

Antibiotic resistance of enteropathogenic bacteria in a teaching hospital in North Khuzestan during a three-year period

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ABSTRACT

Introduction: Gastrointestinal infections affect many people annually. The most common bacterial agents involved in these infections are enteropathogenic bacteria and in the continuation of using broad-spectrum antibiotics, *Clostridium difficile*-associated diarrhea is involved, especially in hospitalized patients. The aim of the present study was to investigate the pattern of antibiotic resistance among enteropathogenic bacteria. **Materials and Methods:** In this cross-sectional study, 163 samples of patients with diarrhea in Dezful Ganjavian Hospital were examined. The samples were cultured in MacConkey, Hektoen enteric agar and GN broth, and cycloserine cefoxitin fructose agar media and incubated under standard conditions. In order to identify enteropathogenic bacteria, biochemical tests and serological confirmatory tests were used. Antibiotic resistance pattern of the isolates was investigated by Kirby-Bauer disk diffusion susceptibility test. **Results:** The frequency of pathogenic bacteria includes 41.1% of *Shigella flexneri*, followed by 41.1% of *S. sonnei*, 6.7% of Enteropathogenic *E. coli*, 5.5% of *Salmonella enterica* Serogroup B, and 5.5% of *Shigella dysenteriae*. The results revealed a total of 46 patients with orders regarding *C. difficile* culture, no *C. difficile* was isolated from the samples. The studied isolates showed the highest resistance to trimethoprim-sulfamethoxazole, and ceftriaxone (88.3%), and the most effective antibiotic in the treatment of patients was ciprofloxacin with 86% sensitivity. **Conclusion:** Susceptibility to antibiotics was different among the isolates, which shows that the early identification of the infection agent and the selection of the correct antibiotic treatment are effective in improving the gastrointestinal infection and preventing the spread of the infection.

Keywords: Antibiotic resistance, *Clostridium difficile*, gastrointestinal infections, *Salmonella spp.*, *Shigella spp.*

Introduction

Gastrointestinal infection leads to thousands of cases of complications and death annually.^[1] Facultative and anaerobic bacteria are involved in the development of these infections.

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One of the main bacteria in hospital diarrhea is *Clostridium difficile* (*C. difficile*), accounting for about 10 to 25% of cases of antibiotic associated diarrhea (AAD).^[2] *C. difficile* is the most important cause of hospital diarrhea among adults. *C. difficile* infections (CDIs) have been reported in 20% of the population of hospitalized patients and 8% of patients with inflammatory bowel disease.^[3-5] More than 500,000 people in the United States are infected with CDIs annually, where increased annually.^[6,7] The long-term use of antibiotics is associated with antibiotic resistance of *C. difficile*.^[8] Clindamycin, ampicillin, amoxicillin, cephalosporins, and fluoroquinolones have been involved in increasing the risk of the infection.^[9] Since 2000, about 12.2% of

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cases of metronidazole therapeutic failure have been reported.^[10] Overall, each of these drugs are capable to change the intestinal microflora and cause inflammatory changes in the bowel, providing an anaerobic environment for the establishment and growth of pathogenic anaerobic bacteria.^[11] Recurrence of clostridial infection after treatment with metronidazole and vancomycin have been observed.^[12] Another group of common gastrointestinal infections (GI) are caused by Enterobacteriaceae such as *Shigella* spp., *Salmonella* spp., and *Escherichia coli* (*E. coli*).^[13,14] *Shigella* spp. is one of the most important leading cause of diarrhea in developing countries, which has dangerous effects, along with *Salmonella* spp.^[15]

The important issue is the resistance of these bacteria to the treatment of antibiotics. There is limited information on the cause of diarrhea in developing countries in terms of geographical and pathogenic characteristics, most reports focus on the prevalence of a pathogen.^[15,16] Numerous studies have shown that resistance among intestinal pathogens in Iran is increasing, which requires restrictions on unnecessary use of antimicrobial drugs in the human population and in veterinary applications.^[16,17] Antibiotic resistance is an important health challenge that has become the essential principle in the study of bacteria.^[18] A comprehensive study of the antibiotic resistance pattern in bacteria is of great importance. Few studies in north of Iran have been conducted to determinate drug resistance isolates of *C. difficile* and GI bacterial infections as hospital pathogens. Therefore, it is necessary to have accurate information about the epidemiology of the disease to implement effective control strategies of the infection caused by these bacteria. Awareness of the drug resistance of GIs in patients' stool specimens can predict the severity of the pathogenic strain and the epidemiological status of the strains to the physicians. The aim of this study was to determine the antibiotic resistance pattern among enteropathogenic bacteria among patients with gastrointestinal complaints.

Materials and Methods

Sampling and culture

In this cross-sectional study, 3280 stool samples from patients referred to the laboratory of Dezful Ganjavian hospital were examined between March 2019 and 2022, Khuzestan, Iran. The stool samples of 3234 patients for the diagnosis of bacterial gastroenteritis (BGE), i.e., *Shigella* spp., *Salmonella* spp., and Enteropathogenic *E. coli* (EPEC) were cultured according to the standard operation procedures. Briefly, the stool samples were cultured on MacConkey agar, Hektoen enteric (HE) agar, and GN Broth (Gram Negative Broth) (Merck, Germany) to investigate causes of BGE (*Shigella* spp., *Salmonella* spp., and EPEC). After 8 hours incubation of GN medium at 35°C, subculture was performed on HE agar medium. Then biochemical diagnostic tests were evaluated on Triple Sugar Iron Agar (TSI), Simmon's Citrate Agar, Sulfur Indole Motility Media (SIM), Urease test, Methyl Red Voges Proskauer (MRVP), Phenylalanine Deaminase (PAD), and Lysine Decarboxylase (Merck, Germany) were used to detect gastrointestinal pathogens, and serological tests were finally applied to confirm the diagnosis of pathogens.

Antibiotic susceptibility testing

The Kirby–Bauer disk diffusion susceptibility test is used to determine the susceptibility or resistance of pathogenic bacteria to Ampicillin (10 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75 µg), Cefixime (30 µg), and Ceftriaxone (30 µg) (Mast, UK) according to the instructions of Clinical and Laboratory Standards Institute (CLSI).^[19]

Anaerobic culture

In 46 patients who suspected *C. difficile* infection at the clinician's request anaerobic culture was performed. In the processing of specimens were used alcohol shock and yeast extract medium, then from the suspension treated on the specific medium of cycloserine, cefoxitin fructose agar (CCFA) enriched with 5% sheep blood, cultured streaky, and incubated in anaerobic conditions, using Anaerocult® A Gas-Pak (Merck, Germany) for up to 2 days, and the plates are checked daily.^[20]

Statistical analysis

In order to statistically analyze the data, SPSS software version 16 and Chi-square and Fisher's tests were used. *P* values < 0.05 were considered statistically significant.

Results

In this study, enteropathogenic bacteria were isolated from 163 stool culture which 60% were male, and the remaining were female and were in the age range of 6 months to 20 years. Most of the samples (99.4%) were hospitalized patients, and one patient was an outpatient. The samples were collected from Children's department (45.4%, 74 patients), Infectious pediatric (39.3%, 64 patients), Internal (11.7%, 19 patients), Neonatal (1.8%, 3 patients), and Intensive Care Units (ICUs) (2 patients). Of the 46 patients who were referred to the diagnostic of *C. difficile* infection, no positive culture was reported. The frequency of pathogenic bacteria includes 67 isolates (41.1%) of *Shigella flexneri*, 67 isolates (41.1%) of *Shigella sonnei*, 11 isolates (6.7%) of EPEC, 9 isolates (5.5%) of *Salmonella enterica* Serogroup B, and 9 isolates (5.5%) of *Shigella dysenteriae*. In microscopic stool examination (S/E), simultaneous isolation of *Shigella* spp. and protozoan gastrointestinal infection was observed in 26 patients, including *Shigella* infection and *Entamoeba histolytica* trophozoite in 22 patients, followed by *Giardia lamblia* (3 patients) and *Blastocystis* spp. (1 patient).

In the antibiotic resistance pattern of enteropathogenic bacteria, the highest bacterial resistance was related to trimethoprim/sulfamethoxazole and ceftriaxone (88.3%), and the most effective antibiotic in the treatment of patients was ciprofloxacin with sensitivity of 86% [Table 1].

Discussion

Due to the occurrence of many accidents such as wars, floods, earthquakes, and other natural disasters caused by climate

Table 1: Antibiotic resistance pattern in enteropathogenic bacteria

Type of antibiotics	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Nalidixic acid	53	32.4	12	7.6	98	60
Ciprofloxacin	140	86	6	3.6	17	10.4
Trimethoprim/Sulfamethoxazole	19	11.7	0	0	144	88.3
Ampicillin	26	16.1	0	0	137	83.9
Cefixime	23	14	0	0	140	86
Ceftriaxone	19	11.7	0	0	144	88.3

change and many other factors that lead to the homelessness of many human populations, access to the health conditions and facilities of the communities is greatly reduced and consequently gastrointestinal infections caused by enteropathogenic bacteria are increasing rapidly in the world and impose high medical costs on the health systems of countries and often cause deaths worldwide.^[15,18] Knowing the pattern of antibiotic resistance is essential to control bacterial infections. This study investigated isolates causing diarrheal infections and their antibiotic resistance. In the present study, the frequency of pathogenic bacteria was as follows: *S. flexneri* (41.1%), *S. sonnei* (41.1%), EPEC (6.7%), *S. enterica* Serogroup B (5.5%), and *S. dysenteriae* (5.5%).

In a similar study on the stool samples of children with clinically suspected gastroenteritis, BGE was confirmed with either *Escherichia coli* O157 (14%), *Salmonella* spp. (43%), and *Shigella* spp. (43%).^[21] In Nuzhat *et al.*,^[22] study predominant species was *S. flexneri*, and in other studies in developed countries was *S. sonnei*,^[23] but in a study in the United States, the frequency of *S. flexneri* and *S. sonnei* was 55% and 45%, respectively.^[24] Ameya *et al.*'s^[14] study found that only 12.6% of cases had *Salmonella* spp. Mahmoudi *et al.*'s^[25] studies in Iran on children isolated 88 (42%) *Salmonella* spp., 85 (40%) *Shigella* spp., 33 (16%) *E. coli*, and 5 (2%) *Candida albicans* from 211 positive stool cultures. Another study isolated enteropathogenic *E. coli* (10.9%), *Shigella* spp. (37.3%), and *Salmonella* spp. (12.7%) in diarrheal fecal samples.^[26] The frequency of *Shigella* isolates in digestive samples varies in studies, so that in East African countries, it has been reported from 0.7% to 20.6% in Sudan and Somalia respectively.^[27] In the present study, *S. enterica* Serogroup B was similar to previous studies. In Abbasi's study, 42.8% of the samples were infected with *S. paratyphi* B and C and 14.3% were diagnosed with *S. typhi*.^[28]

In this study, the studied strains showed the highest resistance to trimethoprim/sulfamethoxazole and ceftriaxone (88.3%), and the most effective antibiotic in the treatment of patients was ciprofloxacin with 86% sensitivity. Ciprofloxacin, norfloxacin, and ceftriaxone are among the most commonly used antibiotics in shigellosis, and fortunately, high sensitivity has been reported in other studies.^[24,27] Investigation of drug resistance mechanisms showed that replacement mutations in *gyrA/B* and *rpoB* genes caused resistance to fluoroquinolones and rifampin.^[29] In another study, the prevalence of isolates with multiple drug resistance was

found to be 40% in *E. coli*, 26.67% in *Salmonella* spp. and 93% in *Shigella* spp. Antibiotic resistance to cotrimoxazole, ciprofloxacin, and azithromycin for *shigella* isolates was 100, 87 and 73%, respectively. While resistance to cotrimoxazole, ciprofloxacin, and azithromycin was found to be 80, 60, and 60% for *E. coli* and 40, 47 and 27% for *salmonella* spp., and resistance to tetracycline was found as 53%.^[30] Another study has shown that isolated *Shigella* and *Salmonella* strains were resistant to common antibiotics such as amoxicillin, erythromycin, chloramphenicol, clindamycin, norfloxacin, ciprofloxacin, cotrimoxazole, and gentamicin.^[14] In our study, high resistance to ampicillin and cotrimoxazole was reported, which is almost consistent with the results of Gaufin's study.^[24] The variety of circulating species and conventional treatment protocols in each region can justify the different drug resistance statistics of countries.

In the present study, *C. difficile* was not isolated from the patients who were referred to the hospital laboratory. Dong *et al.*'s^[31] study showed that rifampin, metronidazole, and vancomycin were most effective against *C. difficile*, and resistance to ciprofloxacin, ceftioxin, clindamycin, tetracycline, and moxifloxacin was reported from 30 to 100%. A study by Larcombe *et al.*^[32] has shown that *C. difficile* is related to the 25% of AAD cases, and other microorganisms such as *C. perfringens*, *Staphylococcus aureus*, and *Klebsiella oxytoca* also play a role in the development of AAD. According to another study, *C. difficile* was isolated from 17% of children with inflammatory bowel disease.^[33] In a study, Alvarez-Perez and colleagues identified 67 genotypes of *C. difficile*. Human origin was significantly associated with resistance to ertapenem, erythromycin, and moxifloxacin. Resistance to clindamycin and erythromycin was more in some ribotypes. Twenty-nine of the isolates showed heteroresistance against metronidazole. Significant differences were observed in the susceptibility profiles of isolates belonging to the same genotype.^[34]

Conclusion

In our study, the examined isolates showed the highest resistance to trimethoprim/sulfamethoxazole, and ceftriaxone, and the most effective antibiotic in the treatment of patients was fluoroquinolones. The accurate and fast diagnosis of bacterial species depends on the use of experienced and committed laboratory personnel, so that they can diagnose the cause of infection and report antibiotic resistance in the shortest possible time using the latest diagnostic and treatment standards. Such measures can be effective in preventing the emergence of resistant isolates of bacteria and controlling infection in society by using the appropriate drug strategy.

Ethics approval and consent to participate

This study was approved by the grants from Dezful University of Medical Sciences and performed in accordance with the principles of the Declaration of Helsinki (Grant No: IR.DUMS.REC.1397.039).

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Conflicts of interest

There are no conflicts of interest.

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