the main food-borne pathogens which could cause gastroenteritis in humans. Contaminated chicken products have been documented as the primary sources of *Campylobacter* transmission to human. This study was done to test raw chicken meat products retailed in local markets in Tehran, Iran for the presence of *Campylobacter coli* and *Campylobacter jejuni* species.
Materials and methods: A total of 70 raw chicken meat samples were collected during a three-month study. All the *Campylobacter* species were identified by biochemical and species-specific polymerase chain reaction (PCR). These isolates were investigated further to examine their potential virulence factors.
Results: *Campylobacter spp*. were detected in 56% of the isolates and identified as *C. coli*. The results indicated that all of the isolates were positive for *cadF, cdtA, iam* genes. On the other hand, none of the isolates were positive for *flaA and pladA* virulence genes.
Conclusion: Overall, the results showed that *Campylobacter* species were common contaminants in chicken meat, which should be screened for the presence of virulence determinants and for their involvement in food-borne diseases.

Keywords: Campylobacter coli, Campylobacter jejuni, PCR, Virulence gene

1. Background

Food-borne zoonotic diseases are considered as a significant reason of morbidity and mortality worldwide. The World Health Organization (WHO) has estimated that over two million individuals die each year from diarrheal diseases mostly caused by eating the contaminated foods (1).Thermophilic *Campylobacter* species is one of the most widespread causes of zoonotic bacterial food-borne diseases causing human bacterial gastroenteritis both in industrialized and developing countries. In addition to enteritis, extra intestinal infections and other infections may occur, including urinary tract infection (UTI), bacteremia, reactive arthritis, and “Guillain– Barre′ syndrome” affecting the peripheral nervous system (2). During the last decade, the incidence of gastroenteritis caused by *Campylobacter* species has been in an increasing trend (3). The main species known in flocks are *Campylobacter jejuni*, and *Campylobacter coli* (4). *Campylobacter* extensively is colonized in the intestinal tract of farm animals and birds (5). Poultry meat contamination is one of the principal causes of human campylobacteriosis infection (6). Chicken meat is considered not only as one of the most prevalent animal-based foods worldwide but also as an imperative source of *Campylobacter* both in industrialized and developing countries (7). The chicken products contaminated with *Campylobacter* species are recognized as the dominant source of campylobacteriosis infection, which emphasize their potential threat to public health.

Although virulence mechanisms in *Campylobacter* spp. are not completely known, a number of putative virulence and toxin genes have been identified so far using the molecular biology methods (7). Bacterial flagellum is one of the most significant virulence factors which are related to motility, adhesion, and invasion. *FlagellinA* (*flaA)* is responsible for chemotaxis and adherence. *Campylobacter* adhesion to fibronectin (*cadF*) is another factor which is responsible for adherence. Virulence genes linked to *Campylobacter* invasiveness are the invasion-associated marker (*iam*) genes, including *Phospholipase A* (*pladA)* and etc (7-9). Several toxins have also been identified in *Campylobacter*, among which cytolethal distending toxin (CDT) has been established to be lethal for host enterocytes (7-8).

2. Objectives

The objective of the present study was to investigate the prevalence rate of *Campylobacter* spp. in chicken meat shops in Tehran, and to characterize the *Campylobacter* strains in order to assess the prevalence rate of five virulence genes (*cadF*, *cdt*A, *iam*, *flaA*, and *pladA*) among the isolates.

3. Materials and methods

3.1.Sample collection and identification

A total of 70 chicken meat samples were selected. These samples were collected during a three-month study (summer 2012) from different shopping centers and retails in Tehran, Iran. All the samples were directly transported to the laboratory in Cary- Blair transport medium (Micromedia, Hungary) improved with 10g.lit^{-1} sodium pyruvate and lower agar content (0.5g.lit^{-1}) on ice (8, 10).

Of each sample, 25g were homogenized in a stomacher Labblender 400 (Seward, London, England) with 225 mL of Campylobacter enrichment broth base added with Campylobacter selective Supplement IV (HIMEDIA, Mumbai, India, FD158). Incubation was completed at 42°C for 24-48 h in a microaerophilic condition provided by gas pack Type C (Merck^{TM}Anaerocult C, Germany). Furthermore, a total of 0.1 mL of the enrichment broth was then streaked onto charcoal cefoperazonedeoxycholate agar (CCDA, Merck, Germany) for selective isolation of *Campylobacter* species (8-9).

Colonies suspected to *Campylobacter* were selected from each selective agar plate and subjected to identification according to the standard microbiological and biochemical tests including microscopic morphology, Gram staining, production of catalase, oxidase, fermentation of glucose, nitrate reduction, and hippurate hydrolysis (7).

3.2. DNA extraction and PCR condition

The DNA was extracted for PCR by the conventional boiling method. Briefly, one colony of each pure culture plate was suspended in 200 µL distilled water and heated at 95°C for 10 min in thermocycler, after which the suspension was centrifuged at 10000 rpm for 10 min, then the supernatants were stored at −20°C and used as template DNA (10-11). The identity of the isolates was confirmed by Polymerase chain reaction (PCR) using primers specific for *cadF*, *asp,* and *hipO* genes which specifically identify *Campylobacter* spp. Including *C. coli* and *C. jejuni* species, respectively (Table 1) (12). The PCR reaction mixture was consisted of 3 mL of each extracted DNA, 2.5 µL of 10x PCR buffer, 0.3 mL of 10mMdNTP mixture, 25 pmol of each of primers, and 0.6µL $MgCl_2$ (50 mM), 1U of Taq DNA polymerase and deionized water to a final volume of 25 µL. The amplification reaction was performed in a thermocycler system (Mastercycler gradient, Eppendrof, Germany). The following PCR conditions were used: initial denaturation at 95°C for 5 min; 30 cycles with denaturation at 95°C for 45s; annealing at 49°C for *iam*, 43°C for *cadF*, 45°C for *pldA* and *flaA* and *cdtA* for 1 min; and extension at 72°C for 1 min; with the final extension at 72°C for 5 min. Finally, the isolates were studied for the presence of five pathogenic genes. Primer sequences were derived from formerly designed primers (Table 1) (8, 12-15). The *C. jejuni* ATCC 29428 and *C. coli* ATCC 43478 strains were used as controls in each PCR assay (9).

Table 1. Primers used for the identification of *Campylobacter* isolates and amplification of virulence genes.

Gene	Product length (bp)	Sequence (5′ to 3′)	References
cadF	400	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	12
hipO	735	GAAGAGGGTTTGGGTGGTG AGCTAGCTTCGCATAATAACTTG	12
Asp	500	GGTATGATTTCTACAAAGCGAG ATAAAAGACTATCGTCGCGTG	12
cdtA	329	ATTGCCAAGGCTAAAATCTC GATAAAGTCTCCAAAACTGC	13
iam	518	GCA CAA AAT ATA TCA TTA CAA TTCACGACTACTATGAGG	14
pldA	913	AAGCTTATGCGTTTTT TATAAGGCTTTCTCCA	15
flaA	1743	TTTCGTATTAACACAAATGGTGC CTGTAGTAATCTTAAAACATTTTG	8

4. Results

4.1. Bacterial isolates

According to conventional biochemical tests used, a total of 39 (56%) cases identified with *Campylobacter* spp. out of 70 chicken samples, were isolated. An amplification band of 400bp (Fig. 1) was obtained for all of the isolates using specific *Campylobacter* spp. primer (*cadF*). Furthermore, all the isolates were identified as *C. coli* strains by conventional methods and confirmed by PCR amplification of *asp* gene and produced a 500 bp band for all the isolates (Fig. 2). None of the isolates were identified as *C. jejuni* gene, and all of them were negative for 735bp band of *hipO* specific PCR primer.

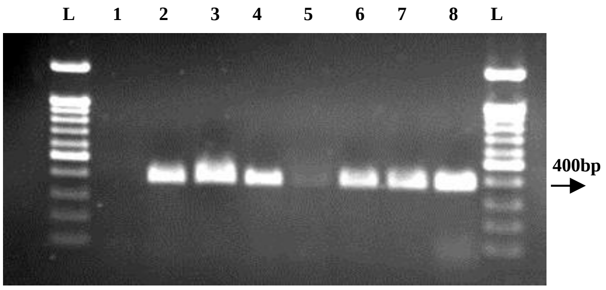

Fig 1. PCR amplification of *cadF* gene (400bp).Lane 1: negative control, lanes 2-7: isolates under study, lane 8: positive control, L: Ladder (DNA ladder).

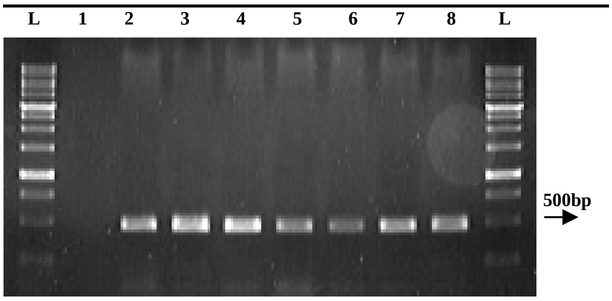

Fig 2. PCR amplification of asp gene (500bp).Lane 1: negative control, lanes 2-7: isolates under study, lane 8: positive control, L: Ladder (DNA ladder).

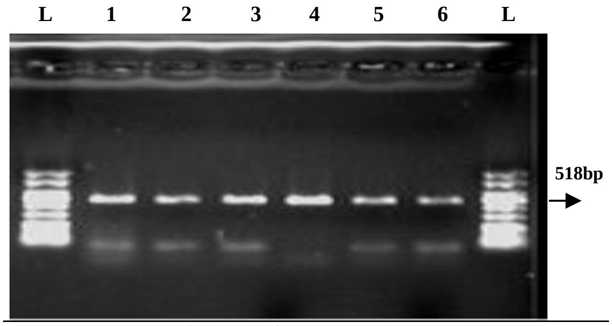

Fig 3. PCR amplification of *iam*gene (518bp).Lane 1: positive control, lanes 2-5: isolates under study, L: Ladder (DNA ladder).

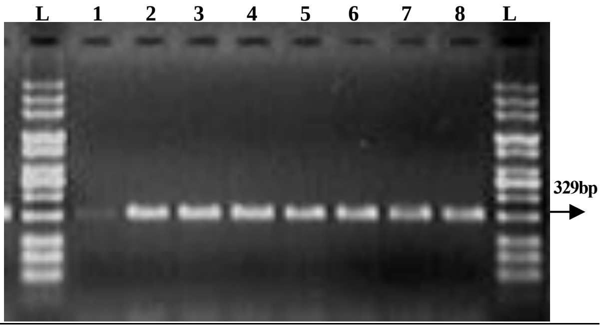

Fig 4. PCR amplification of *cdtA*gene (329bp). Lane 1: negative control, lanes 2-7: isolates under study, lane 8: positive control, L: Low-Range DNA ladder.

4.2. Prevalence of putative virulence genes

The PCR for detection of *cadF*, *cdt*A, *iam*, *flaA*, and *pladA* virulence genes showed that100% of the isolates were positive for *cdt*A, *cadF,* and *iam* genes (Fig. 1, Fig 3-4). On the other hand, none of the isolates were positive for *pladA*, and *flaA* genes.

5. Discussion

Many studies emphasized on the significance of poultry as a reservoir and source of *Campylobacter* infection (16). Generally, *Campylobacter* dwells in the intestines of poultry in a few days after hatching. Broiler herds are disheveled with *C. jejuni* and *C. coli* during the maturing cycle on the farmhouses (7). In this study, *Campylobacter* was isolated from 56% of the chicken meat samples and analyzed. The results observed in this study were in agreement with the findings of other studies conducted by other researchers, including 55% in Iran (17) and 68% in Brazil (18). However, other studies demonstrated higher prevalence rate of *Campylobacter* in poultry meat such as 81% in Italy (19) and 83% in the UK (20).

In this study, all of the isolates were identified as *C. coli* while no *C. jejuni* was found. Many reports from other countries including Reunion Island, Grenada, and Spain indicated that *C. coli* was the predominant *Campylobacter* species in broiler chickens (21-23) while others reported that *C. jejuni* species was more prevalent than other *Campylobacter* species (4, 5). One explanation for this phenomenon is that *C. coli* strains might have developed and substituted the prior *C. jejuni* strains in some slaughterhouse, or it might have occurred because of a discriminatory effect on one population compared to the others as a result of repetitive antimicrobial treatments ordered to the animals at the farmhouses (8, 19). This questionable statement is a common phenomenon in epidemiological investigations done in different times and places.

Investigation regarding the virulence markers of potentially pathogenic bacteria such as *Campylobacter* strains in domestic animals and in food with animal origin is vital to consumers' safety. For this purpose, we investigated the distribution of five virulence-associated genes of *Campylobacter* strains isolated from chicken meat. The present study showed a high prevalence rate for three out of five virulence genes including *cdt*A, *cadF,* and *iam* in all of the isolates. On the other hand, all the isolates were negative for *pladA* and *flaA* genes.

The outcome of the expression of the *cadF* gene is to encode an adhesin- and fibronectin-binding protein engaged in the progression of invasion and to alter microfilament organization in host cells (7). A study conducted by Rozynek et al. (24) showed that all of the obtained isolates had the *cadF* gene. The high prevalence rate (100%) of the *cadF* gene in the present study shows that many strains originating from poultry feces have pathogenic potential properties for humans.

Both of the *pladA* and *flaA* genes are involved in maximal invasion of human intestinal cells. The prevalence rate of virulence-associated genes (*pladA* and flaA) has been reported to be 80-100% in different studies (8). Different prevalence rates reported from different parts of the world show that *pladA* and *flaA* genes frequency are debatable.

6. Conclusion

A high level of contamination with *Campylobacter* spp. was observed in this study; thus, paying attention to hygiene processing and steady microbiological review of poultry meat are important steps in order to diminish the cross contamination. It is also significant to increase consumer's information about the precise handling and cooking of meat in order to avoid cross contamination at home before feeding.

Conflict of Interests

The authors declare that they have no conflict of interests in this investigation.

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Authors' Contributions

All authors contributed equally in this research.

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