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Identification of Campylobacter Jejuni and Campylobacter Coli from Diarrheic Samples Using PCR

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Abstract

Background: *Campylobacter jejune and C. coli are recognized as the most common bacteriological causes of gastroenteritis in humans. In this study Identification of Campylobacter Jejuni and Campylobacter Coli from samples using PCR was explored.*
Methods: *Detection was performed using diarrheal samples collected 8 from 117 children.The genomic DNA of samples was extracted by phenol-chloroform method. All DNA extracts were examined for the presence of C.jejuni and C.coli species based on PCR method.*
Results: *Of 117 diarrheal samples, 35 (29.9%) were found positive for 10 Campylobacter spp using PCR.*
Conclusions: *The results of this study showed that PCR is effective for rapidly screening stool samples for Campylobacter spp, due to its high sensitivity and specificity.*

Keywords: Campylobacter jejuni, Campylobacter coli, Isolation, PCR.
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Introduction

Campylobacter spp are Gram-negative, microaerophilic, anaerobic, and mainly spiral-shaped bacteria. Campylobacter is the leading cause of food-borne bacterial gastrointestinal diseases worldwide.[1,2]The main source of Campylobacter infections is contaminated foods, as these bacteria are normal gut flora in animals such as poultry, pigs, and cattle. Many studies have shown that Campylobacter infections in various countries were linked to high levels of poultry gut colonization by these microorganisms.[3,4]Annually, approximately 400 million cases of Campylobacter-associated gastroenteritis occur worldwide. Campylobacter are known to be the most common causes of bacterial diarrhea across the globe, accounting for 20% to 35% of cases.[5,6]Campylobacterjejuni and C. coli are the most frequent species responsible for diarrheal diseases worldwide. Campylobacter jejuni and C. coli are traditionally differentiated by the hippurate hydrolysis test, as well as other culture-based, serological, and molecular methods.[7]The PCR method has several advantages because it is faster, more sensitive, and more specific than culture-based procedures. In this study, we detected C. jejuni and C. coli bacteria isolated from diarrheal samples using the PCR method.

Materials and Methods

In this study, we acquired 117 diarrheal samples from patients admitted to two pediatric hospitals in Tehran between January 2010 and March 2010. The children ranged from 8 months to 10 years of age. Samples were transferred to the lab in Cary-Blair Transport Medium. Samples were then enriched using Preston enrichment broth supplemented with polymyxin B (2,500 IU/l), rifampicin (5 mg/l), trimethoprim lactate (5 mg/l), amphotericin B (5 mg/l) and 7% defibrinated sheep blood for 24 h at 42°C in microaerophilic conditions. Typical colonies were sub-cultured on Brucella agar after growth was identified by biochemical test.

Suspected colonies on selective media were examined for morphology and motility by phase-contrast microscopy and Gram-staining. Colonies were isolated on blood agar plates containing 7% sheep blood, and incubated under microaerophilic conditions at 42°C for 72 h. The growth period was followed by standard biochemical tests including hippurate hydrolysis, H_2S, catalase, and oxidase.

For hippurate hydrolysis test, a loopful of the colonies isolated on sheep blood agar was added to 0.5 ml of a 1% sodium hippurate solution and mixed by shaking. This was followed by 2 h incubation at 37°C in a water bath. Then 0.2 ml of 3.5% ninhydrin solution in a mixture of acetone and butanol(1:1) was added to each tube on top of the hippurate solution. For color development, further incubation was carried out at 37°C for 10 min. A deep purple color, crystal violet was recorded as a positive result, indicating the presence of glycine, which resulted from the hydrolysis of the hippurate.

To extract the genomic DNA from Campylobacter jejuni and C. coli, bacteria were incubated in modified enrichment broth medium at 37°C for 48 h. The bacterial culture was centrifuged at 3000 rpm for 5 min. The pellet was suspended in 300 µl TE buffer followed by lysis solution containing 10µl lysozyme (10 mg/ml), 200 µl SDS 20%, and 3 µl proteinase K, and was inoculated in 37°C for 1 h. DNA was purified by extraction with an equal volume of pheno, chloroform, and isoamylalcohol (25:24:1) in the presence of 5M sodium perchlorate. A 1/10 volume of 3M sodium acetate and 2 volumes of absolute ethanol were added and incubated in −20°C for 13 h. The DNA was pelleted by centrifugation, washed with 70% ethanol and dried. Finally, the DNA samples were dissolved in 100 µl TE buffer, and to eliminate RNA, 3 µl RNase was added to the tubes, and the tubes were incubated at 37°C for 30 min. The concentration and purity of the DNA

samples were determined spectrophotometrically at A260 and A280 using NanoDrop.

All DNA extracts were examined for the presence of C. jejuni and C. coli species based on PCR amplification of the mapA and ceuE genes, respectively. The oligonucleotide primers used were F, 5'-CTATTTTATTTTTGAGTGCTTGTG-3' and R: 5'-GCTTTATTTGCCATTTGTTTTATTA-3' for the mapA gene, and F: 5'-AATTGAAAATTGCTCCAACTATG-3' and R: 5'-TGATTTTATTATTTGTAGCAGCG-3' for the ceuE gene as described by Denis et al. The PCR reagent, with a final volume of 25 µl, included 1 µl template DNA, 0.5 µl Taq DNA polymerase (5 U/µl), 1 µl of each primer (10 pmol/µl), 1 µl dNTP mixture, 2.5 µl 10x PCR buffer, 1.5 µl MgCl2 (50 mM), and 16.5 µl sterile DDW water. Thermal cycling of amplification mixture was performed using 30 cycles. The PCR program was run at 94°C for 2 min following denaturing for 94°C for 40 s, annealing at 54°C for 40 s, and extension at 72°C for 5 min. The final extension was conducted at 72°C for 5 min. PCR products were electrophoresed in 1% agarose followed by staining with ethidium bromide.

Results

In the culture method, 32 diarrheal samples of 117 (27.35%) were identified as containing Campylobacter spp. In the hippurate hydrolysis test, 25 of 32 samples (78.1%) were positive and 7 samples (21.87%) were negative. Of 117 diarrheal samples analyzed with PCR, 35 (29.9%) were positive for Campylobacterspp, where 27 (77.14%) were C. jejuni and 8 (22.8%) were C. coli. All the culture positive samples were found to be positive using PCR.

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Discussion

Campylobacter spp is the most common cause of diarrhea in children in developing countries,[8] so sequence detection of Campylobacter is important. The culture method for Campylobacter isolation generally requires 5–7 days for confirmation. In the culture method, discrimination between C. jejuni and C. coli is solely based on the hippurate hydrolysis test. Hippurate hydrolysis relies on the ability of the enzyme hippurate hydrolase, produced by microorganisms, to hydrolyze sodium hippurate to benzoic acid and glycine. According to our biochemical test used for strain differentiation, 78.1% of the positive samples belonged to the C. jejuni species, and the remaining 21.87% were C. coli. Van Laverne et al. reported the prevalence of C. jejuni and C. coli isolates to be 79% and 21%, respectively.[9]Similarly, Eyigor et al. determined the prevalence of these two strains was about 67% and 33%, respectively.[10]It seems that PCR is a good substitute for the culture method in the detection of Campylobacter spp. In addition, the PCR method is a rapid genetic assay that can identify and differentiate C. jejuni and C. coli. The high specificity of PCR showed that it was more reliable than the culture method.[11]Different results have also been reported in the literature on the detection of Campylobacter in samples using the PCR technique. Winters et al.[12] and Denis et al.[13] reported 80% and 66.3% of their PCR samples were positive, respectively. On the other hand, Magistrate et al.[14] reported only 5.9% of PCR-detected samples to be positive. Based on our results, PCR was sensitive, fast, and reliable enough to be an appropriate substitute for culture methods, or could be used as a supplementary method when culture methods yield negative results. The rate of Campylobacter isolation from diarrheal children in the present study was 29.9%. The present PCR assay proved to be accurate and simple to perform and could be completed within 3 h. It had the added advantage of detecting the mapA gene in C. jejuni strains which were hippuricase-negative when assessed with phenotypic methods, and therefore usually difficult to differentiate from C. coli with those methods.[15,16]Also, these results showed the significance of Campylobacter as an etiologic agent of gastrointestinal disease in Iran.

In conclusion, although biochemical identification can usually discriminate the two most common species Campylobacter, C. jejuni and C. coli, the PCR method can obtain more rapid results. PCR therefore has an advantage in the timely identification of C. jejuni and C. coli strains.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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