Evaluation of a Multiplex PCR Assay for the Identification of Campylobacter jejuni and Campylobacter coli

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Background: Campylobacter jejuni and Campylobacter coli are identified as the major causes of acute gastroenteritis in humans. Because of the fastidious nature of Campylobacters, many clinical laboratories fail to routinely culture them. The detection of Campylobacter spp. using molecular-based techniques can be useful for diagnostic and epidemiological applications. This study aimed to develop a multiplex PCR assay for the simultaneous detection of C. jejuni and C. coli strains from clinical specimens.

Materials and Methods: During a 19-month period, stool samples were collected from 980 children admitted to a hospital in Tehran, Iran and then examined. The samples were cultured on both Brucella agar and Modified Charcoal-Cetoperazone-Deoxycholate agar (mCCDA) media at 42°C for 48 h. To confirm suspected bacteria, Gram staining and other biochemical tests were carried out. Finally, after extracting DNA from pure cultures using the boiling method, the multiplex PCR assay was performed.

Results: The multiplex PCR assay showed that Campylobacter spp. can be detected using 400 bp target product of cadF. It can also accurately distinguish between C. jejuni and C. coli species with different bands of 735 bp and 500 bp using hipO and asp genes, respectively.

Conclusions: Results showed that the multiplex PCR assay can replace the biochemical assays for differentiating between C. jejuni and C. coli strains in a single-step PCR test.

Keywords: Campylobacter jejuni, Campylobacter coli, Multiplex PCR

1. Background

Campylobacter spp. is a common cause of acute gastroenteritis worldwide (1). Among various pathogenic Campylobacter species in humans, the thermophilic Campylobacter jejuni and Campylobacter coli are recognized as the major cause of human campylobacteriosis (2).

The major reservoir and source of Campylobacters for humans are poultry and poultry products. Undercooked poultry meat, unpasteurized milk, and water can be considered as the sources of human infection with C. jejuni and C. coli (3).

The gastrointestinal manifestations of campylobacteriosis disease include cramps, fever, myalgia, weight loss, acute watery or bloody diarrhea; however, the infection may result in severe extra-intestinal sequelae, especially acute neurological manifestations such as Guillain-Barre syndrome and Miller Fisher syndrome, a cross-reactivity between the bacterial lipooligosaccharides and nervous system gangliosides (4).

Isolation of Campylobacters using the culture method is considered the gold standard for campylobacteriosis disease diagnosis; however, it has several limitations because of the fastidious nature of Campylobacters (5). Furthermore, due to the phylogenetic relatedness of C. jejuni and C. coli species, the identification of Campylobacter at species level using biochemical assays is difficult (6). Therefore, other techniques such as PCR and real-time PCR are used as the methods for Campylobacter identification, showing a very high sensitivity (7-8).

Differentiation of Campylobacter spp. using molecular-based assays can be useful for rapid and specific detection and epidemiological applications. For this purpose, various genes including fliY, cdt, asp, hipO, glyA, ceuE, cadF are used (9).

The asp gene encodes aspartokinase enzyme and is highly specific for C. hippuricase is produced by the hipO gene in C. jejuni (a species-specific hippurate hydrolase enzyme), which is absent in C. coli. The cadF gene also encodes a fibronectin-binding protein that can promote the attachment of the microorganism to eukaryote cells. It has been described as a conserved and genus-specific gene (10-12).

2. Objectives

The aim of this study was to differentiate between C. jejuni and C. coli species using a set of specific primers for asp, hipO, and cadF genes in a multiplex PCR assay.

3. Materials and Methods

3.1. Sampling and growth conditions

During a 19-month period (June 2012-January 2014), 980 children with intestinal signs were admitted to one of the major hospitals in Tehran, Iran. 750 suspected cases to campylobacteriosis were included in this study. After collecting the samples, the stool specimens using Carry-Blair Transport media (Micro Media, Hungary) were transferred to the microbiological laboratory and then cultured immediately on Brucella agar (Merck, Germany) with 5% sheep blood and Modified Charcoal–Cetoperazone–Deoxycholate agar (mCCDA) (Merck, Germany). The plates were incubated at 42°C for 48 h under microaerobic conditions in a sealed jar using gas packs (Merck, Germany).

Both of the media were supplemented with vancomycin, polymyxin B, and trimethoprim (pH 7.2±0.2). Suspected colonies to Campylobacter spp. were confirmed using Gram staining and spiral morphology, catalase and oxidase positive, nitrate reduction positive, and indoxyl acetate hydrolysis positive. For differentiating between them at species level, hippurate hydrolysis test positive (C. jejuni) and negative (C. coli) as well as susceptibility to nalidixic acid (C. coli) were used.

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3.2. DNA extraction and multiplex PCR assay

DNA extraction from pure cultures of Campylobacter isolates was performed by the boiling method. First samples suspended in distilled water and boiled for 15 min at 100°C. The mixture was then centrifuged at 10,000 g at room temperature for 10 min, and the supernatant was used as extracted DNA in the PCR assay.

The multiplex PCR was carried out within a thermal cycler (Eppendorf, Germany) in a final volume of 25 µL containing 3 µL of extracted DNA, 2.5 µL 10X PCR buffer, 1 unit of Taq DNA polymerase, 2 mM MgCl₂, 0.1 µM of each primers (Table 1), 0.2 mM each deoxynucleotide and sterile deionized water.

Amplification conditions for the three genes (asp, hipO, and cadF) were as follow: 95°C for 5 min, followed by 30 amplification cycles; denaturation at 95°C for 45 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 30 seconds. Finally, an additional extension step (5 min, 72°C) was performed. Finally, amplicons were electrophoresed on 1% agarose gel. C. coli ATCC 43478 and C. jejuni subsp. jejuni ATCC 29428 were used as reference strains.

4. Results

Among studied population, 750 children had bacterial systemic and gastrointestinal symptoms including fever, abdominal pain, vomiting, diarrhea with high WBC and RBC count. From a total of 750 cases, 48.5 and 51.5% were male and female, respectively, with an average age of ~6 years. According to the culture results, the number of Campylobacter positive cases was 35, in which 33 cases (94%) were infected by C. jejuni, and 2 cases (6%) were infected by C. coli. About 50% of the bacteria were isolated by each of the media (Brucella agar and mCCDA). Most of the positive cases occurred during the summer season from July to September.

The multiplex PCR assay showed that Campylobacter spp. can be detected using 400 bp target product of cadF. It can also accurately distinguish between C. jejuni and C. coli species with different bands at 735 bp and 500 bp using hipO and asp genes, respectively (Fig.1).

5. Discussion

Campylobacter species have currently been reported as the most common causes of acute bacterial gastroenteritis worldwide both in developing and developed countries. Some studies showed that gastrointestinal infection caused by C. jejuni leads to the high levels of morbidity and mortality. About 2.0 million cases are annually reported to be infected by C. jejuni with a mortality rate greater than 2.000 people (13).

Although biochemical tests such as hippurate hydrolysis are useful for the differentiation of Campylobacters at the species level, they could also be used for the identification of hippurate hydrolysis-negative strains of C. jejuni and false-positive isolates (14). Another disadvantage of conventional tests is that they are extremely time consuming. In this study, the time required for the identification of Campylobacter at the species level from a sample was approximately 4 days.

In addition, the accurate differentiation between two Campylobacter species is important for the following treatment of the disease in human. For example erythromycin is used for treating C. jejuni infection, while C. coli strains are resistant to this antibiotic (6). Therefore, an accurate molecular method can be easily used for clinical diagnosis and appropriate treatment of the disease.

In some previous studies, only one gene was used for differentiating between C. jejuni and C. coli strains. Klena et al. (2004) used divergent and conserved regions of lpxA gene of the lipid A, encoding a UDP-N- acetyl glucosamine acyl transferase, to differentiate between C. jejuni, C. coli, C. upsaliensis and C. lari species by a multiplex PCR. In another study conducted by Shams et al. (2016), conserved regions of the cadF gene were used for the detection and differentiation between C. jejuni and C. coli species. In another study, González et al. (1997) also discriminated between both C. jejuni and C. coli species using the cedE gene (7, 15-16).

Our study is similar to Al Amri et al. (2007) who used a mixture of cadF, hippuricase and aspartokinase genes to distinguish C. jejuni and C. coli by PCR. The same PCR assay was developed to detect isolated strains from Iranian patients (10). Other genes have also been used for distinguishing Campylobacter spp. by multiplex PCR method. For example, Cloak and coworker (2002) designed a PCR method by the cadF gene of pathogenic Campylobacter spp. and two different sets of the specific genes of C. jejuni and C. coli. The lpxA, hipO, and glaA genes were used by Adzity et al. (2011) to differentiate between C. jejuni and C. coli species. In another study, cedE, cadF, and oxidoreductase subunit genes were used for the differentiation purpose (6, 17-18).

In all mentioned studies the multiplex PCRs were able to amplify DNA fragments with different lengths which can be separable on the gel without extra reaction of PCR. Thus, it could be considered as a significant advantage over single-species identification systems for the evaluation of the disease in a large number of clinical specimens.

6. Conclusion

The developed multiplex PCR assay in this report is a sensitive technique and can be useful as a specific tool to simultaneously detect of C. jejuni and C. coli and differentiate them in the field of infectious diseases.

Conflict of Interest

The authors declare they have no conflict of interest.

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Authors’ Contributions

All of authors contribute to this study.

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Table 1. Nucleotide sequences of the primers used in the multiplex PCR assay.

<table>
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<tr>
<th>Primer name</th>
<th>Sequence (5' → 3')</th>
<th>Size (bp)</th>
<th>Targets</th>
<th>Gene</th>
<th>Bacteria</th>
<th>Annealing temperature (°C)</th>
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<td>cadF-F</td>
<td>TGGAGGATTATGATATG</td>
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<td>cadF</td>
<td>C. jejuni &amp; C. coli</td>
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<td>cadF-R</td>
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<tr>
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<td>500</td>
<td>asp</td>
<td>C. coli</td>
<td>43</td>
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<tr>
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<tr>
<td>hipO-F</td>
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<td>hipO</td>
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A multiplex PCR assay for the detection of Campylobacter spp

References


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