The Most Common Microbial Causes of Gastroenteritis in Patients With Clinical Manifestations

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Background: Gastroenteritis is a remarkable hygiene problem worldwide. Bacteria and parasites can cause gastroenteritis-associated disorders.

Objectives: The aims of study were to survey the most common cause of gastroenteritis in patients referred to Imam Khomeini Hospital of Ilam, Iran.

Patients and Methods: This descriptive-analytical study was performed during 2012 to 2013. After collecting 2376 stool samples, standard biochemical and microbiological tests were performed. Susceptibility was tested by disc diffusion method agreeing with clinical and laboratory standards institute (CLSI) guidelines. The protozoa were detected by sediment wet-mount method.

Results: Of 2376 patients, 466 (19.6%) were contaminated with pathogenic bacteria or protozoa. The frequency of microorganisms isolated from the patients were 10.3%, 2.5%, 2.5%, 2.1%, 46.4%, 30.9%, 2.5% and 2.5% for enteropathogenic Escherichia coli, Shigella dysenteriae, Klebsiella pneumonia, Yersinia enterocolitica, Entamoeba histolytica, Giardia lamblia, E. coli and Candida spp., respectively. Sensitivities to ciprofloxacin in E. coli and S. dysenteriae strains were 100% and 91.66%, respectively.

Conclusions: The results showed that some patients were probably contaminated with nonbacterial and nonparasitic agents. All the parasitic isolates were resistant to most antibiotics. Therefore determination of microbial isolates and antibiotic susceptibility is necessary before treatment procedures.

Keywords: Gastroenteritis; Pathogenic Bacteria; Protozoa; Iran; Bacteria; Parasites

1. Background

Acute gastroenteritis is a severe infectious disease which occurs in the gastrointestinal tract (GI). It causes combinations of fever, diarrhea, stomach pain, nausea, vomiting, and abdominal pain and cramping. The signs may start slowly or quickly. The disease commonly passes in less than 24 hours, but can endure for numerous days (1). Diarrhea is a disorder of at least three loose or watery stools every day. It frequently continues for a few days and can result in dehydration because of the fluid loss. However, most incidents of childhood diarrhea are mild; acute cases can lead to important fluid loss which may result in death (2). Acute gastroenteritis or infectious diarrhea is one of the causes of diseases and death in infants and children worldwide, particularly in developing countries. Approximately, 2.5 million deaths occur in Asia, Africa and Latin America in children less than five years old each year (3). In 2004, diarrheal illnesses affected more people than other diseases, even in high-income countries (4). The most common microorganisms that cause acute gastroenteritis include Salmonella spp., Shigella spp., Campylobacter spp., Escherichia coli O157:H7, Listeria monocytogenes, Vibrio cholerae, Yersinia enterocolitica, Rotavirus, Cryptosporidium spp., Entamoeba histolytica, and Giardia lamblia. These organisms can cause potentially severe illnesses which may be mortal, particularly in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks (5). Among the
conditions influencing the progress of gastroenteritis, are poor sanitary circumstances, population explosion, insufficient control of vectors, and infection of the reservoirs. Two protozoa that may cause these symptoms are *E. histolytica*, *G. lamblia* and *Cryptosporidium* species (6). *Giardia* occurs more commonly in developing countries, but this etiological agent causes this type of illness nearly everywhere. It occurs more commonly in persons who travel to areas with high prevalence, children who attend daycare, and males who have sexual intercourse with males. *E. histolytica* is an anaerobic parasitic protozoan, a part of the genus *Entamoeba*. Predominantly infecting humans and other primates, *E. histolytica* is estimated to infect about 50 million people worldwide (7). The accurate knowledge of diarrhea epidemiology and its pathogenesis for recognition of common causes is essential by the broad and minute investigations. This information can be used for the design of efficient approaches for hygiene planning, prevention and treatment. There is limited information about the prevalence of intestinal pathogens in developing countries. The differences in prevalence rates among these pathogens in various regions are potential subjects of several studies.

2. Objectives

The main aim of this study was a survey of the most common cause of gastroenteritis in patients with clinical manifestations, referred to Imam Khomeini Hospital of Ilam, Iran.

3. Patients and Methods

3.1. Clinical Samples and Laboratory Identification

This descriptive-analytical study was performed on fecal samples obtained from gastroenteritis patients during 2012 to 2013 in the Imam Khomeini Hospital of Ilam, Iran. All the clinical specimens were transported to the laboratory by brain-heart infusion broth medium (Merck, Germany). Each sample was evaluated by method of Mahon et al. (8). The media used in this study included selenite-F broth, *Salmonella-Shigella* agar, Skirrow agar, MacConkey agar, triple sugar iron (TSI) agar, and blood agar (Merck, Germany). Cycloserine cefoxitin fructose agar medium was used for *Clostridium difficile* isolates. Diamond’s TP-S-1 (trehalose-6-phosphate synthase 1) medium (Miami, FL, USA) was used for the detection of intestinal protozoa. Thereafter, all the suspected grown colonies were identified by the standard and routine biochemical and microbiological tests.

3.2. Antimicrobial Susceptibility Test

An antibiotic susceptibility test was performed by disc diffusion method on Muller-Hinton agar (Merck, Germany) using antibiotic disks including: ciprofloxacin (CP) (5 µg), nalidixic acid (NA) (30 µg), ceftriaxone (30 µg), amoxicillin (25 µg), gentamicin (10 µg), clindamycin (2 µg) and cotrimoxazole (25 µg) (Mast Co. UK), suggested by the Clinical Laboratory Standards Institute (CLSI) (2012) guidelines (9).

4. Results

Of 2376 samples, 466 strains were obtained, of which 182 (39.1%) and 284 (60.9%) were male and female, respectively. The prevalence of bacterial isolates was higher in males than females. The frequency of microorganisms isolated from the patients were 10.3%, 2.5%, 2.5%, 2.1%, 46.4%, 30.9%, 2.5% and 2.5% for enteropathogenic *Escherichia coli* (EPEC), *Shigella dysenteriae*, *Klebsiella pneumonia*, *Yersinia enterocolitica*, *Entamoeba histolytica*, *Giardia lamblia*, *E. coli* and *Candida* spp., respectively. These results showed that the prevalence of the parasites was higher than the bacteria. The results of the antimicrobial susceptibility test (Table 1) indicated that these bacteria had higher resistance to CP and NA compared to other antibiotics.

| Microorganisms                  | Number of Strains | CP  | NA  | CRO | AMX | GM  | CD  | TMP/SMX |
|---------------------------------|-------------------|-----|-----|-----|-----|-----|-----|---------|
| EPEC                            | 48                | 48  | 41  | 39  | 20  | 38  | 35  | 21      |
| *Shigella dysenteriae*          | 12                | 11  | 10  | 8   | 9   | 10  | 9   | 10      |
| *Yersinia enterocolitica*       | 10                | 9   | 9   | 3   | 1   | 8   | 7   | 2       |

Table 1. Antibiotic Susceptibility Profile in Microorganisms Isolated From Stool Exam

*Abbreviations: EPEC, enteropathogenic *Escherichia coli*; CD, clindamycin; CP, ciprofloxacin; CRO, ceftriaxone; GM, gentamicin; NA, nalidixic acid; and TMP/SMX, cotrimoxazole.

*Values are presented as No. (%).*
5. Discussion

Afección to infectious diarrhea is seen in all age groups in less developed nations. This disease is one of the most important causes of children and newborn mortalities in these nations. Approximately 2.4 - 2.9 million mortalities due to diarrhea have been reported annually. There are different patterns of diarrhea-causing organisms between developed and developing countries. Viruses are major elements of diarrhea in developed nations and bacteria are in the second rank. Moreover, parasites have a negligible role in these countries. In developing countries, viruses play a major role in causing diarrhea and bacteria are in the subsequent rank (but with more prevalence rate than the developed nations). Parasites have been introduced as the third rank cause of diarrhea in developing countries. Among viruses, rotavirus has been known as the cause of diarrhea in more than 50% of children and other viruses play insignificant roles in causing diarrhea (10-12).

In this study, pathogenic agents were detected in 19.6% of the patients. The results revealed that *E. histolytica* was one of the most common causes of gastroenteritis. These findings are in accordance with Hegazi et al. (13) and conflict to Khalili and Sharifi (14) results. *Giardia lamblia* was the second most common organism, followed by *EPEC* and *S. dysenteriae*. Our result showed that females were more susceptible to the acquisition of gastrointestinal (P < 0.05). Most studies in Iran that applied low number of samples have reported that the contamination rate of diarrhea was about 2%-7% (15). In the investigation on diarrhea-affected patients by Ghalavand (16), *Shigella* (18.4%) and *Aeromonas* (4.5%) were the most remarkable organisms in a children medical center among the diarrhea cases. The contamination rate of Rotavirus was reported 55.6% among children of northwest of Iran, reported by Ghorashi et al. (17). Because of the same dissemination pattern of *Cryptosporidium* and *Giardia* through oral-fecal pathway, it seems that contaminated drinking water in urban regions and its exposure to contaminated animals in rural regions may contribute in the dissemination of organisms (18). Sayyari et al. (19) reported the frequency of *G. lamblia* and *E. histolytica* among Iranian people 10.9% and 1.0%, respectively. In a study by Hull et al. (20) it was demonstrated that some non-microbial elements like chemical materials, toxic plants, hypersensitivity, allergy and even stress can elicit microbial gastroenteritis-like intestinal disorders. Therefore, diarrhea may probably be caused by the abovementioned factors in cases without microorganism contamination.

The results showed that more than half of the gastroenteritis agents were not bacteria or parasites and they were probably viruses or other nonbacterial agents. We also found that perhaps some other non-microorganism factors caused gastroenteritis in microorganism-negative cases who had the disease. Interestingly, we did not observe any organisms in a large number of gastroenteritis-affected patients. Our observations indicated that the rate of gastroenteritis caused by parasite was higher than that caused by the bacterial agents. On the other hand, antibiotics do not have an effect on viruses; also, most antibiotics have no effect on parasites, which leads to an increase in the resistance of intestinal bacteria. Regarding the fact that such studies have less been performed in our country, it would be more desirable than ever to perform these types of researches in different parts of Iran because of various epidemiological patterns of protozoa in different locations of this country. Furthermore, it would be a better approach to follow the bacterial resistance patterns overtime to catch more effective treatment trends towards preventing such organisms.

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**Authors’ Contributions**

Study concept and design: Hossein Kazemian. Sample collection and data acquisition: Reza Mohebi. Acquisition of data: Hamid Heidari. Analysis and interpretation of data: Ali Saeedi. Data analysis and final revision: Sobhan Ghaforian. Drafting of the manuscript: Aref Shavalipour. Critical revision of the manuscript for important intellectual content: Abazar Pournajaf. Statistical analysis: Mansour Sedighi. Administrative, technical, and material support: Hossein Kazemian. Study supervision: Hossein Kazemian. Help with the English language version and final revision: Hamidreza Houri.

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Viruses are the most common cause of gastroenteritis in the US. They infect enterocytes in the villous epithelium of the small bowel. The result is transudation of fluid and electrolytes into the intestinal lumen; sometimes, malabsorption of carbohydrates worsens symptoms by causing osmotic diarrhea. In immunocompromised patients, illness may be severe and prolonged, causing substantial electrolyte and fluid loss. Cryptosporidium is usually acquired through contaminated water. It is not easily killed by chlorine and is the most common cause of recreational waterborne illness in the US, accounting for about three fourths of outbreaks. E. coli is the most common and important member of the genus Escherichia. It is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most infections (with the exception of neonatal meningitis and gastroenteritis) are endogenous; that is, the E. coli that are part of the patient’s normal microbial flora are able to establish infection when the patient’s defenses are compromised (e.g., through trauma or immune suppression). This organism is associated with a variety of diseases, including gastroenteritis and extra-intestinal infections such as UTIs, meningitis, and sepsis. A multitude of strains are capable of causing disease, with some serotypes associated with greater virulence. Gastroenteritis is usually caused by viruses. However, bacteria, parasites, and fungus can also cause gastroenteritis. In children, rotavirus is the most common cause of severe disease. In adults, norovirus and Campylobacter are common causes. Eating improperly prepared food, drinking contaminated water or close contact with a person who is infected can spread the infection. It is estimated that there were two billion cases of gastroenteritis that resulted in 1.3 million deaths globally in 2015. Children and those in the developing world are most commonly affected. As of 2011, in those less than five, there were about 1.7 billion cases resulting in 0.7 million deaths, with most of these cases occurring in the developing world. Diarrhea is one of the most common reasons patients seek medical care. In the developed world, it is one of the most common reasons for missing work, while in the developing world, it is a leading cause of death. Infectious agents are the usual cause of acute gastroenteritis. These agents cause diarrhea by several mechanisms, including adherence, mucosal invasion, enterotoxin production, and/or cytotoxin production. Clinical symptoms of C. difficile infection include watery diarrhea, fever, nausea, loss of appetite, and abdominal pain or tenderness. Complications that may result from infection include pseudomembranous colitis, toxic megacolon, perforations of the colon, sepsis, and even death, although it is rare. Bacterial gastroenteritis is caused by a variety of organisms, including Campylobacter, Salmonella, Shigella, Yersinia, Vibrio cholerae, Staphylococcus aureus. Most common bacterial organism pathogen responsible for foodborne gastroenteritis in the US. WBC present (fecal polymorphonuclear leukocytes). Blood present. Complications of noncholera Vibrio infection are common in patients with high levels of free iron (e.g., liver disease, hemochromatosis) or immunocompromise. Septic shock and necrotizing fasciitis associated with Vibrio vulnificus infection (rare). References.