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Prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella* spp. among children with gastroenteritis in an Iranian referral hospital

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ABSTRACT

Background: Gastroenteritis is one of the leading cause of illnesses through the world, especially in developing countries.*Salmonella* and *Shigella* infections are considered as the main public health problems in children. The aim of this study was to detect the prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella* spp. among children with gastroenteritis in an Iranian referral hospital.
Methods: During April 2013 to April 2014, all medical records of children with gastroenteritis admitted to a pediatric medical center were evaluated. Positive stool cultures of children were evaluated and frequency of *Salmonella* and *Shigella* spp. and their antimicrobial susceptibility were detected.
Results: In this study, 676 patients with the mean age of 24.94 months were enrolled. Eighty-eight (42%) *Salmonella* spp., 85 (40%) *Shigella* spp., 33 (16%) *E. coli* and 5(2%) *candida albicans* were isolated from 211 positive stool cultures. Among 85 *Shigella* spp. isolates, *S. sonnei, S. flexneri* and other *Shigella* spp. were isolated from 39 (46%) isolates, 36(42%) and 10(12%), respectively. Among 88 isolated *Salmonella* spp., 36 (41%) isolates were *Salmonella* Serogroup D, 26 (30%) were *Salmonella* Serogroup B, 20 (23%) isolates were *Salmonella* Serogroup C and 6 (7%) were other *Salmonella* spp. isolates.
Thirty-eight percent of *Salmonella* serogroup B were resistant to nalidixic acid, while higher frequency of nalidixic acid resistant was found in *Salmonella* serogroup C and *Salmonella* serogroup D. The higher frequency of ampicillin resistant was found in *Shigella* spp. than *Salmonella* spp. High frequency of cefotaxime resistant was seen in *S. sonei* and *S. flexneri* (77% and 56%, respectively), whereas more than 90% of *Salmonella* serogroup *B, C* and *D* were susceptible to this antibiotic.
Conclusion: In conclusion, *Shigella* and *Salmonella* serogroups can be considered as important etiological agents of acute diarrhea in children. Since the prevalence of antibiotic resistance is increasing in recent years in Iran, further studies on the prevalence, antimicrobial susceptibility pattern and mechanisms of antibiotic resistance in these species is highly recommended.

1. Introduction

Acute gastroenteritis is defined as severe infection of the gastrointestinal tract and is characterized by inflammation in the digestive system, which leads to a combination of diarrhea, dehydration, vomiting, nausea, fever and abdominal pain. Diarrhea is determined as having loose or watery stools, three or more times in 24 h [1].Gastroenteritis is one of the leading causes of illnesses in children throughout the world, especially in developing countries, where a measure of 2.5 million deaths occurs each year in children younger than five years old [2].

Viruses, bacteria and parasites are three major causes of childhood gastroenteritis, which viral agents account for 70 to 80%;

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bacterial pathogens are included in 10–20% and parasites such as *Giardia intestinalis* and *Cryptosporidium* are identified in acute infectious gastroenteritis [3].

Some of the most important bacteria involve in gastroenteritis are *Escherichia coli* O157:H7, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium difficile* [4,5].*Salmonella* and *Shigella* infections are main public health problems around the world, particularly in children. These infections are customary in developing countries where living standards are very low, with poor sanitation and lack of clean water supply [6].

Four species of *Shigella* namely, *Shigella dysenteriae*, *S. flexneri, S. boydii,* and *S. sonnei* are responsible for causing shigellosis in humans. The most prevalent *Shigella* spp. in developing countries is *S. sonnei* and *S. flexneri*. *S. flexneri* had been recognized as the chief reason of shigellosis in Tehran, capital city of Iran until 2003, while after that it was replaced by *S. sonnei* [7]. *Salmonella* infections have also been regarded as an important public health and caused gastroenteritis among children [8,9].

Antibiotic resistance has become a major concern to control diarrhea in children. Therefore, evaluation of the prevalence and resistance patterns of *Shigella* and *Salmonella* spp. in patients with gastroenteritis is necessary [10]. The aim of this study was to detect prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella* spp. among children with gastroenteritis in an Iranian referral hospital.

2. Materials and methods

2.1. Specimen collection

This retrospective study was conducted during April 2013 to April 2014 at Children's Medical Center, an Iranian referral Hospital. A total of 676 stool specimens were taken from 1 month to 13 years children (414 males and 262 females) who were admitted with acute gastroenteritis diseases. The epidemiological information such as age, sex, symptoms (fever, bloody diarrhea) was recorded. Fecal specimen of each patient was collected in a sterile bottle.Samples were transferred to the microbiology lab in Cary Blair transport media.

2.2. Isolation and identification of Salmonella and Shigella spp

Specimens were cultured on xylose-lysine deoxycholate (XLD) agar (Merck, Germany), Eosin Methylene Blue (EMB) agar (Merck, Germany) and Salmonella Shigella (S.S) agar (Merck, Germany), and then incubated at 37 °C for 18–24 h. Small red colonies on XLD and colourless colonies sometimes with black spot were selected, and characterized biochemically using Kligler Iron Agar (KIA), urease tests, motility and IMViC test [11].

2.3. Salmonella serogrouping

Salmonella isolates were serogrouped by slide agglutination tests, according to the manufacturer's guidelines (Baharafshan, Iran). A, B, C and D group specific antisera (Salmonella “O” Antiserum Poly Serogroups A, B, C and D) were used to determine the serogroups.

2.4. Identification of the Shigella spp

Shigella spp. were detected by slide agglutination tests (Baharafshan, Iran). *Shigella* antiserum Poly Group A, B, C and D were used for this test.

2.5. Antimicrobial susceptibility testing

Antibiotic resistance of the isolates against ampicillin, cefotaxime, nalidixic acid and trimethoprim/sulfamethoxazole (Mast, UK) was determined using disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [12]. *E. coli* ATCC 25922 was used as the quality assurance of the test.

2.6. Statistical analysis

Statistical analyses were performed through description evaluation. Microsoft Excel for Windows 2010 was used as a database and SPSS version 18 (SPSS Inc, Chicago, IL, USA) for analysis.

3. Results

Stool specimens from 676 patients were examined. Eighty-eight (42%) *Salmonella* spp., 85 (40%) *Shigella* spp., 33 (16%) *E. coli* and 5(2%) *candida albicans* were isolated from 211 positive stool cultures. *S. sonnei* comprised 46% of the 85 *Shigella* strains (N = 39) isolated, with the remainder being *S. flexneri* (N = 36, 42%) and 12% (N = 10) belonged to other *Shigella* spp. isolates. *Salmonella* spp. were identified as serogroup D (N = 36, 41%), serogroup B (N = 26, 30%), serogroup C (N = 20, 23%) and other *Salmonella* spp. (N = 6, 7%).

The distributions of *Shigella* and *Salmonella* spp. in different seasons are shown in Table 1. Although *Shigella* strains were isolated during the year, most of them were recovered in the autumn 47% *S. flexneri* (N = 17), 61.5% *S. sonei* (N = 24) and, 80% other *Shigella* spp. (N = 8). The high frequency of infection with *Salmonella* serogroup B occurred during summer (N = 8, 31%) and winter (N = 8, 31%). *Salmonella* serogroup C infection was more common in spring (N = 7, 35%). *Salmonella* D infection was more prevalent in summer (N = 11, 31%) and other *Salmonella* spp. was more recovered during autumn (N = 3, 50%) (Table 1).

Table 1
The frequency of *Salmonella* and *Shigella* spp. in both sexes and seasons.

Organism	Male		Female		Spring		Summer		Autumn		Winter		Total
	N	%	N	%	N	%	N	%	N	%	N	%	N
Salmonella serogroup *B*	13	50	13	50	5	19	8	31	5	19	8	31	26
Salmonella serogroup C	16	80	4	20	7	35	3	15	5	25	5	25	20
Salmonella serogroup D	23	64	13	36	7	19	11	31	8	22	10	28	36
Shigella flexneri	16	44	20	56	5	14	5	14	17	47	9	25	36
Shigella sonei	21	54	18	46	1	3	10	26	24	61.5	4	10	39
Other *Shigella* spp.*	6	60	4	40	1	10	1	10	8	80	0	0	10
Other *Salmonella* spp.*	4	67	2	33	1	17	2	34	3	50	0	0	6

*Other *Shigella*: *Shigella dysenteriae* and *Shigella boydii*; Other *Salmonella* spp: *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella Paratyphi* B.