Short Communication

Bacterial entropathogens and antimicrobial susceptibility in children with acute diarrhea in Babol, Iran

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Abstract

Background: Infectious diarrhea is one of common cause of children diarrhea causing mortality and morbidity worldwide. This study was performed to identify the common bacteria and their antimicrobial susceptibility in children with diarrhea.

Methods: Children under 14 years old with acute diarrhea who referred to Amirkola Children's Hospital, Mazandaran, North of Iran, were enrolled during the summer and fall of 2009. From each patient, two fecal specimens were collected. Samples were cultured and bacterial isolation was done by conventional methods. Antimicrobial susceptibility was identified by disk diffusion and micro dilution methods.

Results: One hundred-seventy two patients with the mean age of 41.8±37.6 months were evaluated. The bacteria were isolated in 48 (27.9%) cases. The most common isolated bacteria was E.coli and then shigella in both bloody and nonbloody diarrheal patients. There was a significant difference between bacteria positive specimens and WBC in stool smear (p=0.003). All isolated shigella were susceptible to Ceftizoxime and ciprofloxacin and were resistant to Cefixime. Resistant to Nalidixic acid was seen in 14% of them.

Conclusion: The results show that E.coli was the most frequently isolated pathogen in children with bloody and nonbloody diarrhea. Ceftizoxime is a good antibiotic for shigellosis in children in our area but Cefixime is not appropriate.

Keywords: Diarrhea, Drug, Resistance, Antibiotic, Dysentery.

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Acute diarrhea as a gastrointestinal related symptom may have some different causes such as infection. Infectious diarrhea leads to approximately three million deaths worldwide and 516 deaths in Iranian children younger than 5 years per year (1, 2). The rate of entropathogen isolation in acute diarrhea varied in different studies depending on the sampling methods and microbiological techniques. Some of them, the most common bacterial pathogen is diarrheagenic E. coli (1,3, 4).

Due to the overuse of antibiotics and change in epidemiology and antimicrobial resistance of bacterial agents, a study on local epidemiology of bacterial diarrhea and antimicrobial susceptibility plays an important role in choosing the appropriate antibiotics for empirical treatment. Thus, this study was performed to identify common bacterias and their antimicrobial susceptibility in our region to support choosing a suitable antimicrobial agent.

Methods

This cross sectional study was done on children from 1 month to 14 years old admitted in Amirkola Children's Hospital, North of Iran, with complaints of acute diarrhea due to gastroenteritis during the summer and fall of 2009. Informed verbal consent was taken from the child's parents and then some information such as age, sex, duration of symptoms, bloody diarrhea and preadmission antibiotic consumption were recorded. From each patient, fecal specimen was collected in a sterile container and also with a rectal swab dipped in recto anal mucosa .The microscopic study of each stool smear was done. Each specimen that had more than 5 red blood cells (RBC) or white blood cells (WBC) per each high-power field was defined as RBC or WBC positive.

All samples were transferred to the microbiology laboratory of Babol University of Medical Sciences and a transport culture medium was used up to 2 hours. All samples were cultured and incubated at 37°C for 18 to 24 hours using Eosin Methylene Blue Agar (EMB), Salmonella Shigella (S.S) agar, Selenite-F broth, and Thiosulfate-citrate-bile salts-sucrose (TCBS) agar culture mediums and bacterial isolation done by conventional methods (5).

Antimicrobial susceptibility was identified by disk diffusion and microdilution methods. Both of them were done according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (6) using the ten mentioned antibiotics: amikacin, ampicillin, ceftizoxime, ceftriaxone, cefotaxime, ciprofloxacin, nalidixic acid, cefixime, gentamicin, erythromycin. (The disks were prepared from Patan-Teb Company, Iran and antibiotics for dilution method from Sigma Company, Germany).

The minimum inhibitory concentration (MIC) was defined as the minimum concentration of an antibiotic that is just capable to stop the growth of the organism in vitro. The values were tested using SPSS Version 15. Chi-square, test and Fisher's exact test were used when appropriate. Kappa coefficient was used to study the agreement level between the two methods of antibiogram (microdilution method and disk diffusion). A p-value of 0.05 or less was considered significant.

Results

One hundred seventy-two patients with the mean age 41.8±37.6 months were evaluated. Thirty-three (17.9%) patients had complaint of dysentery and 48 (27.9%) cases had stool culture positive for bacteria in which 49 pathogens were isolated. One sample had two pathogens. The frequency of culture positive cases with respect to different age groups is shown in table1.

Table 1: Frequency of culture positive respect to age groups

Age in years	No. of stool samples	No .of culture positive
≤3	92	16
3-5	40	13
≥5	40	19

P=0.001

Stool smear in 41 (23.8%) patients was positive for RBC of which 19 cases (46%) had positive bacterial culture while only 29 samples of 131 (22.1%) RBC negative stool smear had positive bacterial culture (p=0.005). On the other hand, 11 out of the 33 cases (32.3%) had bloody diarrhea and 38 out of 139 (27.3%) nonbloody diarrhea had positive stool culture (p=0.659). The types of isolated bacteria from bloody and nonbloody diarrhea patients are shown in table 2. There were 107 WBC negative stool smear and 65 WBC positive stool smear of which there were 21 (19.6%) and 27 (41.5%) positive bacterial culture, respectively (p=0.003). E.coli was the most frequent isolated organism in both bloody and nonbloody diarrhea (table 2). Of the 38 isolated E.coli, thirty one were identified as serogroup poly 3 and the frequency of poly 1, 2, 4 were 3, 2, 3 cases, respectively. Six of the 7 isolated Shigella were Shigella sonnei and another case was shigella dysenteriae.

Table 2: Type of isolated bacteria respect to bloody or non-bloody diarrhea

	Bloody diarrhea (n=33)	Non bloody diarrhea (n=139)
Total culture negative	22	102
Total culture positive	11	37
Ecoli	7	30
Shigellasonei	3	3
Shigelladysenteriae	1	0
Salmonella paratyphi	0	3
Salmonella typhi	0	1

The isolated strains of salmonella were S. paratyphi C in two cases, S. typhi in one case and S. paratyphi B in one case. Antimicrobial susceptibility testing with both MIC and disk perfusion methods show highly similar results (table 3). Antimicrobial resistance with respect to different bacteria is shown in table 4. Approximately, all isolated bacteria were resistant to erythromycin and cefixime and susceptible to amikacin and ceftizoxime.

Table 3: Antimicrobial susceptibility testing with both MIC and disk perfusion methods

	Susceptible		Intermediate		Resistant		Kappa(p-value)
	MIC	Disk	MIC	Disk	MIC	Disk	
Ceftriaxone	29	30	8	6	6	7	0.903 (0.000)
Ceftizoxime	40	39	2	1	1	3	0.243 (0.029)
Cefotaxime	29	31	11	6	3	6	0.404 (0.000)
Amikacin	37	30	6	11	0	0	
Gentamicin	16	14	12	16	15	13	0.826 (0.000)
Ampicillin	30	20	9	13	4	10	0.291 (0.005)
Ciprofloxacin	37	41	1	0	5	2	
Nalidixic acid	17	12	17	18	9	13	0.437 (0.000)
Cefixime	4	2	5	4	34	37	0.694 (0.000)
Erythromycin	0	0	14	13	29	30	0.622 (0.000)

Table 4: Antimicrobial resistance of isolated bacteria

Antibiotic	Salmonella typhi N=1(100%)	S. Paratyphi N=2(100%)	S. Dysentery & S. Sonei N=7(100%)	Ecoli N=34(100%)
Ceftriaxone	0	0	2 (28.5)	4 (11.76)
Ceftizoxime	0	0	0	1 (2.9)
Cefotaxime	0	0	2 (28.5)	2 (5.8)
Amikacin	0	0	0	0
Gentamicin	0	1 (50)	3 (49)	12 (35.2)
Ampicillin	0	1 (50)	0	3 (8.8)
Ciprofloxacin	0	0	0	5 (14.7)
Nalidixic acid	0	1 (50)	1 (14.2)	8 (23.5)
Cefixime	0	1 (50)	7 (100)	27 (79.4)
Erythromycin	1 (100)	2 (100)	4 (57)	23 (67.6)

Discussion

In this study, we found bacteria from more than a quarter of stool samples of children with acute gastroenteritis (27.9%). Our findings are in agreement with the results of other studies from the developing countries reported between 16.8 to 33% (1, 4). Contrary to the higher rate of multiple infection involvement in the other developing countries, in our study two enterpathogens were isolated only in one case (3, 6, 7) .This may be related to the environmental and personal hygiene factors in our region. In this study, the frequency of bacterial diarrhea in older age group was significantly higher than the others that differs from the results of the other studies (3, 8) .This may be due to the kind of nutrition and high rate of breast milk feeding in infancy in our region.

E coli was the most common cause of bacterial diarrhea similar to many other previous studies (3, 9-12). In other studies, Shigella was more frequent (4, 13) . E.coli was also the most common cause of bacterial dysentery in our study, but Mota et al. in Uruguayan children showed that the Shigella was the most frequently isolated pathogen in

children with bloody diarrhea (13). Shighella was the second cause of bacterial gastroenteritis in our study with respect to World Health Organization (WHO) planning for gastroenteritis treatment and life-threatening complication of shigellosis, its treatment is important (14).

Shigella sonei were the most common isolated serotypes of Shigella in this study. For many years in Asia and most developing countries as well as in Iran, the most common isolated serotypes of Shigella was S. Flexeneri but reports from India in 2002, Shiraz in 2006 and Tehran in 2008 showed an increase in Shigella Sonei (15-19). We believe that Shigella Sonei is likely becoming more prevalent serotype of Shigella in Iran. The kappa value for all antibiotics except for ciprofloxacin and amikacin demonstrated high agreement between the two methods of antimicrobial susceptibility testing (microdilution method and disk diffusion). We think this difference was related to the quality of the ciprofloxacin and amikacin disk.

All Shigella isolates were susceptible sensitive to ceftizoxime, amikacin and ciprofloxacin but ceftizoxime and amikacin were used for the inpatients and ciprofloxacin had an age- related limitation.

According to the results of our study only 14 % of the Shigella isolates were resistant to nalidixic acid and we think this agent is still the drug of choice for oral empirical treatment in infectious diarrhea. All isolated shigella were resistant to cefixime. However, one limitation of our study was the low number of isolated Shigella. We suggest further studies with larger sample size should be performed on serial antimicrobial susceptibility of bacterial diarrhea focusing on the newer antibiotics such as, azithromycin and other oral antibiotics.

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References

1. Al jarousha AM, El Jarou MA, El Qouqa IA. Bacterial enteropathogens and risk factors associated with childhood diarrhea. Indian J Pediatr 2011; 78: 165-70.
2. Kolahi AA, Nabavi M, Sohrabi MR. Epidemiology of acute diarrheal diseases amongchildren under 5 years of age in Tehran, Iran. Iran J Clin Infect Diseases 2008; 3: 193-8.
3. Moyo SJ, Gro N, Matee MI, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. BMC Pediatr 2011; 11:19.
4. Kansakar P, Baral P, Malla S, Ghimire GR. Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004. J Infect Devctries 2011; 5:163-8.
5. Forbes BA, Sahm DF, Weissfeld AS, Bailey WR. Bailey & Scott's diagnostic microbiology. 12 th ed. USA: Elsevier Mosby 2007.
6. Wikler MA, Cockerill FR, Crraig WA, et al. National Committee for Clinical Laboratory Standards. 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement M100-S17. Availible at: URL: http://www.microbiolab-bg.com / CLSI.pdf. Accessed March 5, 2013.
7. Thapar N, Sanderson IR. Diarrhea in children: an interface between developing and developed countries. Lancet 2004; 363: 641-53.
8. Nimri LF, Elnasser Z, Batchoun R. Polymicrobial infections in children with diarrhoea in a rural area of Jordan. FEMS Immunol Med Microbiol 2004; 42: 255-9.
9. Nair GB, Ramamurthy T, Bhattacharya MK, et al. Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. Gut Pathog 2010; 2: 4.
10. Guerrant RL, Hughes JM, Lima NL, Crane J. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev Infect Dis 1990; 12: S41-50.
11. Nweze EI. Aetiology of diarrhoea and virulence properties of diarrhoeagenic Escherichia coli among patients and healthy subjects in southeast Nigeria. J Health popul nutr 2010; 28: 245-52.
12. Garcia PG, Silva VL, Diniz CG. Occurrence and antimicrobial drug susceptibility patterns of commensal and diarrheagenic Escherichia coli in fecal microbiota

from children with and without acute diarrhea. J Microbiol 2011; 49: 46-52.
13. Mota MI, Gadea MP, Gonzalez S, et al. Bacterial pathogens associated with bloody diarrhea in Uruguayan children. Rev Argent Microbiol 2010; 42: 114-7.
14. World Health Organization (WHO). Management of the child with a serious infection or severe malnutrition. guidelines for care of the first referral level in developing countries, Integrated Management of Childhood Illness (IMCI), Geneva 2000; pp: 55. Availible at: URL: http://whylibdoc.who.int /hq/2000/WHO-FCH-CAH-00.1.pdf. Accessed Sep 17, 2013.
15. Nikkah J, Mchr-Movahead A. Antibiotic resistance among Shigella species isolated in Tehran, Iran. Ann Trop Med Parasitol 1988; 82: 481-3.
16. Moezardalan K, Zali MR, Dallal MM, Hemami R, Salmanzadeh-Ahrabi S. Prevalence and pattern of antimicrobial resistance of Shigella species among patients with acute diarrhoea in Karaj, Tehran, Iran. J Health PopulNutr 2003; 21: 96-102.
17. Ranjbar R, soltandallal MM, Talebi M, Pourshafie MR. Increased isolation and characterization of Shigellasonnei obtained from hospitalized children in Tehran, Iran. J Health Popul Nutr 2008; 26: 426-30.
18. Farshad S, Sheikhi R, Japoni A, Basiri E, Alborzi A. Characterization of Shigella strains in Iran by plasmid profile analysis and PCR amplification of ipa genes. J Clin Microbiol 2006; 44: 2879-83.
19. Dutta S, Rajendran K, Roy S, et al. Shifting serotypes, plasmid profile analysis and antimicrobial resistance pattern of Shigella strains isolated from Kolkata, India during 1995-2000. Epidemiol Infect 2002; 129: 235-43.