

Prevalence and Antimicrobial Resistance of *Campylobacter jejuni*

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Background: *Campylobacter* spp. are Gram-negative bacilli enteric pathogens that pose a major public health problem worldwide. In this genus, the most important species is *Campylobacter jejuni*. This bacterium causes diarrhea as its main symptom, which its intensity varies from mild to severe. Patients' stools may be watery or bloody.

Objectives: In this study, we aimed to determine the prevalence of the species of *Campylobacter jejuni* in Zahedan, a major city in southeastern Iran.

Patients and Methods: Fecal samples from 164 patients with acute diarrhea from Zahedan hospitals were collected from 2011 to 2013. Then the samples were streaked onto a campylobacter selective agar containing supplement and 7% defibrinated sheep blood. Conventional bacteriological tests (such as culture and biochemical tests) were performed to confirm the genus and differentiate at the species level. Finally, disk diffusion method was performed according to the recommendation of Clinical and Laboratory Standards Institute (CLSI) to determine the susceptibility of isolates to antibacterial agents.

Results: Out of 164 samples, 19 (11.6%) were reported positive by culture which confirmed by biochemical tests. Fifteen (78.9%) patients, whose samples were positive, hospitalized in infant ward. Two (10.5%) patients treated as outpatients. Two remaining (10.5%) patients were admitted in internal medicine ward. All of isolated strains were susceptible or moderately susceptible to erythromycin as the drug of choice.

Conclusions: In this study, the prevalence of the disease (11.6%) is found to be more than other parts of Iran. The symptomatic infection mainly affects children younger than 5 years.

Keywords: Drug Resistance; *Campylobacter jejuni*; Diarrhea

1. Background

Gastroenteritis caused by *Campylobacter bacilli* poses a major public health problem worldwide, and its rate has been increasing (1, 2). The genus *Campylobacter* consists of at least 20 species, among them *Campylobacter jejuni* is the major cause of diarrhea in many countries (3).

These bacteria cause diarrhea as their main symptom, which its intensity varies from mild to voluminous. Patients' 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.

The disease develops 2-3 days after the ingestion of contaminated food and the symptoms usually resolve within a week (4-6). Sequelae can occur, including the autoimmune-mediated demyelinating neuropathies such as Guillain-Barre and Miller Fisher syndromes (7).

Campylobacter infection is a zoonotic disease that can be hyperendemic, linked to outbreaks and sporadic

infections. Known risk factors for the disease include ingestion of undercooked poultry and other meats, contaminated food and water or unpasteurized milk and dairy products, direct contact with pets, farm animals, small children, and swimming in lakes and travel abroad. Foods of animal origin, particularly poultry, are significant sources of *C. jejuni*, especially when eaten raw and undercooked or decontaminated following cooking.

Normally, stool samples remain positive for several weeks (8-10). In some countries such as Norway, Romania, Yugoslavia, Hong Kong, and India, *Campylobacter* sp. are the second most common cause of bacterial enteritis (11-14). However much remains to be done about the epidemiology of these pathogens. In some developing countries like Iran, because the routine culture and isolation of *Campylobacter* sp. require special media and incubation temperature, the reports about this pathogen are limited (15).

While most cases of enteritis do not require antimicro-

bial treatment because of their short duration and clinically mild and self-limiting symptoms, antimicrobial treatment is necessary for systemic *Campylobacter* infections, immunosuppressed patients and severe or prolonged illnesses. Treatment appears to be beneficial if it is administered early enough in the course of the disease.

Macrolides, in particular erythromycin, are the usual drugs of choice. However, for the empiric treatment of adults with suspected bacterial gastroenteritis, the preferred drug typically includes a fluoroquinolone (ciprofloxacin) because of its broad scope of efficacy against almost all enteric bacterial pathogens (16). However, acquired resistance to macrolides, fluoroquinolones and other most widely used antibiotics gives rise to a challenge in campylobacteriosis control worldwide (17-20). In developing countries, various patterns of campylobacter susceptibility to antibiotics were described (21).

2. Objectives

In this study, we aimed to determine the prevalence of the species *C. jejuni* in Zahedan, a major city in southeastern Iran. The age and sex of patients and the seasonal distribution of *C. jejuni* and their antibiotic sensitivity patterns were investigated too.

3. Patients and Methods

We collected more than 800 fecal samples from two major hospitals in Zahedan (Boo-Ali and Ali-ibn-Abi-Taleb) and 164 acute diarrhea samples (watery, with leukocyte, mucus and RBC) were screened over a period of 2 years from 2011 to 2013.

No patient had received antibiotic therapy up to 72 hour prior to the collection of the samples. The patients were categorized according to their age, sex, and ward of hospital. The presence of leukocytes, erythrocytes and bacilli bacteria with dart motility in stools was determined microscopically.

To isolate *Campylobacter* sp., each sample was streaked onto a campylobacter selective agar (Lot No: VM800548 643, Merk company, Germany) containing supplement (50 µg/mL polymyxin B, 1 µg/mL trimethoprim and 2 µg/mL vancomycin) and 7% defibrinated sheep blood. These plates were incubated for 24 to 72 h at 42°C in microaerophilic condition.

Typical colonies from each suspected cultures were selected. Conventional bacteriological tests were performed (catalase, oxidase, hippurate hydrolysis, urease, H₂S production, susceptibility to nalidixic acid and cephalothin) to confirm the genus and differentiate at the species level. Various testing methods, such as disk diffusion, broth microdilution, agar dilution and Etest, can be performed to verify the susceptibility of *Campylobacter* sp. to antimicrobial agents.

Although no internationally accepted criteria have been established for the interpretation of minimum inhibitory concentration (MIC) data or resistance break-

points, the agar dilution method (a time-consuming test, rarely carried out as a routine diagnostic) has now been approved by CLSI as the standard susceptibility testing method for *Campylobacter* sp.

In this study, we used disk diffusion method to determine the susceptibility of isolates to antibacterial agents. Isolates were inoculated on Mueller Hinton agar with 5% sheep blood. Disks containing erythromycin (15µg), nalidixic acid (30 µg), and cephalothin (30µg) (Mast company, Germany) were placed on the inoculated plates. The plates were then incubated in a microaerophilic atmosphere at 42°C for 24 to 72 h. Susceptibility categorization was carried according to the recommendation of CLSI (22).

4. Results

Out of 800 fecal samples, 164 diarrheic samples were screened. Among 164 patients (83 [50.6%] male; and 81 [49.4%] female), 127 patients (77.4%) were younger than 5 years, 12 (7.3%) 6 to 15 years old, 11 (6.7%) 16 to 30 years old, 11 (6.7%) 31 to 60 years old and 3 (1.8%) older than 60 years.

Out of all samples, 19 (11.6%) were positive by culture and then confirmed by biochemical tests. Fifteen (78.9%) patients, whose samples were positive, hospitalized in infant ward. Two (10.5%) patients treated as outpatients and the remaining patients (10.5%) admitted in internal medicine ward. All of the patients with *C. jejuni* infection suffered from acute gastroenteritis symptoms but no one had systematic symptoms. All of isolated strains were susceptible or moderately susceptible to erythromycin as the drug of choice.

5. Discussion

Acute gastroenteritis, though common, can be lethal and therefore constitutes one of the most common challenges faced by medical practitioners in the developing countries. Etiological agents can be viral, bacterial, or protozoan. Bacterial agents can be either enteropathogenic, toxigenic, or both. Among the bacterial agents, thermotolerant *Campylobacter* spp. (*Campylobacter jejuni*, *Campylobacter coli*) are the most frequent cause of intestinal infections worldwide.

Prevalence of *C. jejuni* varies in different part of Iran: Tehran, 8% (23); Semnan, 9.8% (24); and Shiraz, 9.8% (15). In this study, the prevalence (11.6%) is more than other parts of Iran because of different reasons such as level of hygiene, nutrition, weather, and multi-cultural population in this city. These data show similarity to other studies in other developing countries such as China, Bangladesh, Thailand, Egypt, Jordan, Nigeria (1) and Pakistan (25) in which *C. jejuni* has been a common enteropathogen.

Campylobacter jejuni could infect all age groups but clinical presentation may differ. Enteritis caused by *C. jejuni* is the infection most frequently observed before the development of Guillain-Barre and Fisher syndromes, making *Campylobacter* infection a major public health issue. Like other studies, the symptomatic

infection mainly affects children younger than 5 years, particularly in developing countries such as Bangladesh, Thailand, Egypt and Nigeria (1).

In this study peak incidence of *Campylobacter* infection associated with diarrhea was observed in 0 to 5 age too. Other researchers have shown that *Campylobacter* enteritis can occur in adults as well as children (1), but in current study prevalence of *C. jejuni* in adults was low. Because the diagnosis of these bacteria by culture, serologic, molecular and biochemical tests are not routine in our laboratories, true prevalence of *Campylobacter* spp. is more than we think.

The most commonly used drugs are erythromycin (the first choice therapy) and fluoroquinolones (26). The increasing level of macrolides resistance to *C. jejuni* is becoming a major public health concern in some parts of the world (27). Furthermore, life threatening systemic diseases caused by *Campylobacter* sp. is diagnosed more and more.

Thus, it is imperative to investigate the clinical impact of macrolides resistance in *Campylobacter* infections. In the current study, all isolates were susceptible or moderately susceptible to erythromycin. Unlike some surveillances (20, 23, 28-34), we did not find macrolide-resistant strains.

Since *C. jejuni* is one of the most important enteropathogens, further studies are still needed in order to reveal the geographic prevalence and resistance distributions of these microorganisms in Iran.

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