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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS IN MICROORGANISMS ISOLATED FROM THE DIARRHEAL CHILDREN IN ILAM, IRAN

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ABSTRACT

Diarrhea is one of the remarkable hygiene problems in the world. Despite reducing the diarrheal mortality rates for 3 million children per year through Oral Rehydration Therapy (ORT), mortality reduction among patients is still substantial.

Objectives: The aim of this study is, detection of bacterial and parasitic agents isolated from diarrheal children's stool and their antibiotic susceptibility profiles. This study also aims to introduce appropriate solutions for reducing of diarrhea causative agents.

Material and Methods: This study was performed in less than ten years old children with clinical symptoms of gastroenteritis. Direct examination, routine biochemical and microbiological tests and antibiotic susceptibility tests were performed on each sample.

Results: Out of 2568 samples, 602 (19.6 %) cases had been contaminated by pathogenic bacteria or parasites. 234 patients (38.8 %) were male and 368 patients (61.2 %) were female. 43.6% children were contaminated to *Entamoeba histolytica*. Males were infected more than females. 93.3% of *E.coli* and 91.6% of *Shigella dysenteriae* were sensitive to Ciprofloxacin.

Conclusions: The results of this study demonstrate that some cases of gastroenteritis were caused by non-bacterial and non-parasitic agents. Therefore, parasitic contaminations were more prevalent than bacterial infections. Enteropathogenic *E. coli* was the most isolated bacteria which ciprofloxacin could be regarded as the most effective antibiotic.

INTRODUCTION

Diarrhea is a digestive system disorder and is a common cause of children mortality in developing countries. It is estimated that 3-5 million cases of diarrhea throughout the world leads to death annually. The mortality rate of diarrhea is significant in less than five year old children. The broad spectrum of bacteria, viruses, and parasites are causative agents of diarrhea (Bueris *et al*., 2007; Aranda *et al*., 2007). In spite of enormous advances in medical sciences diarrhea still kills millions of people especially in developing countries. According to UNICEF, reducing the diarrheal mortality rates for 3 million children per year can occur using Oral Rehydration Therapy (ORT) (Thomas *et al*., 1993; Bhan *et al*., 1989) but mortality reduction among patients is still substantial. Despite some achievements have been gained by implementing the Diarrheal Diseases Control Program in Iran. It is one of the most important causes of growth abnormalities and mortality among children younger than five years of age (Thomas *et al*., 1993; Sachdev *et al*., 1991). There are many infectious and non-infectious causes of diarrhea. A variety of pathogenic viruses, bacteria, and parasites are among the infectious agents. High population density, poor sanitation and malnutrition are predisposing factors to diarrhea (Gurwith *et al*., 1981). *Shigella* sp., *Salmonella* sp., *enteropathogenic E. coli, Aeromonas, Vibrio cholerae, Campylobacter* sp. and *Yersinia enterocolitica* are the most common causes of bacterial diarrhea. *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*,

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and *Blastocystis hominis* are the most important parasitic causes of diarrhea .The prevalence rate of diarrhea differs from lower than 1% to higher than 30% all over the world (Casemore *et al*., 1990). Because of high mortality rate among Iranian children as a result of diarrheal diseases, a comprehensive study is necessary to find the prevalent bacterial and parasitic agents. The aim of this study is, detection of bacterial and parasitic agents isolated from diarrheal children's stool and determination of antibiotic susceptibility patterns. This study also aims to introduce appropriate solutions for reduce of diarrhea causative agents.

MATERIALS AND METHODS

Patients and sample collection

This descriptive-analytical study was performed as a survey of intestinal bacterial and parasitic pathogens isolated from stool samples of children younger than 10 years of age with gastroenteritis manifestation over the year 2013. The children had been referred to laboratory for parasitical examination and microbiological diagnosis. Samples were collected three or more days after a diarrhea onset.

Microscopic examination

Microscopic examination was done for each sample in order to eggs, worm larvae and parasites observation by light microscope (×40) using two wet mount slides counting saline and iodine.

Bacterial isolation and identification

MacConkey Agar (Merck Co, Germany) was used as an isolation of intestinal pathogenic bacteria such as *Salmonella* sp., *Shigella* sp., Enteropathogenic *E. coli*, and *Yersinia* sp. Each stool sample was cultured on the Salmonella Shigella agar, Macconkey agar, and Ampicillin-contained Blood agar (Merck Co, Germany) and incubated in 37°C for 24h. In order to isolation of *Salmonella spp.* and *Shigella spp.* XLD agar (Xylose lysine deoxycholate agar) (Merck Co, Germany) was used. All suspected grown colonized were analyzed by routine biochemical and microbiological tests such as oxidase, catalase, citrate, glucose, lactose and mannitol fermentation, motility, urease, gas production, and SH_2 production and specific polyvalent anti-serums assay.

Antimicrobial susceptibility tests

The Antimicrobial susceptibility tests was performed by Disc diffusion method on Mueller-Hinton agar (Merck, Germany) (Soltan *et al*., 2005) for eight antibiotics including; Ciprofloxacin (5µg), Nalidixic acid (30µg), Ceftriaxone (30µg), Cephalexin (30µg), Ceftazidime (30µg), Carbenicillin (100µg), Amoxicillin (25µg), and Tobramycin (10µg) (mast., UK) according to the of Clinical Laboratory Standards Institute (CLSI) guideline (CLSI., 2013).

RESULTS

Out of 2568 samples, 602 (19.6 %) cases had been infected with pathogenic bacteria or parasites. 234 (38.8 %) and 368 (61.2 %) were male and female respectively. The mean age of the population studied was 6.7 years. 103 (17.1 %) and 499 (82.8 %) pathogenic bacteria and parasites were isolated from the all patients, respectively. The most common pathogenic spp. were; *Entamoeba histolytica*, *Candida*, enteropathogenic *E. coli*, *Giardia lamblia*, *Entamoeba coli*, *Shigella dysenteriae*, *Klebsiella pneumonia*, and *Yersinia enterocolitica* (Table 1).

Table 1. Frequency of pathogenic bacterial and parasitic isolates

Pathogen Type		Total No.	Percentage
	Enteropathogenic *E. coli*	53	8.8
	Shigella dysenteriae	18	2.9
	Klebsiella pneumonia	18	2.9
	Yersinia enterocolitica	14	2.3
Bacteria			
	Entamoeba histolytica	263	43.6
	Giardia lamblia	26	4.3
Parasite	*Entamoeba coli*	21	3.4
Yeast	*Candida species*	189	34.1

Table 2. Antimicrobial susceptibility profils

Bacteria	Enteropathogenic *E. coli* (n=53)		*Shigella dysenteriae* (n=18)		*Klebsiella pneumonia* (n=18)		*Yersinia enterocolitica* (n=14)	
Antibiotic	N	P (%)	N	P (%)	N	P (%)	N	P (%)
Ciprofloxacin (CP)	50	94.3	16	88.8	17	94.4	13	92.8
Nalidixic acid (NA)	41	77.3	15	83.3	17	94.4	9	64.2
Ceftriaxone (CRO)	39	73.5	8	44.4	8	44.4	3	21.4
Cephalexin (CN)	16	30.1	9	50	9	50	8	57.1
Ceftazidime (CAZ)	31	58.4	3	16.6	5	27.7	7	50
Carbenicillin (CB)	22	41.5	3	16.6	7	38.8	3	21.4
Amoxicillin (AMX)	20	37.7	9	50	3	100	1	7.1
Tobramycin (TBO)	7	13.2	8	44.4	9	50	5	35.7

Susceptibility test analysis showed that EPEC, *S. dysenteriae*, *K. pneumonia* and *Y. enterocolitica* were highly sensitive to Ciprofloxacin and Nalidixic acid. The results of antimicrobial susceptibility test are listed in Table 2.

DISCUSSION

Diarrhea is one of the most common illness in all age groups and one of the most important causes of mortality and

morbidity among children and neonates. Approximately 2.4 to 2.9 million deaths are reported annually. There are different patterns of risk factors for cause of diarrhea between developed and developing countries. In the developed countries Viruses are prior than bacteria in diarrhea incidence among children. Problems caused by parasites in acute diarrhea can be ignored because of this organisms have less dispersion (Lane *et al*., 2002). In developing area, viruses have the first cause of diarrhea and bacteria are in the second rank (but possessing high prevalence than developed countries), also parasites possess the third rank among diarrhea-producing agents. Role of Candida species is negligible in childhood diarrhea with good nutrition. In this study non-pathogenic organism and candida species, were isolated from the children which had been diagnosed with gastroenteritis. Candida-positive cases were remarkable (Table 1). Ghalavand *et al.* (2007) reported 27.8% cases of diarrhea caused by bacterial agents (Ghalavand *et al*., 2007). Soltan *et al.* (2005) reported the most common agents of diarrhea. 18.4% and 4.5% of *Shigella* and *Aeromonas,* were isolated from the patients, respectively (Soltan *et al*., 2005).

As giardiasis is transmitted via oral-fecal route, it seems that contaminated drinking water at urban regions and its exposure to contaminated animals at rural areas may contribute to its dissemination (Khalakdina *et al*., 2003). Based on Hull *et al.* and Burkhurt *et al* studies, it has been shown that some non-microbial elements like chemical materials, toxic plants, hypersensitivity, allergy and even stress may cause microbial-like gastroenteritis (Khalakdina *et al*., 2003; Burkhart *et al*., 1990) that agreement with our study because of some of the children who had been diagnosed with gastroenteritis via clinical manifestations by physicians, were not contaminated to any bacteria or parasites. Despite the proven role of some microorganisms in gastroenteritis, it is still hard to conclude that in our cases, diarrhea is probably caused by above mentioned non-microorganism factors. But the role of viruses cannot be readily ignored. More investigation is needed to be performed to clarify the role of non-bacterial and non-parasitic factors in gastroenteritis. Amiri and his colleague showed the prevalence of parasitic contaminations among the diarrhea-contracted children in Noshahr and concluded that the prevalence of *Giardia lamblia*, *Entamoeba histolytica*, and *Entamoeba coli* were 51%, 19.6, and 9.3 respectively (Amiri *et al*., 2014).

Gharveriani *et al*. showed the prevalence of bacteria in 405 cases that the frequency of *Y. enterocolitica*, *V. cholerae*, EPEC, *Shigella* and *Plesiomonas* were 3.2%, 2.7%, 1.4%, 0.4% and 0.2%, respectively (Gharveriani *et al*., 2007). The frequency of *Y. enterocolitica*, *Shigella*, and EPEC in the mention study is in contrast with the present study. This contrast may be due to the different geographical area and level of hygiene. Our results showed that prevalence of parasites such as *E. histolytica* was considerably high. Perhaps it is because of the ignorance of parasite treatment and bacterial infections always be cured. According to Shiva *et al.* prescriptions for gastroenteritis treatment were not recommended and were at odds with guidelines of World Health Organization (WHO) in 70% of cases (Shiva *et al*., 2006). Present study highlighted the role of bacteria and parasites as a major causative agent in gastroenteritis. However, less than half of the gastroenteritis causing microorganisms could occur with other causative agents like viruses. Also, we realized that some non-microbial factors can cause gastroenteritis. Interestingly, we observed that *Salmonella* had low frequency, but *E. histolytica* and *Giardia* species were more prevalent. These findings are not similar to previous studies. Ongoing study manifested, that among bacterial agents, EPEC was the major bacterial cause of gastroenteritis. Also, *E. histolytica* was the most-prevalent agent among parasites. In the study it was observed that the rate of parasitic made gastroenteritis was higher than bacterial gastroenteritis. On the other hand it should be regarded that antibiotics are ineffective on viruses and parasites and their aberrant prescription would result in resistance of intestinal bacteria. It is necessary that all of the cases with diarrhea and certain clinical signs should be assessed through susceptibility test prior to antibiotic therapy.

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