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.
INTRODUCTION

Campylobacter is a genus of gram-negative microaerophilic 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). Cytotoxins 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 deoxyribonucleic 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...
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).

### RESULTS

Gram-negative bacilli with wet, shiny and

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide sequence of primers</th>
<th>Length of PCR product</th>
</tr>
</thead>
<tbody>
<tr>
<td>hipO</td>
<td>F:5'GAAGAGGGTTTGGGTGGTGTG3' R:5'AGCTAGCTTCGCCATAATAACTTG3'</td>
<td>735bp</td>
</tr>
<tr>
<td>cdtC</td>
<td>F:5'TGGATGATAGCAGGGGATTTTAAC3' R:5'TTGCACATAACCAAAAGGAAG3'</td>
<td>555bp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTP mix (10mM)</td>
<td>1µl</td>
</tr>
<tr>
<td>MgCl₂(50mM)</td>
<td>1µl</td>
</tr>
<tr>
<td>10X PCR Buffer</td>
<td>2.5µl</td>
</tr>
<tr>
<td>Primer Forward</td>
<td>1µl</td>
</tr>
<tr>
<td>Primer Reverse</td>
<td>1µl</td>
</tr>
<tr>
<td>Taq DNA Polymerase</td>
<td>0.8 µl</td>
</tr>
<tr>
<td>DNA Template</td>
<td>3µl</td>
</tr>
<tr>
<td>D.D.W</td>
<td>14.7 µl</td>
</tr>
<tr>
<td>Total Volume</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Process</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Initial denaturation</td>
<td>95 °C</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td>Second</td>
<td>Denaturation</td>
<td>94 °C</td>
<td>1 min</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Annealing</td>
<td>53 °C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>72 °C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Final extension</td>
<td>72 °C</td>
<td>1 min</td>
<td>1</td>
</tr>
</tbody>
</table>

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. Hipurate 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.

REFERENCES


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.


