Brief Report

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

Hossein Kazemian [1,2]; Aref Shavalipour [3]; Hamid Heidari [4]; Ali Saeedi [5]; Sobhan Ghafourian [1];

Reza Mohebi [1]; Hamidreza Houri [3]; Mansour Sedighi [6]; Abazar Pournajaf [6,*]

[1]Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, IR Iran
[2]Department of Medical Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, IR Iran
[3]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[4]Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
[5]Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[6]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, IR Iran

Corresponding author: Abazar Pournajaf, Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, IR Iran. Tel: +98-9358529694, E-mail: abazar_pournajaf@yahoo.com

Received: April 15, 2014; Revised: April 28, 2015; Accepted: May 6, 2015

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* (EPEC), *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, particu-larly 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 labora-

tory 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.

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

Microorganisms	Number of Strains	Antimicrobial Resistance [b]						
		CP	NA	CRO	AMX	GM	CD	TMP/SMX
EPEC	48	48 (100)	41 (85.4)	39 (81.2)	20 (41.6)	38 (79.2)	35 (72.9)	21 (43.7)
Shigella dysenteriae	12	11 (91.66)	10 (83.3)	8 (66.6)	9 (75)	10 (83.3)	9 (75)	10 (83.3)
Yersinia enterocolitica	10	9 (90)	9 (90)	3 (30)	1 (10)	8 (80)	7 (70)	2 (20)

[a] Abbreviations: EPEC, enteropathogenic *Escherichia coli*; CD, clindamycin; CP, ciprofloxacin; CRO, ceftriaxone; GM, gentamicin; NA, nalidixic acid; and TMP/SMX, cotrimoxazole.
[b] Values are presented as No. (%).

5. Discussion

Affection 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 gastroenteritis (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.

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 Ghafourian. 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.

References

1. Nelson EAS, Kwan E, Leung SF, Poon KH, Chow CB, Sin WK, et al. Guidelines for the management of acute diarrhea in young children. *H. K. J. Paediatr.* 2003;**8**:203–36.
2. American Medical A, Centers for Disease C, Center for Food S, Applied Nutrition F, Drug A, Prevention., et al. Diagnosis and management of foodborne illnesses: a primer for physicians. *MMWR Recomm Rep.* 2001;**50**(RR-2):1–69.
3. Leung A, Prince T. Oral rehydration therapy and early refeeding in the management of childhood gastroenteritis. *Paediatr Child Health.* 2006;**11**(8):527–31.
4. Ram PK, Crump JA, Gupta SK, Miller MA, Mintz ED. Part II. Analysis of data gaps pertaining to Shigella infections in low and medium human development index countries, 1984-2005. *Epidemiol Infect.* 2008;**136**(5):577–603.
5. Heseltine P. Has resistance spread to the community? *Clin Microbiol Infect.* 2000;**6 Suppl 2**:11–6.
6. Upcroft P, Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. *Clin Microbiol Rev.* 2001;**14**(1):150–64.
7. Calzada F, Yepez-Mulia L, Aguilar A. In vitro susceptibility of Entamoeba histolytica and Giardia lamblia to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *J Ethnopharmacol.* 2006;**108**(3):367–70.
8. Mahon CR. Gasterointestinal infection and food poisoning. *Mahon CR and manuselis Text book of diagnosis microbiology.* 2 ed. Philadelphia: W.B. Saunders Co; 2000. pp. 945–67.
9. Clinical and Laboratory Standards Institute. In: Performance standards for antimicrobial susceptibility testing; 16th infor-

mational supplement. Clinical and laboratory institute, editor. wayne, Penncylvania: 2012.

10. Amenu D. Antimicrobial Resistance for Enteric Pathogens Isolated from Acute Gastroenteritis Patients. *World J Nat Appl Sci* . 2014;**1**(1):1–14.

11. Yongsi HB. Pathogenic microorganisms associated with childhood diarrhea in low-and-middle income countries: case study of Yaounde - Cameroon. *Int J Environ Res Public Health.* 2008;**5**(4):213–29.

12. Hall GV, Kirk MD, Ashbolt R, Stafford R, Lalor K. Frequency of infectious gastrointestinal illness in Australia, 2002: regional, seasonal and demographic variation. *Epidemiol Infect.* 2006;**134**(1):111–8.

13. Hegazi MA, Patel TA, El-Deek BS. Prevalence and characters of Entamoeba histolytica infection in Saudi infants and children admitted with diarrhea at 2 main hospitals at South Jeddah: a re-emerging serious infection with unusual presentation. *Braz J Infect Dis.* 2013;**17**(1):32–40.

14. Khalili M, Sharifi M. Study of the Prevalence of Causative Bacterial&Protozoal Agents of in Stool Samples of 470 Gastroenteritis Patients Referring to the Nikoopour Clinic in Yazd, Iran. *J of Shahid Sadoughi Uni of Med Sci* . 2004;**12**(1):35–43.

15. Fallah M, Haghighi A. Cryptosporidiosis in children with diarrhea submitted to health centers in the west of iran (hamedan). *Medical Journal of The Islamic Republic of Iran (MJIRI).* 1996;**9**(4):315–7.

16. Ghalavand Z. . *[Prevalence of Aeromonas, Plesiomonas in children with diarrhea admitted to the Medical Center of Tehran].* [MS thesis.]. Tehran: Tehran University of Medical Sciences; 2001.

17. Ghorashi Z, Behbahan AG, Oskouei SA. Rotavirus enteric infection in children of northwest Iran. *Pediatr Infect Dis J.* 2011;**30**(7):616–8.

18. Khalakdina A, Vugia DJ, Nadle J, Rothrock GA, Colford JJ. Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area. *BMC Public Health.* 2003;**3**:11.

19. Sayyari AA, Imanzadeh F, Bagheri Yazdi SA, Karami H, Yaghoobi M. Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. *East Mediterr Health J.* 2005;**11**(3):377–83.

20. Hull BP, Spence L, Bassett D, Swanston WH, Tikasingh ES. The relative importance of rotavirus and other pathogens in the etiology of gastroenteritis in Trinidadian children. *Am J Trop Med Hyg.* 1982;**31**(1):142–8.