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Campylobacter jejuni and *Campylobacter coli* in Children With Acute Diarrhea in Health Centers of Hamadan, Iran

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Abstract

Background: Enteritis caused by *Campylobacter* is considered as the most common acute bacterial diarrhea around the world. In most cases, infection occurs as a result of consuming contaminated water or food, especially raw meat of fowls.

Objectives: The purpose of the present study was to determine the prevalence and antibiotic resistance of *campylobacter* species among pediatrics of Hamadan city, Iran.

Patients and Methods: A total of 120 stool samples from children less than 10 years old were examined from January 2013 to December 2014 in Hamadan, Iran. The samples were incubated in Campy-Thio enrichment medium for 1 - 2 hours and then cultured on a specific medium; after that, the suspected colonies were analyzed for *Campylobacter* spp. identification by conventional tests. The identified species by biochemical methods were confirmed by polymerase chain reaction (PCR). Antimicrobial susceptibility testing was performed by disk agar diffusion (DAD) method.

Results: Twelve (10%) *Campylobacter* spp. from 120 stool samples were isolated including *C. coli* and *C. jejuni*. In the antibiotic susceptibility test, the most frequent resistance was observed to ciprofloxacin 8 (88.8%), followed by 7 (77.7%) resistant strains to tetracycline, 7 (77.7%) to erythromycin, 6 (66.6%) to clindamycin, 5 (55.5%) to meropenem, 4 (44.4%) to gentamicin, 3 (33.3%) to nalidixicacid and only 1 (11.1%) to chloramphenicol.

Conclusions: *Campylobacter* is responsible for some important clinical problems such as enteritis and is also associated with meningitis and hemolytic-uremic syndrome. It is imperative to monitor the prevalence and antibiotic resistance of *Campylobacter* spp. as well as other the zoonotic bacteria.

Keywords: Polymerase Chain Reaction, Antibiotic Resistance, *Campylobacter coli*, *Campylobacter jejuni*

1. Background

In the last three decades, different species have emerged within the genus of *Campylobacter* as human and animal pathogenic bacteria in clinic and veterinary setting. The intestine of warm-blooded animals and in particular birds is the normal habit of *Campylobacter* species. In many developed and developing countries, the most frequently identified cause of human gastroenteritis is *Campylobacter* species (1), particularly *C. jejuni* and *C. coli*, which are responsible for the majority of human enteritis (2). It has been demonstrated in many studies that the major cause of human infections is *C. Jejuni* (3) which is transmitted by the fecal-oral route through contaminated food and water resources (4). In addition, it has been found that zoonotic *campylobacters* are associated with threatening complications in life, such as Guillain-Barre syndrome which is a serious neurological disease with symptoms such as meningitis, hemolytic-uremic syndrome, reactive arthritis, and flaccid paralysis (5-7). There are numbers of enteritis cases in humans, especially in the developed world. Recently, enteritis due to *Campylobacter* has exceeded to those enteritis caused by *Shigella* and *salmonella*. In addition, the campylobacteriosis incidence is often underestimated in developing countries, because of lack of adequate laboratory infrastructure (7). According to the global estimates, the most common causes of bacterial diarrhea are known as *campylobacters* across cases. When people consume contaminated water, food and milk, the mentioned disease occurs. According to a conducted research, another cause of *campylobacter* has also been found as poultry meat, sporadically up to 70% of (8). Therefore, it is a difficult approach at the level of species to detect *campylobacter*. It is necessary to differentiate campylobacter species and this approach is usually carried out by conventional diagnostic method.

If there are some suspected stool samples, using combination of culture on selective agar (containing antibiotics) and biochemical tests at 42°C under microaerophilic condition for up to 72 hours must considered; hippurate hydrolysis is also used for *campylobacter* confirmation (9). In using conventional methods for *campylobacter* detection, there are some problems such as long incubation time, uncertainly in results, and detection of some atypical strains. Using molecular methods like polymerase chain reaction (PCR) have gained special significance in recent years. This study was designed to investigate identification, prevalence and antibiotic resistance of *Campylobacter* species among pediatric settings.

2. Objectives

The purpose of this study was to detect the prevalence and antibiotic resistance of *Campylobacter* species among pediatrics of Hamadan city, Iran.

3. Patients and Methods

In the present study, 120 rectal swaps were collected from children less than 10 years old from January 2013 to December 2014 in Hamadan, Iran.

Firstly, the samples were enriched on Campy-Thio medium for 1 - 2 hours and then cultured on a selective medium and *Campylobacter* selective agar, which contained 5% defibrinated sheep blood, trimethoprim, polymyxin, and vancomycin. For further investigation, the suspected colonies were examined using Gram staining technique and under microaerophilic conditions at 42°C for 48 hours. There are some supplementing tests like hippurate hydrolysis, catalase activities, oxide tests, and also susceptibility to 30 μg discs of nalidixic acid and cephalothin (all the antibiotics were from MAST Co., England).

3.1. DNA Extraction

The *Campylobacter* DNA was prepared by suspending a loop of overnight colonies in 1.5 mL tubes containing 500 μL of sterile distilled water, followed by boiling for 10 - 15 minutes and then centrifuging at 14,000 g for five minutes (10).

3.2. Antimicrobial Susceptibility Test

The antibacterial susceptibility testing of *Campylobacter* isolated strains were examined using disk diffusion method, according to the clinical and laboratory standards institute (CLSI) guidelines (CLSI references). Ciprofloxacin (5 μg), amikacin (10 μg), gentamicin (10 μg), tetracycline (30 μg), erythromycin (15 μg), chloramphenicol (30 μg), and meropenem (10 μg) (Mast Group Ltd, Merseyside, UK) were used for antimicrobial susceptibility testing.

3.3. Polymerase Chain Reaction

The primers used for the identification of *Campylobacter* species were the *mapA* gene of *C. jejuni* (5) and the random gene of *C. coli* and the sequences are presented in Table 1 (8). The PCR conditions were as follows: a final volume of 20 μL containing 10 μL of 2x Taq premix Mastermix (Parstous Biotech Co., Iran), 5 μL sterile double-distilled water, 1 μL of forward (F) primer, 1 μL of reverse (R) primer, and 3 μL of DNA sample. The DNA samples were amplified by an initial denaturation step for two minutes at 94°C followed by 35 cycles of 94°C for 40 seconds, 54°C for 40 seconds, and 72°C for one minute, and a final cycle of 72°C for seven minutes in a Bio-Rad thermal cycler. After the amplification, 5 μL of each amplification product was combined with 1 μL of 6x DNA loading dye buffer (Parstous Biotech Co., Iran) and was subjected to electrophoresis in a 1.5% (w/v) agarose gel stained with DNA safe stain (SinaClon Co., Iran). The 100-bp molecular size marker (SinaClon Co., Iran) was run concurrently. Electrophoresis was carried out in 1x TBE (Tris, boric acid, EDTA) at 80 V for one hour. The amplified products on the agarose gels were visualized and photographed using UV transilluminator (Vilbert Lourmat Co., Japan).

4. Results

Twelve (10%) of 120 samples were positive by conventional culture method and biochemical activity such as hippurate activity from suspected colonies; but in PCR method, 9 samples (7.5%) were positive and confirmed as *Campylobacter*, of which 6 (66.6%) were positive with PCR, belonging to *C. jejuni* and 3 (33.3%) were confirmed as *C. coli* (Figure 1). The results from the hippurate hydrolysis tests were positive in eight cases, but negative in rest of the samples. The PCR results showed that 5% of all the samples were *C. jejuni* and 2.5% were *C. coli*. The results of antibiogram test showed a high percentage [8 (88.8%)] of *Campylobacter* isolates to be resistant to ciprofloxacin, 7 (77.7%) to tetracycline, 7 (77.7%) to erythromycin, 4 (44.4%) to gentamicin, 6 (66.6%) to clindamycin, 5 (55.5%) to meropenem, 3 (33.3%) to nalidixicacid, and only 1 (11.1%) to chloramphenicol, as presented in Table 2.

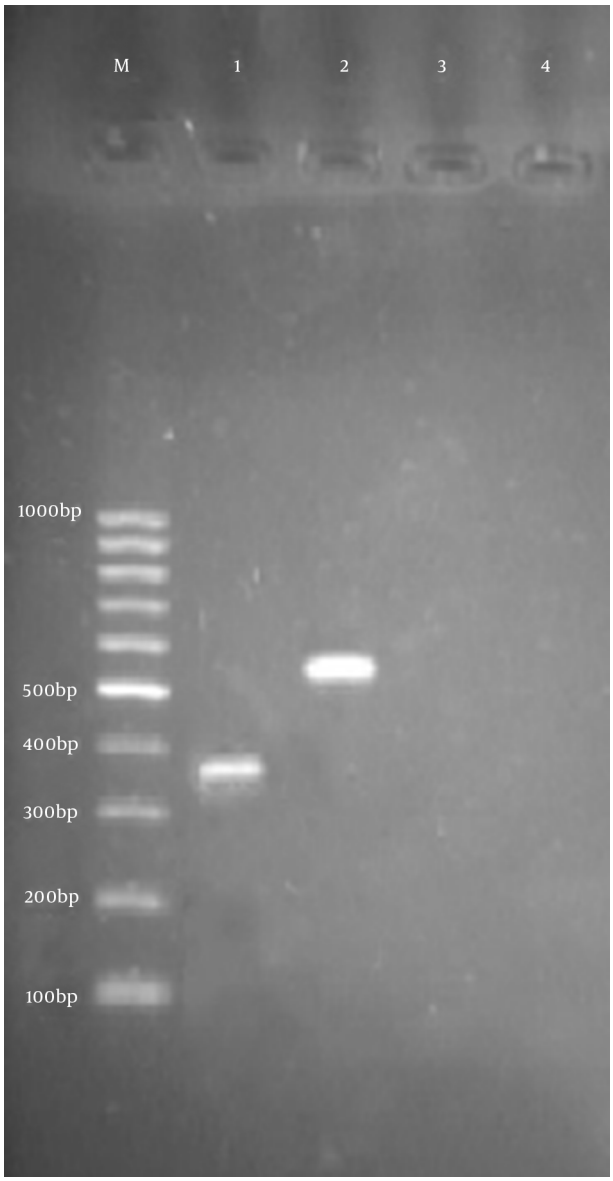
Table 1. Primers Used for Polymerase Chain Reaction

Target Gene/Primer	Sequences	Amplification Length, bp	Reference
mapA		589	(8)
CJ F	CTATTTTATTTTTGAGTGCTTGTG		
CJ R	GCTTTATTTGCCATTTGTTTTATTA		
Random		364	(11)
CC F	AGG CAA GGG AGC CTT TAA TC		
CC R	TAT CCC TAT CTA CAA TTC GC		

Table 2. Results of Antimicrobial Susceptibility of *Campylobacter coli* and *Campylobacter jejuni* Using Disk Diffusion Method

	Erythromycin	Tetracycline	Ciprofloxacin	Gentamicin	Clindamycin	Meropenem	Nalidixic acid	Chloramphenicol
***C. coli* (n = 3)**	3 (100)	2 (66.6)	2 (66.6)	1 (33.3)	2 (66.6)	2 (66.6)	1 (33.3)	0
***C. jejuni* (n = 6)**	4 (66.4)	5 (83.3)	6 (100)	3 (50)	4 (66.6)	3 (50)	2 (33.3)	1 (16.6)
***Campylobacter* spp. (n = 9)**	7 (77.7)	7 (77.7)	8 (88.8)	4 (44.4)	6 (66.6)	5 (55.5)	3 (33.3)	1 (16.1)

Figure 1. Gel Electrophoresis for Results of Polymerase Chain Reaction Amplification of *Campylobacter* spp.



Lanes M and 4, 100 bp DNA ladder; lane 1, positive specimen for *C. coli* (364 bp); lane 2, positive specimen for *C. Jejuni* (589 bp); lane 3 and 4, negative controls (samples without template).

5. Discussion

Campylobacter was isolated in 1970s from the feces of patients with gastrointestinal diseases (12). In many studies, it was demonstrated that *C. jejuni* was the major cause of human infections (3) and could be transmitted through contaminated food and water by the fecal-oral route (4). In an outbreak of waterborne *campylobacter*, water was a common vehicle and affected thousands of individuals (11); sewage was also reported as the most likely contamination source (13). The purpose of the current study was to investigate the prevalence of *Campylobacter* spp. in Hamadan.

In this study, the prevalence of *Campylobacter* spp. in children less than 10 years old was 7.5%, which is similar to other studies in different parts of Iran: Zahedan, 11.6% (14); Shiraz, 9.8% (15); Tehran, 8% (16); Semnan, 9.8% (17). Wasfy (18) reported the prevalence of *Campylobacter* from acute enteric infections as (17%) in Egypt. The annual percentage of detection of *Campylobacter* was averaged 2.3%, identical to that reported from Kuwait (19), half of that reported in similar researches in Saudi Arabia and Jordan (8, 17), but over twice of that reported in Israel (20).

In eight isolates, the hippurate enzyme activity was false-positive despite the absence of the *mapA* gene (specific for *C. jejuni*) in the PCR products. On the other hand, in two isolates, the hippurate enzyme activity was false-negative despite the presence of the *mapA* gene in the PCR. Al Amri (19) also compared hippurate biochemical activity with PCR results and reported that in 17 isolates the hippurate enzyme activity was false-positive by PCR and seven isolates showed false-negative hippurate enzyme activity despite the presence of the hippuricase gene in the PCR. Hurd (21) also found that in most laboratories thatused culture-based methods, different methods wereused. He reported 106 testing algorithms among 214 laboratories with a complete profile; only 16 laboratories were fully adherent toexisting guidelines (21).

The true prevalence of *Campylobacter* spp. is more, because it is not usual to diagnose these bacteria in routine laboratories. *Campylobacter* infection is considered as a major public health issue, particularly in developing countries such as Nigeria, Egypt, Thailand, and Bangladesh (22). In this study, a high rate of resistance was shown through antibiotic resistance to erythromycin [7 (77.7%)], ciprofloxacin [8 (88.8%)], and tetracycline [7

(77.7%)]. Furthermore, it was found that 8 (88.8%) of nine isolated *Campylobacter* spp. Were multidrug resistant strains and they were resistant to ciprofloxacin and tetracycline and one of the strains (*C. jejuni*) was resistance to eight antimicrobial agents used in this study; 5 (55.5%) of the isolates exhibited multi-resistance and also there was resistance to ciprofloxacin, tetracycline and erythromycin. A much higher rate was reported by Northern Ireland than was reported in some European countries; for isolates from broiler chickens, multi-resistance was very low (0.8% for *C. jejuni* and 0% for *C. coli*) (23).

In our study, resistance tonalidixic acid was 3 (%33.3, which is similar to Ibrahim et al. (24) report (23%) and two different studies from Thailand (77% (25) and 84% (26)), whereas resistance to erythromycin was 77.7% whichconflicts with Salehi et al. (0%) (14) and Ibrahim and Wardak (0%) (24, 27) reports, but is similar to Nonga and Muhairwa reports (28) which showed that 20% - 50% of isolates were resistant to erythromycin and gentamicin. The resistance rate of our isolates to tetracycline was 77.7% that conflicts with the resistance rate reported in Poland (13.7%), but is similar to the results of Alberta in Canada (50%) and Spain (72%) (20, 29). All the isolates of *C. jejuni* were resistance to ciprofloxacin, whereas Wardak et al. (27) reported 55.9%. Hoge et al. (26) reported an increase in ciprofloxacin resistance among *Campylobacter* species from 0 in 1991 to 84% in 1995 in Thailand, which is similar to our report. In general, resistance to fluoroquinolone and macrolide is influenced by several factors such as veterinary use of macrolides and fluoroquinolones.

In our study, we studied the range of antibiotic sensitivity that could be used for the treatment of *Campylobacter*. Ciprofloxacin, tetracycline and erythromycin were resistant in most of the times, whereas chloramphenicol showed low resistance; this implies that chloramphenicol is safer to use with its high sensitivity rates. Regarding this food-borne infection, due to frequent isolation in animals, food concerns have increased (30). There are many studies have considered two main reasons about the increasing antibiotic resistance problem in *Campylobacter* strains. In treatment of human, there is a frequent improper usage of antibiotics and this treatment is particularly used in cases of infections whose courses are self-limiting. The excessive use of these substances is the second and very important reason for infections treatment and preventions in veterinary medicine. In the poultry case, the use of antibiotics may lead to the emergence of resistant strains of *Campylobacter* and transmission via contaminated food to humans. In addition, growth promoters such as antibiotics are used in many countries in food animals. These results showed that there is mandatory need for monitoring the prevalence and antibiotic resistance of zoonotic bacteria in animals, food, and also humans.

We need to monitor the zoonotic bacteria prevalence and antimicrobial resistance in livestock, humans, and foods. Important information has been provided for surveillance reporting of antimicrobial resistance and it has been directed for action support to reduce the resistance occurrence.

Footnotes

Authors' Contribution: Mohammad Reza Arabestani contributed in designing of the project to prepare the manuscript, Mohammad Yousef Alikhani and Iraj Sedighi contributed in designing of the project, Sahar Rastyani, Sima Kazemi and Hamed Farhadi Kohan contributed in sample collecting and practical working.

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