Prevalence of *cdt* Gene in *Campylobacter jejuni* Strains Isolated from Surface Waters of Rasht, Iran

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ABSTRACT

Background and Objective: *Campylobacters* are infectious zoonotic agents, and among the main bacterial causes of gastroenteritis in humans. Studies have shown that *Campylobacter jejuni* is of the main causes of infection among humans. Detection of these infectious agents in water resources is of great importance for maintaining the health of humans. Therefore, the aim of this study was molecular detection of *C. jejuni* strains in surface water samples collected from Rasht, Iran.

Methods: This cross-sectional descriptive study was performed on 45 surface water samples collected from the city of Rasht. After culture and isolation of bacteria, the molecular detection of *C. jejuni* was carried out using hipO-specific primers. Presence of cytolethal distending toxin (cdt) gene in positive samples was evaluated by polymerase chain reaction using *cdtC*-specific primers.

Results: Of 45 samples, seven (15.5%) were positive for *C. jejuni* contamination, five of which (71.4%) had the *cdtC* gene.

Conclusion: The prevalence of toxin-producing *C. jejuni* in surface waters of Rasht is notable. Therefore, it is recommended to take necessary measures for controlling the spread of this microorganism.

Keywords: *Campylobacter jejuni*, Surface water, *cdt* gene, PCR.

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INTRODUCTION

Campylobacter is a genus of gram-negative microarrophytic bacilli, and one of the most important causes of gastroenteritis in humans (1). *Campylobacter jejuni* is the most common human clinical isolate, and the main cause of childhood diarrhea around the world (2). It is also an important factor in occurrence of Guillain-Barré syndrome (3). In many European countries, the rate of campylobacteriosis has been higher than salmonellosis (4, 5). Cytolethal distending toxin (CDT) is one of the virulence factors produced by *Campylobacters*. Some gram-negative bacteria including the *Campylobacter* spp. can produce this toxin. In 1988, Johnson and Lori were the first that reported CDT production by *Campylobacter* spp.

In a study, 41% of 718 *Campylobacter* isolates produced CDT (6). The toxin consists of three protein subunits: cdtA, cdtB, and cdtC (7) with molecular weight of 30, 29 and 21 kDa, respectively. These subunits have a tendency to associate with the outer membrane of the bacteria and contribute to delivery and activity of the toxin by simultaneous presence at the target site (6, 8). CdtA is the host-cell binding agent, while cdtB is the active part of halotoxin with dexoyribonucleic activity, requiring the presence of the cdtC subunit (9, 10). This subunit leads to formation of active toxins by connecting the other two subunits. The association between the presence of the *cdt* gene and the tissue invasion potential of *C. jejuni* has been demonstrated (11). Cdt causes morphological changes in the cell, damages the DNA, blocks cell proliferation in the G2 phase and prevents transition of cell cycle into the mitosis phase, leading to apoptosis (6, 12, 13). Some studies have reported the presence of the *cdt* gene in almost all *C. jejuni* isolates (14).

Campylobacteriosis is a common disease with a worldwide distribution among humans and animals (15). Poultry and poultry products are the most important sources of *C. jejuni* (16). Epidemiological studies have shown the high prevalence (40 to 100%) of *Campylobacter* spp. in chicken, duck and turkey (17-20). *C. jejuni* is isolated from flowing water, rivers, turkey slaughterhouse wastewater and even seawater (16, 18). The prevalence of *C. jejuni* in rivers and ponds has been variable, reaching up to 70% (8). Molecular screenings indicate that the presence of *Campylobacter* in water is due to contamination by wild and domestic animal feces (17, 19, 20). Considering the importance of the disease and bacterial screening in surface waters, and the role of *cdt* in the pathogenesis of this bacterium, it is essential to determine the frequency of this gene in *C. jejuni* isolates from high-risk areas. Therefore, the aim of this study was to determine the level of contamination with *C. jejuni* in surface water samples collected from the suburbs of Rasht, and evaluate the frequency of the *cdt* gene among positive samples.

MATERIAL AND METHODS

Sample collection and bacterial isolation

This cross-sectional descriptive study was conducted on 45 water samples collected from the rivers around the city of Rasht in autumn, 2014. One-liter samples were collected from the river (distance of one meter and depth of 30 cm) in sterile containers. For isolation of bacteria, the samples were centrifuged at 6000 rpm for 15 minutes. The resulting precipitate was transferred to Preston agar, and then incubated under microaerophilic conditions at 42 °C. After 48 hours, 100 µl of the medium was transferred to the charcoal agar medium. The plates were incubated under microaerophilic conditions at 42 °C for 48 hours. Then, all suspected colonies were examined microscopically. Biochemical tests including oxidase, catalase and hippurate hydrolysis were performed on the microbial colonies to detect *Campylobacter* spp. (21, 22).

Extraction of bacterial genomic DNA

After culture of bacteria in Preston broth without antibiotics, and incubation at 42 °C on rotary incubator at 60 rpm for 24 hours, extraction and purification of genomic DNA were performed using AccuPrep® kit (Bioneer) according to the manufacturer's instructions. The purity of the extracted genome was assessed by electrophoresis on 1% agarose gel.

Polymerase chain reaction (PCR)

A primer specific for the hipO chromosomal lucos of the bacterium was used for the molecular identification of *C. jejuni* (23). The characteristics of the primers used, the amount of reagents required for the PCR reaction and the thermocycler conditions for the amplification of the *hipO* gene are given in

Tables 1-3, respectively.Positive samples for *C. jejuni* were examined for the presence of *cdtC* gene using a specific primer for the gene (24) (Table 1). The amount of reagents used for the PCR reaction and the temperature conditions of the thermocycler were similar to the previous step, except for the annealing temperature of the *cdtC* primer (42 °C).

RESULTS

Gram-negative bacilli with wet, shiny and flattened grayish colonies. These motile, catalase positive, oxidase positive and hippuricase positive isolates were identified as *C. jejuni*.

A 735 bp fragment was produced in the PCR process using the *C. jejuni*-specific primer, which confirmed the results of the biochemical tests. Presence of the *cdtC* gene was detected in five of the seven (71.4%) *C. jejuni*-positive samples by identifying a 555 bp PCR product (Figure 2).

Table 1- Characteristics of the forward and reverse primers used in the study

Gene	Nucleotide sequence of primers	Length of PCR product
hipO	**F:5'GAAGAGGGTTTGGGTGGTG3' R:5'AGCTAGCTTCGCATAATAACTTG3'**	**735bp**
cdtC	**F:5'TGGATGATAGCAGGGGATTTTAAC3' R:5'TTGCACATAACCAAAAGGAAG3'**	**555bp**

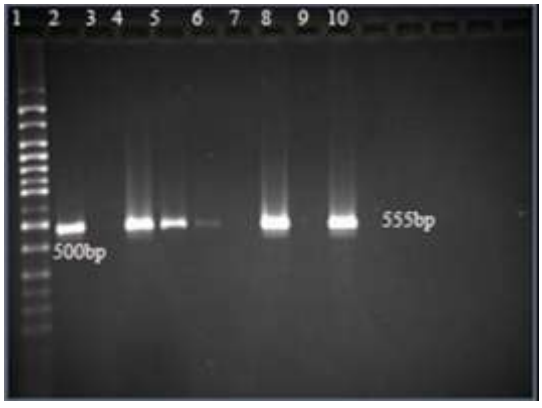
Table 2- The amounts of reagents used for the PCR reaction

Reagents	Amount
dNTP mix (10mM)	**1µl**
$MgCl_2$(50mM)	**1µl**
10X PCR Buffer	**2.5µl**
Primer Forward	**1µl**
Primer Reverse	**1µl**
Taq DNA Polymerase	**0.8 µl**
DNA Template	**3µl**
D.D.W	**14.7 µl**
Total Volume	**25 µl**

Table 3- Thermocycler temperature programs for the amplification of the hipO gene

Step	Process	Temperature	Time	Number of cycles
First	**Initial denaturation**	**95 °C**	**5 min**	**1**
Second	**Denaturation**	**94 °C**	**1 min**	**35**
	Annealing	**53 °C**	**1 min**	
	Extension	**72 °C**	**1 min**	
Third	**Final extension**	**72 °C**	**1 min**	**1**

Figure 2- Detection of the *cdtC* after gel electrophoresis of PCR products. Column 1: 100 bp marker, Column 2: positive control, Column 3: negative control (PCR without DNA template), Columns 4, 5, 6, 8, 10: samples containing the *cdtC* gene, Columns 7 and 9: samples lacking the *cdtC* gene.



DISCUSSION

We determined the frequency of *C. jejuni* in rivers around the city of Rasht, and the frequency of *cdtC* gene among the identified bacteria. Bacterial culture and PCR of hipO chromosomal locus of the bacterium were used to identify *C. jejuni* in the samples. Hippurate hydrolysis test has a high specificity for detection of the bacterium, while positive results are observed in isolates containing the *hipO* gene.

In this study, 15.5% of the examined water samples were contaminated with *C. jejuni*. Limited number of studies in Iran has investigated the prevalence of *Campylobacter* in surface waters. In study of Ghane et al., *C. jejuni* was identified in 2.66% of samples collected from the Caspian Sea using culture and PCR methods (21). The prevalence of this bacterium in water resources vary in different parts of the world. According to study of Dungan et al. in the United States, the level of contamination with *C. jejuni* in pond water samples is reported to be 70% (25), while study of Meinersmann et al. in Georgia found that only 7.5% of the samples are contaminated with *C. jejuni* (26). Van Dyke et al. evaluated the prevalence of *Campylobacter* in 344 surface water samples collected in Canada, and reported that 69.8% of the samples were positive for *Campylobacter* spp. in the PCR method (18).

According to our results, 71.4% of the positive samples for *C. jejuni* contained the *cdtC* gene. Several studies have reported the high prevalence of the *cdt* gene in *C. jejuni* isolates. of *cdtA, B, and C* genes to be 58.3% in 10 clinical isolates of *C. jejuni* (27). In study of Talukder et al. in Bangladesh, 97.5% of *C. jejuni* isolates had the *cdtA, B, C* genes. The mentioned study reported that the genes are present in strains causing diarrhea in humans, indicating the pathogenic potential of *Campylobacter* spp. (28). In a study in Brazil, 83.3% of *C. jejuni* isolates from poultry and vegetable samples contained the *cdt* gene (29). In Iran, Shojaei et al. detected the *cdt* gene in 93% of *C. jejuni* isolates from intestine of poultry, in 65% of *C. jejuni* isolates from turkey intestine, and in 67% of isolates from the intestine of quail (30).

CONCLUSION

The results of this study indicate that the frequency of potentially virulent *C. jejuni* is high in the rivers of the suburbs of Rasht, Iran. Considering the role of surface waters in the dissemination of campylobacteriosis, it is recommended that some preventive measures be taken for controlling the spread of this microorganism.

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CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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