The First Isolation of Campylobacter jejuni
from Caspian Sea's Water

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Abstract

The major purpose of this study was isolation, identification and characterization of Campylobacter spp. from Caspian Sea’s water in north of Iran. Sampling from the water was conducted within 4 seasons. Campylobacter spp. was isolated using standard method then identified by Phenotyping tests. Finally, the identification of strain was verified by PCR method. After conducting of the Phenotyping methods and their confirmation with molecular technique, seven strains of Campylobacter jejuni were identified. With regard to the obtained results, prevalence of this bacterium in the coastal waters of the Caspian Sea’s was evaluated as 2.66 percent. The result obtained from this study is, in fact, regarded as the first report from the isolation of Campylobacter jejuni from Caspian Sea. Knowledge of the state of the entry and time span of the survival of the Campylobacter in the water
environments to control the water quality and prevention from the disease is of importance.

**Keywords:** Campylobacter, Isolation, Caspian Sea, Iran

1. **Introduction**

Campylobacter’s are gram negative bacteria, which are seen in curve and S-shaped [1]. These bacteria have single and polar flagella in one or both end of the cell [2]. Campylobacter’s are microaerophilic, non proteolytic, non saccharolytic and non lipolytic, therefore to energy obtain from oxidation of amino acid or tricarboxilic acid [3, 4]. These bacteria commonly live in intestinal track of warm blood animals [4, 5].

Campylobacter’s are one of the most common causes of diarrhea in world wide. According to world health organization estimated 2-35% of diarrhea induced by campylobacter’s. So that, annually 400-500 million peoples in the word, which infected for these bacteria [6]. Disease in human being occurs with consumption of contaminated water and animal products viz., meat and milk [7, 8]. Studies showed that 95% of Campylobacteriosis in human induced by \textit{C.jejuni}, 4% by \textit{C. coli} and 1% by other species [9, 10].

Waters play important role in transmission of \textit{Campylobacter} to animal and human. These bacteria through of urine and feces of animal and birds into the water [11]. Several studies showed that \textit{Campylobacter jejune} isolated from surface water [12-14], rivers [15, 16], ground water [17], rivers sediments [18], lakes [19] lagoons [20], sea waters [21-23], sea sands [24] as well as contaminated water and soil to sewage [20]. This is why reported existence of \textit{Campylobacter jejune} in untreated drinking water [15, 17]. Hence, surface water appropriate source to transmission of this bacteria to Human beings [14].
Therefore, based on foregoing evidence *Campylobacter* infections are considered a worldwide problem of public healthy, causing considerable suffering and no more information is available concerning to isolation and frequency of occurrence of this bacterium from Caspian sea, in order to achieve minimum information concerning to the same.

2. Materials and methods

2.1. Sample collection

In total, 263 water samples were obtained from south coastal Caspian Sea in one year. The water samples were collected in 500 ml sterile bottles (falcon tube), from one deep on surface sediment and transported to the laboratory at ambient temperature and stored at 4°C. In order to keep the temperature low, all test tubes containing the water samples were placed in the full-of-ice flask. Sampling time was from 8 a.m. to 2 p.m., and the maximum time of transferring the samples to laboratory was one hour. The environmental features, including atmospheric condition, pH, temperature and salinity of water have been taken into consideration.

2.2. Sample processing and isolation

at first, the tubes containing water sample were centrifuged in 4000 rpm (ALC, Italy) within 10 minutes; then, supernatant was discarded and 1 to 2ml of it was used in order to isolate the bacterium. In the next step, water sample was transferred to the brain heart infusion broth (Merck-Germany) by the 0.45 micrometer filter. The tubes were incubated at 37°C for 48 h under microaerophilic conditions.

After this period a one loop was taken from the bacterial suspension and the spread culture was carried out on the CAMP medium (Merck-Germany), enriched by the defibrinated blood of sheep. All cultured plates were placed in an incubator
at temperature of 37°C for 72 hours and under microaerophilic conditions in order to isolate the *Campylobacter* spp.

2.3. Identification and biotyping of *Campylobacter* spp

*Campylobacter* identification was performed by subjecting all the suspected colonies to microscopic examination, gram staining, glucose fermentation, oxidase and catalase test. The isolates were characterized by using standard *Campylobacter* phenotypic identification tests recommended by Atabay & Corry [25]. At the end, the PCR method was carried out in order to confirm the Phenotyping results.

2.4. DNA extraction and PCR method

DNA was extracted from suspected colony using phenol chloroform method. The purity of the extracted DNA was analyzed based on absorbance of the extracted DNA at 260 and 280 nm wavelengths by biophotometer (Eppendorf-Germany). Specific Primers produced by TAG Copenhagen (Denmark) were used to amplify 16s rRNA for campylobacter gene. The sequences of forward and reverse primers were 5’-GGTTAAGTCCCGCAACGAGCCGC-3’ and 5’-GGCTGATCTACGATTACTAGCGAT-3’, respectively. Each reaction was performed in a total volume of 25 µl contained 14.5 µl of molecular biology-grade water (sigma aldrich company ltd.), 2.5 µl of 10×PCR buffer (cinagen-Iran), 1 µl of 10 pmol each forward and reverse PCR primers, 1 µl of a 10 mM dNTPs (cinagen-Iran), 0.5 µl of smar taq polymerase (cinagen-Iran), 1 µl of 50mm MgCl2 (cinagen-Iran) and 5 µl of DNA template. Non-template control (NTC) tube contained the same PCR reagents as above but had 5 µl of water substituted for template. PCR amplification conditions on thermocycler (Biorad-Germany) were as follows: 94°C for 4 min, followed by 30 cycles of 94°C for 60 s, 52°C for 45 s and 72°C for 45 s, with a final extension at 72°C for 5 min and storage at 4°C.
All PCR products were run on a 1.5% (w/v) agarose gels with a 1kb DNA ladder (Fermntas-Russia). Aliquots of PCR products were electrophoresed at 75 V for 40 min; DNA was visualized using ethidium bromide and photographed after UV transillumination with Uvidoc (England).

3. Results

Sampling from the Caspian Sea’s water was conducted within 4 seasons. Average water salinity of the Caspian Sea was 12.85 (ppt) in the various seasons. The minimum and maximum water temperature in the various seasons was reported 7 and 24°C, respectively. Also, water pH was ranged from 5 to 8 (Table 1).

After conducting of the Phenotyping methods and their confirmation with molecular technique, seven strains of *Campylobacter jejuni* were identified. With regard to the obtained results, prevalence of this bacterium in the coastal waters of the Caspian Sea’s was evaluated as 2.66 percent (table 2).

Of 7 isolated strains, 2 strains in spring and 5 strains in summer were identified, respectively. It is required to mention that the isolation of bacterium was not performed in the autumn and winter seasons. In all cases which strains of *Campylobacter jejuni* were isolated, the sea had the calm state so that the isolation of bacterium was not successful in the stormy and wavy state.

4. Discussion

As mentioned above, *C. jejuni* cause of zoonotic infection, transmitted water and foods [26]. These bacteria identified from surface water, water cannal, swage and sea water [22, 27]. Amongst, intestinal tract pathogens include, *Escherichia, Salmonella, Vibrio*, and *Campylobacter jejuni* is also transmitted by swage. This species, despite that they are transferred to water through the birds’ feces, but the
swage is the main source of transferring the pathogen to rivers and surface water [28-30].

Isolation and identification of *C. jejuni* from Caspian Sea was not reported. The results obtained from present study achieved during 4 season from 263 samples were collected of which 7 strain of *C. jejuni* isolated. This is evidently could concluded that there exist this bacteria in coastal water of Caspian Sea.

In addition, within the studies conducted by Jose *et al* (1990) on the presence of the *Campylobacter* in the sea waters of Spain, 25 strains (13%) were isolated out of 192 collected Samples [31]. Within another research under the topic of the identification of the Campylobacter’s in the water resources of the Africa’s west conducted by deer guard in 2004, they could report the prevalence of the *Campylobacter jejuni* in these waters up to 13.6% in this research [14]. In a research carried out in Israel in 1999, it was specified that, out of 115 collected samples, 52 strains (45%) from the sample of the swimming beaches of this country are contaminated with *Campylobacter* [24].

The main way of the entry of these bacteria in to the Caspian Sea’s water can be thought the house hold sewages and surface waters pouring into the sea.

Within the studies under the title of identifying Campylobacter’s from the environmental samples in the north of Iran carried out by Ghane et al., 2010 they reached this conclusion that one of the main sources of *Campylobacter* in this geographical region in surface waters and rivers. Of 65 samples of the surface waters and 27 samples of the sewage, they identified 22 strains (33.84%) and 2 strains (7.4%) *Campylobacter*, respectively which were the highest prevalence related to the species of the *Campylobacter jejuni* [32].

The existence of this bacterium in the sea water can be attributed to the presence of the seagulls as well because this bacterium doesn’t create a specified disease in the gulls. But can enter in to sea water through feces [33, 34].

The existence of the environmental contamination and swage assists noticeably the survival of the species of this bacterium so that they are found more greatly in
the contaminated environments. This is because of providing the nutrients for the microorganisms. This affair shows that there exists a direct relationship between the sewage. Infections and growth of the Campylobacters in the waters, including the seawater [35].

The results obtained from this study is, in fact, regarded as the first report from the isolation of campylobacter jejuni from Caspian sea. Knowledge of the state of the entry and time span of the survival of the campylobacter in the water environments to control the water quality and prevention from the disease is of importance.

REFERENCES


Isolation of C. jejuni from Caspian Sea


Fig. 1. Agarose gel (1.5%) analysis of a PCR diagnostic test. Lane M: size marker 1 kb, Lane 1: positive control, Lane 2-4: positive sample, Lane 5: Negative control.
Table 1. Temperature, pH and Salinity of Caspian Sea in 4 season sampling

<table>
<thead>
<tr>
<th>seasons</th>
<th>Temperature</th>
<th>pH</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>7-11</td>
<td>6/5-7</td>
<td>12/80</td>
</tr>
<tr>
<td>Winter</td>
<td>5-11</td>
<td>5-6/5</td>
<td>12/85</td>
</tr>
<tr>
<td>Spring</td>
<td>7-18</td>
<td>6/5-8</td>
<td>12/95</td>
</tr>
<tr>
<td>Summer</td>
<td>13-24</td>
<td>7</td>
<td>13/00</td>
</tr>
</tbody>
</table>

Table 2. Frequency of occurrence of *C. jejuni* isolated from Caspian sea

<table>
<thead>
<tr>
<th>seasons</th>
<th>No. sample collected</th>
<th>Isolated number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Winter</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>Spring</td>
<td>67</td>
<td>2 (0.76%)</td>
</tr>
<tr>
<td>Summer</td>
<td>94</td>
<td>5 (1.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>263</td>
<td>7 (2.66%)</td>
</tr>
</tbody>
</table>

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