Occurrence of *Campylobacter jejuni* in Shiraz, Southwest Iran

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Key Words

Prevalence • *Campylobacter jejuni* • Acute diarrhea

Abstract

Objective: To investigate the occurrence of *Campylobacter jejuni* among other bacterial agents of acute diarrhea. **Subjects and Methods:** Over a period of 3 months, 114 specimens of acute diarrhea were collected from patients aged 2–58 years referred to Nemazi Hospital, Shiraz, Iran. The samples were tested for bacterial pathogens causing acute diarrhea. **Results:** Of the 114 samples, 40 proved to be positive for bacterial enteric pathogens: *C. jejuni* 11 (9.6%), *Salmonella* spp. 18 (15.8%) and *Shigella* spp. 11 (9.6%). Maximum prevalence of *C. jejuni* was observed in patients aged 11–15 years. Significantly more females than males were infected with all diarrheal agents ($p < 0.05$). **Conclusion:** This study confirmed the presence of *C. jejuni* as a significant cause of gastroenteritis in Shiraz, Southwest Iran.

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Introduction

Acute gastroenteritis, though common, can be lethal and therefore it constitutes one of the most common challenges faced by medical practitioners in the developing world. Etiological agents can be viral, bacterial, or protozoan. Bacterial agents can be either enteropatho-

genic, toxigenic, or both. Among the bacterial agents, thermotolerant campylobacters (*Campylobacter jejuni*/*Campylobacter coli*) are the most frequent cause of intestinal infections worldwide [1–5]. These motile, Gram-negative, slender, curved, enteropathogenic, microaerophilic rods cause diarrhea as the main symptom, which can vary from limited to voluminous. Stools may be watery or bloody. Another frequent digestive tract symptom is abdominal pain, but vomiting is uncommon. Fever, headache, asthenia, and anorexia can also be present and may precede diarrhea [2, 5–7]. In the guidelines from the Practice Parameters Committee of the American College of Gastroenterology, it is recommended that stool cultures be carried out when adults present with severe or persistent diarrhea, or bloody stools and oral temperature 38.5°C [8].

Campylobacter enteritis may occur in all age groups but clinical presentation can differ according to age [9, 10]. *C. jejuni* enteritis is the infection most frequently observed before the development of Guillain-Barré and Fisher syndromes, making *Campylobacter* infection a major public health issue [11, 12].

Foods of animal origin, particularly poultry, are significant sources of *C. jejuni*, especially when eaten raw and undercooked or recontaminated following cooking. Normally, the disease develops 2–3 days after the ingestion of contaminated food and the symptoms usually resolve within a week. The disease tends to recur if no treatment is given; however, it is not identified by a routine

Fig. 1. Distribution of patients with positive stool cultures for bacterial pathogens by age range.

coproculture. Stool samples remain positive for several weeks. Treatment appears to be beneficial if it is administered early enough in the course of the disease [13–15].

Campylobacter isolation is the standard for the diagnosis of this type of bacterial infection. However, routine culture and isolation for *Campylobacter* requires special media and incubation temperature and is not performed in the laboratories of our hospitals. Although there are a few reports of the prevalence of infectious diarrheal agents including parasites [16, 17], bacteria [18] and viruses [19] in Iran, information about *Campylobacter* enteritis is limited in our country [20] and there is no available data about *Campylobacter* epidemiology in this region. Thus, in this cross-sectional study, we aimed to determine the prevalence of the two species of *C. jejuni* and *C. coli* among other bacterial agents of acute diarrhea in Shiraz, as a major city in Southwestern Iran.

Subjects and Methods

A total of 114 stool samples were collected from 70 female and 44 male patients with age ranges 2–42 years and 5–58 years, respectively. All patients had acute diarrhea and were referred to the emergency department of Nemazi Hospital, Shiraz, Iran, between July 10 and October 10, 2004.

Nemazi Hospital is the main referral center and the biggest educational hospital of Shiraz University of Medical Sciences,

Shiraz, serving a population of about 4 million in the Fars province and 1.3 million in Shiraz. Diarrhea was defined as per guidelines [8]: three episodes of passing loose stools per day, for at least 2 days. Patients on antibiotic therapy 3 days before sample collection were excluded from this study. A detailed history of patients: frequency of stools, its content, contact with animals and the nature of food taken were recorded. The samples were collected in clean plastic containers containing Cary-Blair medium and transported to the microbiology laboratory, Biology Department, School of Science, Shiraz University. The samples were processed immediately in accordance with standard laboratory methods [2].

The samples were preserved by chilling before bacterial culture. In addition to other routine stool culture procedures [3], samples were examined microscopically and were inoculated on Campy-BAP medium and Butzler's medium for *Campylobacter* isolation, as described before [21, 22]. Campy-BAP medium comprised brucella agar (Himedia, Mumbai, India), 5% (v/v) sheep blood, 10 µg/ml vancomycin, 0.25 µg/ml polymyxin B, 25 µg/ml trimethoprim, 2 µg/ml amphotericin B and 15 µg/ml cephalothin. Butzler's medium contained Mueller-Hinton agar (Himedia), 5% (v/v) sheep blood, cefoperazone 30 µg/ml, rifampicin 10 µg/ml, amphotericin B 2 µg/ml [21, 22]. The plates were then incubated at 42–44°C under microaerophilic conditions. The growth after 48 h was provisionally identified by colonial morphology, oxidase test, Gram staining and motility. The organisms were identified to species level by hippurate hydrolysis, urease test, nitrate reduction, catalase test, H₂S production and susceptibility to disks containing cephalothin and nalidixic acid [3].

Chi-square tests were used to compare differences between the groups (significance at $p = 0.05$).

Results

The 114 samples yielded bacterial pathogens in 40 cases (35%): female 29 (72.5%) and male 11 (27.5%). A majority (80%) of these patients were older than 11 years and the remaining 20% were under 10 years of age (fig. 1).

The most frequent pathogen was *Salmonella* spp. in 18 samples (15.8%) while *Shigella* spp. and *C. jejuni* isolations were 11 (9.6%) each. No dual or triple infection with other bacteria was observed. The sex distribution data revealed that all of the three agents of diarrhea were more frequent in females than males ($p < 0.05$, table 1). The mean age of children with *C. jejuni* infection was 11 years with peak incidence from 11 to 15 years and the male:female ratio was 1:2.7.

All of the patients affected by *C. jejuni* had abdominal pain. Of the 11 patients with *C. jejuni* isolates, 9 (82%) had symptoms of watery diarrhea and vomiting. 7 (64%) had fever with or without other complaints. Five (45%) diarrheal stool samples had blood and 2 (18%) mucus. A history of chicken meat consumption was noted in a majority of the cases (82%).

Table 1. Distribution of the bacterial pathogens in diarrheal stools by gender

Gender	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>C. jejuni</i>	Total
Female	14 (12.3%)	7 (6.1%)	8 (7.0%)	29 (25.4%)
Male	4 (3.5%)	4 (3.5%)	3 (2.6%)	11 (9.6%)
Total	18	11	11	40

Discussion

In this study, *C. jejuni*-associated diarrhea was detected in a proportion of samples similar to that of *Shigella* spp. which caused diarrhea (9.6%). There are few reports of campylobacteriosis in Iran, mostly in children [20, 23]. Results of this study showed the presence of *C. jejuni* among other enteropathogens ($p < 0.05$). Similar to other studies in other developing countries, *C. jejuni* has been a common enteropathogen in China, Bangladesh, Thailand, Egypt, Jordan, Nigeria [9] and Pakistan [4]. Estimates for campylobacteriosis in the general population in developing and developed countries are similar. Thus, in previously documented studies *Campylobacter* was recovered in about 4 to 40% of diarrheal patients, both in developed and developing countries [2–4, 24–29]. Relatively, the figure in this study was 9.6%; although it is smaller than that for *Salmonella* spp., it suggests the importance of detecting *Campylobacter*-associated infections in Iran.

In this study a higher proportion of females suffered from all types of bacterial gastroenteritis, including *Campylobacter* gastroenteritis (table 1) consistent with a previous study [1] but not with other reports [2, 3, 15], where *Campylobacter* spp. were isolated more frequently in males rather than females. If the converse is true, the greater incidence in females might be due to handling/preparing food.

In the younger age group (11–15 years), a peak incidence of *Campylobacter*-associated diarrhea was observed. Though it is to be noted that a majority of all diarrheal patients in the current study were between 11 and 20 years old, other studies have shown that symptomatic infection mainly affects children younger than 5 years, particularly in developing countries such as Bangladesh, Thailand, Egypt and Nigeria [9], other researches have shown that *Campylobacter* enteritis can occur in adults as well as children [9]. A study in Saudi Arabia has shown

Campylobacter enteritis to be an important cause of bacterial enteritis both in adults as well as in children [10].

Presence of *C. coli* was not detected in this study probably due to small sample size.

Campylobacter-associated infection caused abdominal pain as the most common symptom among our patients; whereas watery diarrhea, vomiting and fever were frequent, bloody and mucoid diarrhea were less frequent. However, in developed countries, bloody diarrhea and fever are common consequences of *C. jejuni*/*C. coli* infection [5, 7], but in developing countries such severe manifestations of the infection are still uncommon [30].

Further epidemiological studies in various areas of Iran are needed to ascertain the extent of occurrence of *Campylobacter* infections. This would enable recommendations for routine testing for *Campylobacter* in the laboratories.

Conclusion

This study confirmed the presence of *C. jejuni* as a significant cause of gastroenteritis ($p < 0.05$) in Shiraz, a major southwest Iranian city.

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