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Isolated and non-isolated enteric pathogens in children with diarrhea and related laboratory characteristics

Hossein Dahifar, MD.[1], Aboulfazl Ghorbani, MD.[2], Manijeh Ghods, PhD.[3]

Department of Pediatrics, Shohada-e- Tajrish Hospital, Shahid BeheshtiUniversity of Medical Sciences, Tehran, Iran.

Abstract

Background: Diarrhea has been recognised as a major public health problem worldwide. A prospective study was performed to determine the etiology, seasonal and age prevalence, relevant laboratory investigations, sensitivity of isolated microorganisms to current medication, and practical approaches to the diagnosis and management of diarrhea in Iran, as a developing country.

Methods: All infants and children under age five (n=825, mean age 18.9) admitted to Tehran Children's Hospital, Tehran, with diarrheal symptoms during the period of April 2005 to March 2006 were included in the study; 371 approximately age-matched controls (mean age 19.1 months) from the same hospital but not having diarrhea formed the control group.

Results: The most frequent isolated pathogen was Escherichia coli (18.9%), followed by Shigella spp (0.7%), and Salmonella spp (0.4%). Prevalence of diarrheic children with either isolated or non-isolated pathogens were 66.5% in the colder seasons and 54.4% in warm seasons. E. coli was more prevalent in children younger than two years old while Sigella spp and Salmonella spp were common to all ages. Fecal leukocytes were associated with 100% of isolated Escherichia coli, 19.4% of non-isolated organisms, 2.5% of Shigella spp, 0.5% of Salmonella spp and none in controls. Escherichia coli was also associated with fecal red blood cells (29.4%), as were Shigella spp (83%) and Salmonella spp (33.3%). White blood cell counts, polymorphonuclear cells, band cells, erythrocyte sedimentation rate and C-reactive protein measurements had no diagnostic value. Amikacin was the global choice of antimicrobial treatment for Shigella spp in (99%) of cases and for Escherichia coli in (91%) of isolated cases. Only 70% of patients infected by Salmonella showed sensitivity to Gentamycin.

Conclusion: Diarrheal diseases in either isolated or non-isolated pathogens were more prevalent in the colder seasons and in children younger than two years of age. For differentiation of bacterial from non-bacterial etiology, we had to wait for laboratory reports and then decide for antibiotic administration. The antibiotic most sensitive to Echerichia coli and Shigella was Amikacin, and Gentamycin was the most sensitive drug for Salmonella.

Keywords: Diarrhea, Escherichia coli, Shigella, Salmonella, Amikacin.

Introduction

Infectious diarrhea is a major cause of morbidity and mortality in infants and young children worldwide [1]. Infectious diarrhea can be classified into two groups, 1) invasive, when fecal leukocytes are present in stool and 2) noninvasive, when fecal leukocytes are absent or only scattered in stool [2]. The main etiology of

1. **Corresponding author**, Associate Professor of Pediatrics, Shahid Beheshti University of Medical Sciences. Address: No 50, Koohestan 8, Pasdaran Ave, Tehran, Iran. Tel/ Fax: +9821 22289518, email: dr_dahifar@yahoo.com.
2. Pathologist, Booali Hospital, Pathology Department, Marivan, Iran.
3. Medical Microbiologist, Sandwell and West Birmingham Hospital, Birmingham, England.

the diarrhea is related to the wide range of bacteria, enteroparasites and viruses. By lack of clinical microbiology investigations in most of the laboratories, the cause of diarrhea in children remains largely unknown. Although bacterial diarrhea diseases are often self-limited, specific antibiotic treatment may shorten the illness in normal hosts and prevent serious complications such as sepsis and protracted diarrhea in young infants or in children with underlying conditions such as immunosuppression or malnutrition [3]. When examination of the stool for fecal leukocytes is positive, it is likely that the patient has an invasive or cytotoxin-producing organism which disrupts or destroys the gastrointestinal epithelium [4]. The followings aspects were considered during the study period:

1)To show the limitation of laboratory examination as a considerable problem for pediatric practitioners to determine the right choice of treatment. 2) Determination of bacterial pathogenicity in diarrhea and its relationship with age and season. 3) To distinguish the bacterial form of diarrhea from nonbacterial forms by simple routine laboratory tests. 4) Sensitivity evaluation of isolated microorganisms to the current available antibiotics.

Methods

Patients

The population consisted of children up to 5 years of age, who were involved with acute diarrhea and vomiting from April 2005 until March 2006 in Tehran Children's Hospital. Most of the patients were admitted to the hospital for symptoms of fever, vomiting, diarrhea and dehydration. The relevant clinical information was collected by filling the questionnaire for each patient. The requested data included age, sex, duration of diarrhea and history of antibiotic therapy prior to the clinic visit. Diarrhea was defined as an episode of $\geq$ 3 loose macroscopically non-bloody stools for 24h. Vomiting was defined as a forceful expulsion of gastric contents occurring at least twice in a 24h period. Children who had only vomiting without diarrhea, respiratory illness, prior antimicrobial therapy for 5 days, less than 24h hospitalization, mixed organisms isolation, chronic diarrhea, parenteral diarrhea, (e.g. acute otitis media and pyelonephritis) incomplete laboratory records and discharged without physician orders were excluded from the study.

Controls

Stool samples were selected randomly from children without diarrhea presenting at or admitted to Tehran Children's Hospital. These controls were treated at the same period for other illnesses without receiving any antibiotics in the last two weeks prior to the date of hospital admission.

Microbiological studies

Fresh stool specimens were collected from symptomatic patients after their admission and were cultured straight away for bacterial isolation on EMB agar [5]. Samples were macro/microscopically examined for blood, mucus, red blood cells (RBC) and leukocytes. Fecal leukocyte and RBC examination were performed by placing a small fleck of fresh stool which was diluted by one drop of saline on a clean glass slide. A sample was considered positive in the presence of more than 10 leukocytes or RBC. Other specific methods for differentiation of five strains of diarrheagenic E. coli were not available. In order to isolate Salmonella and Shigella spp SS agar media was employed. Campylobacter and Yersinia enterocolitica were not considered in our study, since these microorganisms are not routinely checked for and related investigations and their growth requires specific media. Other laboratory evaluations such as complete blood count (CBC), differentiation of white blood cells, polymorphonuclear (PMN) cells, band (B) cells, erythrocyte sedimentation rate (ESR), qualitative C-reactive protein (CRP), and bacterial sensitivity to

various antibiotics were also performed by using antimicrobial disk susceptibility tests (Padtan Teb Co. Tehran, Iran). For sensitivity evaluation, a variety of antibiotics such as Ceftriaxone, Ceftizoxime, Amikacin, Gentamycin, Ampicillin, Trimethoprim-sulfamethoxazole and Nalidixic acid were tested via disc diffusion method.

Statistical analysis

The collected data were analysed by SPSS software, version 11.5. The results were expressed as mean ± standard deviation (SD). The significance level was set at $P<0.05$. The comparison of mean values was conducted by using t- test.

Results

From the total number of 1987 children with diarrhea who were hospitalized during a 12-month period, 825 were entered in our study. Stool samples of 371 children not having diarrhea but with other diseases were examined as control, at the same time with approximately similar age. The mean ± SD age of children with diarrhea were 18.9 ± 13 (range 3 to 60) months and control children 19.1 ± 9.8 (range 4 to 60) months. The number of children in different age categories is shown in Table 1. Among the children with diarrhea, the female-to-male ratio was 0.8, and among the control children, it was 0.76.

Enteric pathogens were isolated from the samples of 165 (20%) of 825 children with diarrhea. No pathogenic bacteria were isolated from the 371 controls. The most common isolated enteropathogen was E. coli =156 (18.9%) (of these, 60 (37.9%) were female), Shigella spp= 6 (0.7%) (of these, 3 (50%) were female), and Salmonella spp= 4 (0.4%) (of these 2 (66%) were female). There was a significant increased prevalence of diarrheagenic E. coli in children with diarrhea. Analysis of age strata data showed a significant association with diarrhea for E. coli organisms within the first two years of age, whereas, Shigella and Salmonella spp had no particular age pattern. The occurrence of recognized enteric pathogens and non-isolated pathogens from children is shown in Table 2.

Age (mo)	*No. (%) of diarrheal children*	*No. (%) of control children*
3-12	367(44.6)	151(40.7)
13-24	306(36.9)	109(29.3)
25-36	67(8.1)	53(14.3)
37-48	51(6.2)	32(8.6)
49-60	34(4.2)	26(7)

Table1. Age strata of 825 children with diarrhea and 371 control children without diarrhea.

The seasonal prevalence of diarrhea in children was 33.4% in warm seasons and 66.5% in the colder seasons. Of these, 25.4% and 58.9% had non-isolated pathogens respectively. Fifty-nine (37.8%) of 156 children with isolated E. coli organisms in their stool were ill during the hot seasons.

Two-hundred one (24.3%) of 825 children with diarrhea had leukocytes in their stool samples. Of these, 156 (77.6%) had E. coli, 39 (19.4%) had no isolated organism, and 5 (2.5%) and 1 (0.5%) had Shigella and Salmonella spp isolated from stool samples of children respectively. Leukocytes were not found in stool sam-

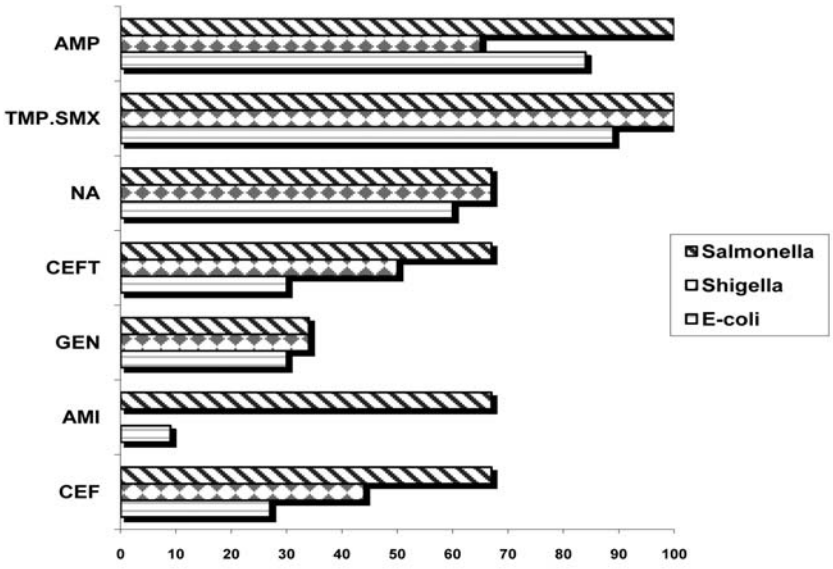
	No. (%) of children with diarrhea			
Age group (month)	Non-isolated pathogen	E. coli	Shigella	Salmonella
3-12	296 (44.9)	63 (40.4)	-	1(0.6)
13-24	250 (37.9)	55 (33.2)	2(1.2)	1(0.6)
25-36	60 (9)	14 (8.4)	-	-
37-48	32 (4.9)	14 (8.4)	-	1(0.6)
49-60	22 (3.3)	10 (6.4)	4(2.4)	-

Table 2. Major enteric isolated and nonisolated pathogens from 825 children with diarrhea according to age.

Variables	*Total children No (%)*	*Non-isolated pathogen*	*E. coli*	*Shigella*	*Salmonella*	*Controls*
Fecal WBC	201(24.3)	39 (19.4)	156 (77.6)	5 (2.5)	1 (0.5)	-
Fecal RBC	52 (6.3)	-	46 (88.4)	5 (9.6)	1 (1.9)	-
WBC/mm^3	8019±3278	7831±3192	8776±3523	9766±4912	4800±1044	8652±2181
PMN cells%	53±20	57±20	53±20	48±23	64±24	52±21
B cells%	0.06±2.4	0.7±2	0.4±3	3.8±6	0.3±2	0.5±4
ESR mm/h	21±12	14±12	17±13	19±16	8+2	18±12
+	76 (49.6)	56 (39.6)	20 (13.2)	-	-	32 (52.4)
CRP no (%) ++	72 (47)	50 (32.6)	22 (14.3)	-	-	28 (45.9)
+++	5 (3.2)	3 (1.9)	2 (1.3)	-	-	1 (1.6)

Table 3. laboratory characteristics of 825 children with isolated, non-isolated pathogens and 371 control children.

ples of control children. Fifty-two (6.3%) of 825 children with diarrhea had RBC in their stool samples. From this population, 46 (88.4%) children were infected by E. coli, 5 (9.6%) had Shigella and from one patient (1.9%) diarrheagenic Salmonella was isolated. RBC was not seen in stool samples of diarrheal children with non-isolated pathogens or in controls. The total mean ± SD of white blood cells in all children with diarrhea were 8019± 3278.6/mm^3. The differences and comparison of non-isolated pathogen and isolated E. coli, Shigella, Salmonella and control children are shown in Table 3. There were no significant differences among children with isolated E. coli, Shigella, non-isolated pathogens and control children (P>0.05), whereas in Salmonella the difference was significant (P<0.05). The mean±SD of polymorphonuclear (PMN) cells in all children with diarrhea was 53± 20.9%. There was no significant difference among isolated E. coli, Shigella, Salmonella, non-isolated pathogen and control children (P>0.05). The total mean± SD of band (B) cells in all children with diarrhea was 0.06±2.4%. Except for Shigella, there were no significant differences among isolated E. coli, Salmonella, non-isolated and control children (P>0.05).



CEF=ceftriaxone, AMI= amikacin, GEN= gentamycin, CEFT=ceftizoxime,
NA= nalidixic acid, TMP-SMX= trimethoprim-sulfametoxazole, AMP= ampicillin.

Fig. 1. Antibiotic resistance among 165 enteric isolated pathogens from 825 children with acute gastroenteritis.

The total mean±SD of erythrocyte sedimentation rates in all children with diarrhea was 21 ± 12 mm/hr. There were no significant differences among isolated E. coli, Shigella, non-isolated pathogens and control children (P>0.05); however, there were significant differences among diarrheagenic Salmonella and the remainder of children (P<0.05). C-reactive protein was positive from 1+ to 3+ in 153 (18.5%) of 825 children with diarrhea. There was significant difference among non-isolated pathogens with isolated E-coli, Shigella, Salmonella and control children (P<0.05).

The sensitivity of isolated enteric organisms to antibiotics is shown in figure 1.

Overall, 525 children (69.1%) of 825 with diarrhea received antibiotics. Of these, 363 (69.2%) were non-isolated pathogen children, 156 (29.7%) E. coli, 4 (0.8%) Shigella, and 2 (0.4%) Salmonella. Resistance to trimethoprim-sulfamethoxazole (TMP-SMX) was recorded in 88.5%, 100%, and 100% of infected children with E-coli, Shigella and Salmonella, respectively. These organisms were 40.3%, 33.3% and 33.3% resistant to nalidixic acid, respectively. All of Shigella, 91% of E. coli and 33.3% of Salmonella isolates were sensitive to Amikacin. All of Salmonella, 50% of Shigella and 83.4% of E. coli isolates were resistant to Ampicillin, respectively.

Discussion

The prevalence of diarrhea in the examined city, i.e. Tehran, Iran, with a known etiology was 62.2% in the colder seasons, which is not in agreement with reports from other developing countries [6-9]. Shigella and Salmonella spp were isolated at a high frequency during the colder seasons. This result differs with the other studies' reporting Shigellosis and Salmonellosis as being more prevalent during warm seasons [6,10].

In our study, there was a high frequency of non-isolated pathogens (58.9%) during the colder seasons which we think the etiology of these diarrheic children may be either viruses or enterotoxigenic E. coli (ETEC) and enteropathogenic E. coli (EPEC). Rotavirus has been reported as the main common virus associated with diarrhea in young African children [11-13]. The present study demonstrated a high frequency of non-isolated organisms during the colder seasons. If we suppose rotavirus is the etiologic agent, it would differ with other investigations showing a peak in the incidence of rotavirus during the warm season in different areas of Africa [13,14]. Furthermore, we found leukocytes in 5.9% of stool samples of non-isolated organism which is in agreement with other reports of rotavirus-infected stools [14,15]. In contrast, Ryder [16] in Bangladesh found no leukocytes in similar cases. Vargas [6] reported enteropathogenic and enterotoxigenic E. coli as a predominant pathogen in the warm season. Therefore, our non-isolated pathogen from stool samples of diarrheic children may be due to either virus or enteropathogenic and enterotoxigenic E. coli. Additionally, because there were no leukocytes or RBC in stool samples of control children and nonisolated organism children, their etiology must be one of the pathogenic E. coli such as enteropathogenic or enterotoxigenic E. coli. Therefore, we should also consider the virus as a cause of diarrhea.

Albert [17] in Dhaka reported rotavirus diarrheagenic pathogen in 24.8% of children younger than 12 months of age, whereas in our study the majority of children with non-isolated organism were within the first two years of life. If we compare other laboratory examinations such as WBC, PMN , ESR and CRP in isolated diarrheagenic pathogens and control children, there would be no significant differences among them. Therefore, we cannot use them for differentiation of bacterial from nonbacterial pathogens.

In this study, 118 (75.6%) of 156 children with E. coli organism in their stool samples were younger than two years of age. We do not exactly know what E. coli strain has produced

diarrhea. In addition, 43 (27.3%) of 156 children had both leukocytes and RBC in their stool samples. Therefore, we only can suppose that the pathogenic organism may be enteroinvasive E. coli or Shiga producing E. coli. Parat [18] in southern Israel reported that 0.2% of 1496 stool samples harboured enteroinvasive E. coli. Ochoa [19] reported that in the developing world, just 5% of sporadic diarrhea episodes and 20% of bloody diarrhea cases may be caused by EIEC strains. Albert [9] did not isolate EIEC and EHEC from any children with diarrhea. Thus, there are various reports that differ with our study results.

The second most common pathogenic isolated organism was Shigella which was more frequent in the colder seasons. The prevalence of Shigella in Vargas' study [6] was 20% in 451 children with diarrhea and was higher during the warmer season. Thirty-three (2.7%) of 1197 children with diarrhea with various ages from Central Africa Republic [14] were infected by Shigella , while in our study 0.7% of cases were infected by Shigella in various ages. The last pathogenic organism in our study was Salmonella with no specific age pattern in the colder seasons. In another investigation [6] Shigella prevalence was 1.4% in warm seasons. The only significant laboratory examination in our study was leukopenia. The most valuable laboratory investigation for differentiation of bacterial from nonbacterial cause for diarrhea was fecal leukocytes and to a less extent RBC in stool samples; this is supported by Huicho's study [20] as well. Other studies [21] preferred fecal lactoferrin to fecal leukocyte and occult blood.

In Tehran, like other cities in Iran, the resistance of enteric pathogens to commonly used oral antibiotics is high. 50% of all Shigella spp were resistant to ampicillin, 100% to trimethoprim-sulfamethoxazole, and 67% to nalidixic acid. Whereas in other studies, 57% of all Shigella spp were resistant to ampicillin, 82% to trimethoprim-sulfamethoxazole and 0% to nalidixic acid [22]. Sixty percent of all E. coli isolated from our study's samples were resistant to nalidixic acid, 89% to trimethoprim-sulfamethoxazole and 84% to ampicillin; while in a similar study, resistance was 0%, 61% and 78%, respectively [23]. In this study, the resistance of E. coli, Shigella and Salmonella to ceftriaxone was 27%, 66% and 33% respectively. This resistance in other studies was 6%, 0% and 0%, respectively [23]. Therefore, by its high degree of activity against a wide range of gastrointestinal pathogens, amikacin was identified as an excellent choice for the empirical treatment of most common pathogens in hospitalized children with diarrhea.

Conclusion

This study concluded that non-isolated pathogens are the predominant cause of diarrhea in children younger than two years old in Iran during the colder seasons. E. coli was the most common isolated organism from the stool samples of the children. There were no specific laboratory examinations to demonstrate or differentiate the known and unknown etiology, except fecal leukocyte and RBC. These two indicators can guide the pediatric practitioners in order to make the decision towards the diagnosis and management of diarrhea, with recommendation of amikacin as the most common sensitive drug to the isolated organisms.

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