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Prevalence Of Bacterial , Parasite And Non Growth Enteric Pathogens Associated With Diarrhea In Pediatric Outpatients And Inpatients Settings

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ABSTRACT:

The frequency of bacterial enteropathogens in children with diarrhea in a developing country in an ambulatory private clinic (site 1) and hospitalized children (site 2) is unknown and to establish guidelines for determining bacterial cultures and pediatricians to administrate antibiotic.

Methods: Children less than five years of age with diarrhea were prospectively enrolled in two sites and their stools were examined for diarrheogenic bacteria and parasites.

Results: A total of 183 and 529 stool samples of children with diarrhea examined in site 1 and 2 respectively for bacterial enteric pathogen and parasites. Of the 183 stool samples examined in site 1, 28 (15.3%) detected enteropathogen including Escherichia coli 10 (5.5%), Salmonella spp 17 (9.3%), Shigella spp 1 (0.5 %), Entamoeba histolytica 5 (2.7%), and 148 (80.8%) were culture negative. Of the 529 stool samples in site 2, E.coli 18 (3.4%), Entamoeba histolytica, 5 (0.9%) and 506 (95.6%) did not show any pathogen.

Conclusion: The most prevalent bacterial pathogen in site 1 was Salmonella spp and E.coli in site 2. The etiology of diarrhea in each geographic area are different from other areas and all pediatricians should know information about them.

KEYWORDS: Gasteroenteritis, Escherichiacoli, Salmonella spp, Children.

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INTRODUCTION

Diarrhea is an important cause of morbidity and mortality in children from developing counties[1]. In the United State, diarrhea annually results in an estimated 2.1-3.7 physician visits by children and 220.000 hospitalizations of children; the average total cost (including office visits, medications and lost parental wages) per episode was $ 289 in 1993[2]. The main etiology of diarrhea is related to wide range of bacteria, enteroparasites and viruses. In many hospitals or medical laboratories in our developing country lacking modern technologies for differentiation various Escherichia coli, isolation of some parasites, viruses and those bacteria need specific media. The purpose of this prospective study was to determine etiologies of acute gastroenteritis in a developing country and maybe guidelines for physicians who visit the children in such as our countries.

Material and methods

This study was carried out in Tehran, Iran in a private ambulatory pediatric clinic (pegah children clinic) staffed by various pediatrician (site 1) and hospitalized children Tehran children hospital staffed by various pediatrician (site 2). Children presenting with acute diarrhea at site 1 and 2 from October 2010 to September 2011 were enrolled in the study. Patients were eligible if they were younger than 5 years old and if parents agreed to submit stool specimen from their children in site 1. in site 2, all patients have been visited by various on duty pediatricians that they did not any information about study. All characteristics of patients and laboratory examinations were assessed after completion of stool examination and patients discharged from hospital. This study was approved by ethic committees of both sites.

Laboratory methods and data management: Stool specimens were collected in a polyethylene jar and sent it to the regional medical laboratory center in both sites.

Laboratory investigation was in the following sequences, presence of red blood cells and while blood cells by wet mount, bacterial culture and parasite examination.

Detection of Bacteria: Conventional culture techniques were used to identify common bacterial enteric pathogens. Submitted stool samples were inoculated to sheep blood agar (macromedia), xylose lysine deoxycholate agar (Kardan Azma) for isolation Salmonella – Shigella, sodium selenit broth (pronadisa), for the selective isolation of Salmonella in feces, Eosin Methylene blue agar (Kardan Azma) for isolation of gram positive or negative pathogens and occult blood with the use of alcohol piramidon method. All media were incubated at $37^{\circ}C$ in air. These culture techniques are sufficient to isolate and detect Salmonella spp, Shigella spp and Escherichia coli.

Detection of parasites: Fecal materials (taken particularly from areas with blood and mucus) were examined for the presence of parasites be viewing them under microscope that included direct wet mount for detection of parasites and protozoan cysts.

Analysis: Statistical analyses were performed using spss test.

RESULTS:

Patients presented mainly with watery

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diarrhea in both sites were evaluated by pediatricians. Dehydration were detected in all patients who hospitalized and no dehydration in all outpatient children. One hundred eighty three stool specimens from site 1, 93 (50.8%) girls and 90 (49.2%) boys with mean ages of 20±16.8 and 15±15.6 months in girls and boys respectively and 529 patients from site 2, 231 (43.7%) girls and 298 (56.3%) boys with mean ages of 19.6±10.4 and 17.7±8.9 months in girls and boys respectively were examined (P not significant). The characteristics of patients are shown in table 1.

Table 1: Demographic features of children with diarrhea.

Characteristics	No of children		
	Site 1 (N = 183)	Site 2 (N = 529)	Total (N = 712)
Gender			
male	90 (49.2)*	298 (56.3)	388 (54.5)
female	93 (50.8)	231 (43.6)	324 (45.5)
Age			
< 1 yr	71 (38.8)	277 (46.7)	318 (44.6)
1-2 yr	43 (23.5)	170 (32.1)	213 (29.9)
2-3 yr	26 (14.2)	63 (11.9)	89 (12.5)
3-4 yr	14 (7.6)	30 (5.6)	44 (6.1)
4-5 yr	19 (1.4)	8 (1.5)	27 (3.7)
Median age (mo) range	20 (3.6)	15 (3-60)	17.5 (3-6)
Mean age (mo ± SD)	23.8 ± 9.15	19.6 ± 13.2	22.5 ± 12.3

* Number in parentheses, percent.

Twenty eight (15.3%) of 183 stool samples collected from site 1 and 18 (3.4%) of 529 stool samples collected from site 2 showed at least one enteropathogen.

The mean ± SD ages of patients with positive enteropathogen in site 1 and 2 were 21.3±24.6 (range 4-60) and 24.5±23.6 (range 4-60) months respectively. There were not significant

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differences among two sites (P not significant).

The prevalence of different enteropathogens in site 1 were Escherichia coli (E-coli) 10 (5.5%), Salmonella spp 17 (9.3%), Shigella spp 1 (0.5%), Entamoeba histolytic 5 (2.7%) and 148 (80.8%) did not show any pathogen. The prevalence of different enteropathogens in site 2 include E.coli 18 (3.4%), Entamoeba histolytic 5 (0.9%) and 506 (95.6%) did not show any pathogen. The most commonly isolated enteropathogens from stool samples were Salmonella spp in site 1 and E.coli in site 2 with no significant different in age groups.

The proportion of nonisolated enteropathogen in site 1 and 2 were significantly higher (80.8%) and (95.6%) respectively. The total prevalence of stool samples with known etiologies were 28 (15.3%) and 18 (3.4%) in site 1 and 2 respectively which 28 (58.3%) of them were during cold weather.

The prevalence of parasite detected in stool patients of both sites were 10 (1.4%) of total patients which was equal in each sites with no significant differences in seasons.

Characteristics of stool examination: All stool samples in two sites analyzed for white blood cells with gram – stained smear and/or wet mount. White blood cells were detected in 46 (6.46%) of 712 stool samples. All 46 stool samples were associated with positive bacterial cultures. Of these, 7 (25%) had red blood cells in stool examination with positive E-coli culture and also 4 (8.6%) of 46 with positive Salmonella spp culture had high white blood cells and red blood cells in their stool samples. Whereas 12 (26%) of 46 stool patients with positive salmonella culture had not white and red blood cells. Also, in the stool patients with nonisolated enteric pathogens did not find white and red blood cells.

Ten (1.4%) of 712 stool samples, 5 samples from each site were associated with Entamoeba histolytic that all were associated with white and red blood cells in their stool samples and there were not significant differences during seasons (Table 2).

Table 2: Microbiology results by sites.

	No positive / total tested		
			Total
	Site 1	Site 2	

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Bacterial pathogens	28 / 183 (15.3)*	18/529 (304)	46/712 (6.4)
Escherichia coli	10 (5.5)	18 (3.4)	28 (8.9)
Salmonella spp	17 (9.3)	-	17 (9.3)
Shigella spp	1 (0.5)	-	1 (0.5)
Parasites	5 / 183 (2.7)	5/529 (0.9)	10/712 (3.6)
Entamoeba histolytica	5 (2.7)	5 (0.9)	10 (3.6)
Non pathogens or parasites	148 (80.8)	506 (95.6)	654 (91.8)

* Number in parentheses, percent.

DISCUSSION:

The prevalence of cases of diarrhea in two different sites in Tehran, Iran with a known etiology was 15.3% and 3.4% in site 1 and 2 respectively. Whereas, Klein et al[3] reported 7.3% prevalence in outpatient setting in an emergency department in Seattle and also Koopman et al[4] reported 5% prevalence in an inpatient pediatric setting and a 2% prevalence in outpatients pediatric setting in Ann Arber, MI. Other studies have demonstrated higher prevalence bacterial enteric pathogen in outpatient settings. Specifically, Dewitt et al[5] reported 15% of children evaluated in a pediatric outpatient clinic in New Haven were infected with bacterial pathogen. Donna M. Donno et al[6] reported 5.3% of isolated enteropathogens in their patients stool in two sites. As our study demonstrated the prevalence of isolated enteric pathogen in our patients is approximately similar but there is difference between our studies with their studies. They could demonstrated all type of enteric pathogen such as detection of E-coli virulence factor by polymerase chain reaction or specific media and technique for isolation of Campylobacter, Aeromonas spp, yersinia spp, Clostridium difficile toxin and finally viruses that are the most common enteropathogen in children with diarrhea. But only medical laboratory in our country can isolate E-coli, Shigella, Salmonella and parasites. They do not conduct more new technique or to use specific media because they are expensive and also the population or social security insurance organization can not pay cost of laboratory examinations. Moreover, there are some factors influence in examination results such as geographic area, diagnostic techniques used, the population or countries's economic conditions, period of studies and prevalence of bacterial pathogen in that areas.

The most common pathogens recovered in our stud was Salmonella in site 1 and E-coli in site 2 whereas in Donna's[6] study was Campylobacter jejuni and in Martha Vargas's[7] study was E-coli (33.5%) and also 7-50% of E-coli in Guerrant's[8] study from developing countries and 1-7% from developed countries. As

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we made mention, the main differences between present study and other study should be probably related to geographic area, health and hygien, family education, economy and new technologies used. In some countries like Iran, general medical laboratories do not differentiate virulence factors of enteropathogenic bacteria or other technologies.

As all investigators or pediatricians know, E-coli organism is one of normal intestinal bacterial flora in healthy children without white or red blood cells in their stool samples but in our studies, all stool samples with E.coli positive cultures were associated with high white blood cells and in addition 7 (28.6%) of them had red blood cells in their stool samples which suggest enteroinvasive E-coli or shiga-toxin producing E-coli. These organisms induce colonic lesions with ulcerations, hemorrhage, mucosal and submucosal edema and infiltration by polymorphonuclear leukocytes. The clinical features of diarrhea are seldom distinctive characteristics to allow definite diagnosis and routine laboratory studies such as peripheral white blood cells count and so on have very limited value and heavily depends on laboratory studies that are not available in our hospital or outpatients clinic. So, we clinicians only can suppose diagnosis based on clinical manifestations, fecal leukocytes and red blood cells which should be one of enteric pathogen. Our results did not show Campylobacter spp during one year in both sites but Vargus[7] demonstrated high prevalence of Campylobacter during dry season and other investigators did not find seasonal differences[9]. The diagnosis of Campylobacter enteritis is usually confirmed by identification of the organism in cultures of stool or rectal swab and sometimes selective media.

Our study only demonstrated Salmonella spp in sixteen (9.2%) of 183 stool samples in site 1 during one year whereas Vargas[7] study showed 5 (1.44%) of 348 patient in dry season but in our study only one case of Shigella spp were recovered during cold weather in site 1 and nothing in site 2. This result is despite of previous report that showed Shigellosis was more prevalent during dry season[10]. Present study demonstrated nonisolated organism to be the most common findings in both sites. We think it should be due to viruses or organisms that our laboratories could not detect because of unavailable new technologies or media and high expensive instruments and low income.

The present study identified Entamoeba histolytica is the most common parasite induced diarrhea in both sites. Guerrant[8] reported 2-15% of their patients infected with E-histolytica in developing areas. Amebiasis may be associated with bloody or invasive diarrhea in which fecal leukocytes are often absent. Whereas our stud showed high leukocytes in stool samples of all patients infected with E- histolytica. But among parasites causes diarrhea, Cryptosporidium in increasingly recognized in both developed and developing areas[11]. Our study did not recognize such organism.

CONCLUSION:

The present study is one of several studies for detection bacterial pathogen in children with diarrhea in outpatients and inpatients setting in a developing country and can be a guideline for pediatricians to decide antibiotic administration or not. The pediatrician should emphasize on the presence of certain risk factors such as white blood cells or red blood cells and parasites by

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microscopy suggest a bacterial infection. In addition, diarrheogenic E.coli was the predominate enteropathogen in site 2, Salmonella in site 1 and parasite was equal.

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