

A Survey of the Epidemiology and Antibiotic Resistance Patterns of Enteropathogens Isolates in an Iranian Hospital

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Received 2015 August 25; Revised 2015 November 21; Accepted 2015 December 8.

Abstract

Background: Infectious diarrhea is one of the most frequent diseases among children, especially in developing countries.

Objectives: The aim of this study was to determine the etiological agents and drug resistance patterns of common enteric pathogens isolated in an Iranian 1000-bed tertiary care hospital.

Patients and Methods: In a retrospective study, we analyzed the etiology and drug resistance patterns of enteric pathogens associated with diarrheal cases. The study was carried out in the Milad hospital of Tehran over two years, from April of 2012 to January of 2014. Stool specimens from patients with diarrhea (n = 7321) were examined for enteric pathogens using routine microbiological culture methods. Strains of *Salmonella*, *Shigella*, and enteric pathogenic *E. coli* (EPEC) were serotyped and their susceptibility to commonly used antimicrobial agents was determined by a disk diffusion method, as recommended by the clinical and laboratory standards institute (CLSI) guidelines.

Results: Enteric pathogens were isolated from 310 (4.23%) of the patients. The most frequently isolated microorganisms included enteropathogenic *E. coli* (EPEC), *Salmonella*, and *Shigella* spp. The majority of the isolates of EPEC were resistant to commonly used antibiotics such as ampicillin (85.61%), cefixime (79.41%), and nalidixic acid. Resistance among other enteric pathogens was also prevalent. About 45.70% of the *Salmonella* isolates were resistant to chloramphenicol, and 87.95% were resistant to sulfamethoxazole/trimethoprim. Resistance of the *Shigella* isolates to nalidixic acid in comparison to the resistance recorded in previous studies was higher.

Conclusions: The results show that enteric bacteria, including EPEC, *Salmonella* spp., and *Shigella* spp. are the major causative agents of diarrhea in the hospital. The emergence of antimicrobial resistance among enteric pathogens is an important problem for public health. Considering the threat of emerging antimicrobial resistance among enteric pathogens, a better surveillance system is necessary.

Keywords: Enteric Pathogens, Drug Resistance

1. Background

Intestinal tract infections, especially acute diarrheal diseases, are the second most important medical problem after respiratory tract infections (1). Diarrhea is a syndrome that is not frequently differentiated by specific agents. There are more than 20 viral, bacterial, and even parasitic agents responsible for diarrhea (2). It is estimated that 1.3 billion cases of acute diarrhea occur annually worldwide, and more than two million children younger than five years old die as a result of such occurrences each year. Eighty percent of these deaths are among children under two years of age. Predisposing factors such as malnutrition, low socioeconomic conditions, and poor hygiene are the major risk factors for such deaths in developing countries (3-8).

Among the bacteria responsible for diarrhea, *Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter* spp., *Vibrio cholerae*, *Aeromonas hydrophila*, and *Plesiomonas* spp. are

the most common agents of diarrhea (2, 5, 6). In spite of fluid and electrolyte replacement as a method of choice for the treatment of diarrheal diseases, antimicrobial therapy is recommended for suspected cases of *Salmonella*, *Shigella* and *V. cholerae* (7). The prescription of antibiotics for treatment of diarrheal diseases has a crucial role in reducing morbidity and mortality of the diseases; however, misuse and overuse of antibiotics in the treatment of diarrhea has resulted in antibiotic resistance (8). Therefore, the study of the prevalence and patterns of drug resistance among common enteric pathogens in each country is of essential importance for effective epidemiology surveillance and successful management of such cases in patients (3, 4, 7). However, although there are a few studies in the Islamic Republic of Iran on diarrheal diseases, there is still insufficient data on bacterial enteric pathogens and antimicrobial resistance patterns.

2. Objectives

The aim of this study was to determine the prevalence and drug resistance patterns of common bacterial agents of diarrhea over two years in an Iranian 1000-bed tertiary care hospital.

3. Patients and Methods

3.1. Study Design and Sampling

This study was carried out at Milad hospital located in Tehran. Milad hospital is a 1000-bed tertiary care hospital affiliated with a social security organization. From April of 2012 until January of 2014, all patients with acute diarrhea admitted to Milad hospital were the subjects of the study. Diarrhea was defined as involving three or more loose, liquid, or watery stools, or at least one loose and bloody stool passed within 24 hours (9). The demographic data of all patients, including sex, age, and clinical information, was obtained using questionnaires for outpatients, and in the case of hospitalized patients, all necessary data was derived from the patients' files. Stool specimens were collected by trained nurses using sterile containers and transported to the microbiology laboratory as soon as possible.

3.2. Laboratory Study and Pathogen Detection

First, the samples were examined microscopically for pus cells, red blood cells (RBCs), leukocytes, cysts, and ova of parasites. Stool samples were cultured in Hektoen enteric agar, XLD agar, MacConkey's agar, and blood agar for identification of each of the *Shigella*, *Salmonella*, and *E. coli* strains. All microbiological media were purchased from the Pronidosa company (Spain).

All plates were incubated at 35°C for 24 - 48 hours, and were then examined for the growth of enteric pathogens. Suspected colonies of enteric pathogens were identified according to standard microbiological procedures such as gram stains and various other biochemical reactions with other substances, including indol, lysine iron agar, triple sugar iron agar, oxidase, and methyl red, in addition to the application of the Voges proskauer test, citrate reaction, and the utilization of glucose and lactose (10, 11). *Shigella*, *Salmonella*, and EPEC strains were serotyped using commercially available antiserum (obtained from the Bahar Afshan company in Tehran, Iran).

3.3. Susceptibility Testing

The disk diffusion method was used to determine the

susceptibility of isolates of *Salmonella*, *Shigella*, and EPEC species to different antimicrobial agents using commercially available disks (ROSCO Denmark), as recommended by the CLSI guidelines (12). The antibiotic disks used in the testing of *Salmonella*, *Shigella*, and EPEC were nalidixic acid (NA, 30 µg), sulfamethoxazole/trimethoprim (SXT, 23.75/1.25 µg), ampicillin (Amp, 10 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (C, 30 µg), ceftriaxone (CRO, 30 µg) and cefixime (CFM, 30 µg). In this study, the defining criterion for multidrug resistance (MDR) was resistance to > 2 of the antimicrobial agents belonging to a different structural class. The quality control organisms in susceptibility testing were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and, *S. aureus* ATCC 29213. All of the control strains were provided by the Iranian reference health laboratory. We used the SPSS statistical software package version 24 for the analysis of our data.

4. Results

Out of 7321 stool specimens, 310 (4.26%) of the cultures were positive for enteric pathogens. Of the 310 positive stool specimens, 179 (57.74%) were from males and 131 (42.26%) were from females. Out of the 310 patients, 186 were outpatients and 124 were hospitalized (Table 1). The age distribution of patients is shown in Table 2. All cases of enteropathogenic *E. coli* (EPEC) were isolated from patients under 10 years old. Sixty five (78%) and 57 (72%) of patients affected by *Salmonella* and *Shigella*, respectively, were also children. The other important isolated enteric pathogens included *Salmonella* spp. with 83 isolates, and *Shigella* spp. with 79 isolates. Out of 83 isolates of *Salmonella*, group D *Salmonella* accounted for 29 isolates; out of 79 *Shigella* strain isolates, 53 isolates (67.08) were *S. sonnei*, followed by 20 *S. flexneri* (25.31%), and *S. boydii* and *S. dysenteriae*, each with 3 isolates.

The frequency of isolated organisms is shown in Table 1. Two strains of *Aeromonas hydrophila* were also isolated from the patients. The majority of isolates of EPEC were resistant to antibiotics such as ampicillin (85.61%), cefixime (79.41%) and nalidixic acid (59.58). Resistance among other enteric pathogens was also prevalent. About 45.70% of *Salmonella* isolates were resistant to chloramphenicol, and 87.95% were resistant to sulfamethoxazole/trimethoprim. The third generation of cephalosporins, including ceftriaxone, cefixime, and ciprofloxacin, were the most effective antibiotics against *Salmonella* isolates. Resistance of *Shigella* isolates to nalidixic acid in comparison to other antibiotics was high. The results of susceptibility testing of prevalent enteric pathogens in response to different antibiotics are summarized in Table 3.

Table 1. Sex, Inpatient, and Outpatient Distribution

Microorganism	Male	Female	Outpatients	Inpatients
<i>Salmonella</i> spp.	50	33	52	31
<i>Shigella</i> spp.	38	41	42	37
EPEC	89	57	91	55
<i>A. hydrophila</i>	2	0	1	1

Table 2. Distribution of Enteric Pathogens According to Age

Age Group	EPEC	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>A. hydrophila</i>
≤ 10	146	65	57	0
10 - 19	0	2	9	0
20 - 29	0	5	5	0
30 - 39	0	6	5	0
40 - 49	0	4	1	2
50 - 59	0	1	1	0
60 - 69	0	0	1	0
70 - 79	0	0	0	0
80 - 89	0	0	0	0

Table 3. Drug Resistance Patterns of Common Enteric Pathogens Isolated From Diarrhea Patients^a

Antibiotics	<i>Shigella</i> spp. (n = 79)		<i>Salmonella</i> spp. (n = 83)		EPEC (n = 146)	
	S	R	S	R	S	R
Chloramphenicol	65 (82.27)	14 (17.72)	45 (54.21)	38 (45.78)	126 (86.30)	20 (13.69)
Nalidixic acid	39 (49.36)	40 (50.63)	73 (87.95)	10 (12.04)	59 (40.41)	87 (59.58)
SXT	62 (78.48)	17 (21.51)	12 (14.45)	71 (85.54)	52 (35.61)	94 (64.38)
Ceftriaxone	72 (91.13)	5 (6.32)	81 (97.59)	2 (2.40)	65 (06)	51 (34.93)
Ciprofloxacin	70 (88.60)	2 (2.53)	81 (97.59)	2 (2.40)	NT	NT
Cefixime	73 (92.40)	6 (7.59)	80 (96.38)	3 (3.61)	30 (20.54)	116 (79.45)
Ampicillin	64 (81.01)	15 (18.98)	36 (43.37)	47 (56.62)	21 (14.38)	125 (85.61)

Abbreviations: R, resistant; SXT, sulfamethoxazole/trimethoprim; S, susceptible.

^aValues are expressed as No. (%).

5. Discussion

In the present study, enteropathogenic bacteria from 320 stool specimens were isolated from patients suffering from diarrheal diseases. Enteric pathogenic bacteria, including EPEC, *Salmonella* spp. and *Shigella* spp., are the most common bacterial agents of infectious diarrhea in Iran and other developing countries (9). EPEC is among the most common pathogens infecting children under five years of age in developing countries, but the prevalence may vary depending on differences in the study population, age group, diagnostic criteria, and diagnostic tools used (13-15). Various studies carried out in Iran and abroad have shown a significant association between EPEC and children's diarrhea compared to the controls (16). In this study, EPEC, with 146 isolates, was the predominant organism responsible for diarrhea. The second most frequently isolated organism was *Salmonella* spp. *Salmonella* serogroup D, including *Salmonella enteritidis*, was the predominant *Salmonella* spp. responsible for *Salmonella*-associated diarrhea. The findings here are in agreement with other studies that have been conducted in other parts of the world (17, 18).

Diarrhea caused by *Shigella* is a prevalent infectious disease, especially in developing countries. Among the *Shigella* serogroups, *S. sonnei* is a major cause of shigellosis in developed countries, while in developing countries,

S. flexneri and *S. dysenteriae* are the predominant serogroups. Recent studies in Iran have shown that *S. sonnei* is becoming the predominant serogroup, which is aligned with the findings in this study (19, 20).

The emerging high rates of resistance to multiple antimicrobial agents among enteric pathogens is an important public health challenge in Iran. Recent studies have shown a high rate of drug resistance among enteric pathogens in the country (4). In this study, many isolates of EPEC were resistant to commonly used antibiotics. Resistance of *Salmonella* and *Shigella* was also prevalent in this study, and therefore proper measures should be taken by the health authorities to combat the seriousness of this public health issue.

In this study, two strains of *Aeromonas hydrophila* were isolated from patients suffering from diarrhea. *A. hydrophila* is a causative agent of a number of human infections. It has been recognized as a food and water borne pathogen. In spite of a number of virulence factors produced by the *A. hydrophila* species, its association with diarrheal diseases has not been clearly determined (21); for instance, in a study which was carried out in western Iran, *A. hydrophila* was isolated in patients with diarrhea and also asymptomatic healthy persons (22).

The present study addresses some important issues about diarrheal infections and their most common bacterial etiologic agents at Milad hospital in Tehran. The results have shown that enteric bacterial infections caused by EPEC, *Salmonella*, and *Shigella* are prevalent in Milad hospital. Considering the threat of emerging antimicrobial resistance among these enteric bacterial pathogens and antimicrobial susceptibility patterns, the establishment of a more effective surveillance system is necessary.

Acknowledgments

We thank the personnel of the microbiology laboratory of Milad hospital in Tehran, Iran for their technical assistance.

References

- Ranjbar R, Dallal MMS, Pourshafie MR, Aslani MM, Majdzadeh R. Serogroup distribution of shigella in Tehran. *Iran J Public Health*. 2004;**33**(3):32-5.
- Samal SK, Khuntia HK, Nanda PK, Satapathy CS, Nayak SR, Sarangi AK, et al. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. *Jpn J Infect Dis*. 2008;**61**(5):350-5. [PubMed: 18806340]
- Jafari F, Shokrzadeh L, Hamidian M, Salmanzadeh-Ahrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. *Jpn J Infect Dis*. 2008;**61**(4):269-73. [PubMed: 18653967]
- Esmaili Dooki MR, Rajabnia R, Barari Sawadkahi R, Mosaiebnia Gatabi Z, Poornasrollah M, Mirzapour M. Bacterial enteropathogens and antimicrobial susceptibility in children with acute diarrhea in Babol, Iran. *Caspian J Intern Med*. 2014;**5**(1):30-4. [PubMed: 24490011]
- Rahouma A, Klena JD, Krema Z, Abobker AA, Treesh K, Franka E, et al. Enteric pathogens associated with childhood diarrhea in Tripoli-Libya. *Am J Trop Med Hyg*. 2011;**84**(6):886-91. doi: 10.4269/ajtmh.2011.11-0116. [PubMed: 21633024]
- Dallal MM, Khorramzadeh MR, MoezArdalan K. Occurrence of enteropathogenic bacteria in children under 5 years with diarrhoea in south Tehran. *East Mediterr Health J*. 2006;**12**(6):792-7. [PubMed: 17333824]
- Kansakar P, Baral P, Malla S, Ghimire GR. Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during 2002-2004. *J Infect Dev Ctries*. 2011;**5**(3):163-8. [PubMed: 21444984]
- Okon KO. Enteropathogens Associated with Childhood Diarrheal Cases seen at a tertiary hospital in Nguru, Yobe State Nigeria. *British Microbiol Res J*. 2014;**4**(5):583-91.
- Bonkougou IJ, Haukka K, Osterblad M, Hakanen AJ, Traore AS, Barro N, et al. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. *BMC Pediatr*. 2013;**13**:36. doi: 10.1186/1471-2431-13-36. [PubMed: 23506294]
- Delost MD. *Introduction to diagnostic microbiology: a text and workbook*. 1st ed. Maryland: Mosby; 1997.
- Forbes BA, Sahm DF, Weissfeld AS, Bailey WR. *Bailey & Scott's diagnostic microbiology*. 12th ed. USA: Mosby; 2007.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 21th Informational Supplement. Wayne: Clinical and Laboratory Standards Institute. 2011.
- Petri Jr WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest*. 2008;**118**(4):1277-90. doi: 10.1172/JCI34005. [PubMed: 18382740]
- Haghi F, Zeighami H, Hajjahmadi F, Khoshvaght H, Bayat M. Frequency and antimicrobial resistance of diarrhoeagenic *Escherichia coli* from young children in Iran. *J Med Microbiol*. 2014;**63**(Pt 3):427-32. doi: 10.1099/jmm.0.064600-0. [PubMed: 24281909]
- Hart CA, Batt RM, Saunders JR. Diarrhoea caused by *Escherichia coli*. *Ann Trop Paediatr*. 1993;**13**(2):121-31. [PubMed: 7687108]
- Jafari A, Aslani MM, Bouzari S. *Escherichia coli*: a brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iran J Microbiol*. 2012;**4**(3):102-17. [PubMed: 23066484]
- Ranjbar R, Giammanco GM, Farshad S, Owlia P, Aleo A, Mammia C. Serotypes, antibiotic resistance, and class 1 integrons in *Salmonella* isolates from pediatric cases of enteritis in Tehran, Iran. *Foodborne Pathog Dis*. 2011;**8**(4):547-53. doi: 10.1089/fpd.2010.0736. [PubMed: 21204690]
- Hamidian M, Tajbakhsh M, Tohidpour A, Rahbar M, Zali MR, Walther-Rasmussen J. Detection of novel gyrA mutations in nalidixic acid-resistant isolates of *Salmonella enterica* from patients with diarrhoea. *Int J Antimicrob Agents*. 2011;**37**(4):360-4. doi: 10.1016/j.ijantimicag.2010.12.013. [PubMed: 21371866]
- Tajbakhsh M, Garcia Migura L, Rahbar M, Svendsen CA, Mohamadzadeh M, Zali MR, et al. Antimicrobial-resistant *Shigella* infections from Iran: an overlooked problem? *J Antimicrob Chemother*. 2012;**67**(5):1128-33. doi: 10.1093/jac/dks023. [PubMed: 22345385]
- Rahbar M, Deldari M, Hajia M. Changing prevalence and antibiotic susceptibility patterns of different *Shigella* species in Tehran, Iran. *The Internet J Microbiol*. 2007;**3**(2).
- Ghaghesh KS, Ahmed SF, El-Khalek RA, Al-Gendy A, Klena J. *Aeromonas*-associated infections in developing countries. *The Journal of Infection in Developing Countries*. 2008;**2**(02):081-98.
- Aslani MM, Alikhani MY. The role of *Aeromonas hydrophila* in diarrhea. *Iran J Public Health*. 2004;**33**(3):54-9.