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Prevalence and Antibiotic Susceptibility of *Shigella* Species Isolated From Pediatric Patients in Tehran

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Abstract

Background: Dysentery accounts for a significant proportion of morbidity and mortality cases, especially in children with diarrhea in developing countries. Shigellosis remains a public health problem in Iran.

Objectives: The objectives of this study were to investigate the presence and the frequency of *Shigella* spp. among children younger than 10 years with diarrhea and to determine the antimicrobial resistance patterns of these isolates in Tehran, Iran.

Patients and Methods: This cross-sectional study was conducted in two pediatric hospitals in Tehran, from October, 2013 to October, 2014. Nine hundred and thirty-eight (n = 938) stool samples were collected (one stool per patient) from children less than 10 years of age. The stools were primarily cultured on MacConkey agar and xylose lysine deoxycholate (XLD). (Selenite-F broth is also used as an enrichment medium for the isolation of *Shigella* spp.) All of the isolates were confirmed as *Shigella* species by biochemical and serological tests. Molecular confirmation of *Shigella* isolates was determined by polymerase chain reaction (PCR) using specific primers for the *ipaH* gene. The antibiotic susceptibility of the *Shigella* isolates was tested using the Kirby-Bauer disc-diffusion method.

Results: Amongst 938 stool samples, 36 were *Shigella* spp. *S. sonnei* (61.1%, n = 22), which was the most common, followed by *S. flexneri* (27.8%, n = 10), *S. boydii* (8.3%, n = 3), and *S. dysenteriae* (2.8%, n = 1). Furthermore, most *Shigella* isolates showed resistance to amoxicillin (83.3%), tetracycline (61.1%), and co-trimoxazole (83.3%). No resistance was found against ciprofloxacin and imipenem. This study showed that *S. sonnei* is currently the predominant species in Tehran, Iran.

Conclusions: Because resistance to antibiotics changes constantly, *Shigella* strains should be under surveillance in order to monitor local susceptibility and subsequently formulate policies for the use of antimicrobial drugs.

Keywords: Shigellosis, Antimicrobial Resistance, Pediatrics, Iran

1. Background

Shigellosis still accounts for a significant proportion of morbidity and mortality cases, especially in children with diarrhea in developing countries. The global burden of shigellosis has been estimated at about 165 million cases annually, of which 163 million are in the developing world. It has been estimated that over one million deaths occur yearly due to shigellosis (1). Shigellosis is primarily a disease of poor, crowded communities that do not have adequate sanitation or clean water. The genus *Shigella* comprises four species: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Of these species, shigellosis is predominantly caused by *S. flexneri* in developing countries, especially in Asia, whereas *S. sonnei* is predominant in industrialized countries (2-4).

Effective antibiotic treatment against shigellosis alleviates dysenteric syndrome, fever, and abdominal cramps, reduces the duration of pathogen fecal shedding, interrupts further transmission of disease, and reduces the risk of potentially lethal complications. The Centers for Disease Control and Prevention (CDC) have recommended that sensitivity testing be conducted to guide the selection of appropriate antimicrobial therapy for Shigellosis. Because antibiotic resistance patterns have varied widely at different times and in different places, supervising resistance patterns is necessary to guide the selection of proper empirical treatment. However, *Shigella* species have become progressively resistant to most widely-used antibiotics such as ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole. Furthermore, the emergence of resistance to third-generation cephalosporins in *Shigella* is a matter of great concern, mainly in developing countries (5, 6).

The problem of resistance to antimicrobial agents is not

unique to Iran, where easy and unrestricted access to antibiotics diminishes the value of such agents for those patients who actually need them (7). There are several reports showing that the prevalence of resistance against different antibiotics is growing among *Shigella* strains in Iran.

2. Objectives

The objectives of this study were to investigate the presence and the frequency of *Shigella* spp. among children younger than 10 years with acute diarrhea and to determine the antimicrobial resistance patterns of these isolates in Tehran, Iran.

3. Patients and Methods

This cross-sectional study was conducted in two pediatric hospitals in Tehran, from October, 2013 to October, 2014. Nine hundred and thirty-eight (n = 938) stool samples were collected (one stool per patient) from children less than 10 years of age. These children suffered from acute diarrhea and had experienced more than three episodes of watery, loose, or bloody stools per day. The mean age of patients with acute diarrhea was 4.1 ± 3.4 years. After gross and microscopic examination, the stool specimens were primarily inoculated on MacConkey agar and xylose lysine deoxycholate (XLD). Selenite-F broth was also used as an enrichment medium for the isolation of *Shigella* spp. Colonies morphologically resembling *Shigella* were further evaluated with conventional biochemical tests using triple sugar iron (TSI), urea agar slant, methyl red (MR), sulphur indole motility (SIM) media, and Simmon's citrate agar slant.

Molecular confirmation of *Shigella* isolates was determined by polymerase chain reaction (PCR) using specific primers for the ipaH gene. PCR was performed in 25 μl volumes containing 1× PCR buffer (50 mmol/L KCL, 10 mmol/L Tris, pH 9), 2.5 mmol/L MgCl2, 0.2 mmol/L of each primer with 0.5 U TaqDNA polymerase (CinnaGen Co., Iran), and 3 μl of crude DNA extract. The cycling conditions for the PCR reactions were 93°C for five minutes, followed by 32 cycles of 93°C for 30 seconds, 58°C for 30 seconds, and 72°C for one minute. A final extension of 72°C for six minutes was also employed. The PCR products were run on 1% agarose gels, stained with ethidium bromide, and visualized under UV transillumination. Serotyping of the isolates was also performed by slide agglutination using *Shigella* specific antisera (Denka Seiken, Tokyo, Japan).

Antibiotic susceptibility of the *Shigella* isolates was tested using the Kirby-Bauer disc-diffusion method on Muller-Hinton agar plates following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for the following antibiotics (8): amoxicillin (10 mg), cefotaxime (30 mg), ceftazidime (30 mg), ciprofloxacin (5 mg), co-trimoxazole (25 mg), imipenem (10 mg), and tetracycline (30 mg) (Hi-Media, India). To verify that susceptibility test results were accurate, the *E. coli* ATCC 25922 strain was used as a quality control according to CLSI guidelines. The significance of differences between percentages of antimicrobial resistance of *Shigella* species was determined by the chi-square test or the Fisher's exact test. P-value < 0.05 was considered statistically significant.

All ethical issues were considered. Life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of all research subjects were protected in this study.

4. Results

Out of 938 stool samples, 36 (3.8%) were positive for *Shigella* species. As for other bacterial pathogens, *Salmonella* spp. were isolated from 10 (1%) specimens. Enteropathogenic *E. coli* (EPEC) was also recovered from 40 (4.26%) patients. We defined the EPEC strains by classical EPEC serogroups.

The prevalence of *Shigella* among patients was 3.8%. The distribution of *Shigella* species was as follows: 61.1% (n = 22) *S. sonnei*, 27.8% (n = 10) *S. flexneri*, 8.3% (n = 3) *S. boydii*, and 2.8% (n = 1) *S. dysenteriae*. The patients comprised 20 (55.6%) males and 16 (44.4%) females. Twenty *Shigella* strains (55.6%) were isolated from patients between two and five years of age. However, 30.5% (n = 11) of the total positive samples were obtained from children aged five to 10 years (Table 1). The most frequent antibiotic resistance was observed against amoxicillin and co-trimoxazole, with resistance rates of 86.1% and 83.3%., respectively. None of the isolates were resistant to ciprofloxacin or imipenem. Antimicrobial resistance varied by species (Table 2); most of the *S. flexneri* isolates were resistant to amoxicillin (90%), co-trimoxazole (90%), and tetracycline (70%), whereas resistance to these antibiotics was much less common among *S. sonnei* isolates (81.8%, 81.8%, and 59%, respectively). In other words, *S. flexneri* was more resistant to antibiotics than *S. sonnei*. However, this difference was not significant (P value > 0.05). Fever (94.4%, 34/36), nausea (86.1%, 31/36), tenesmus (97.2%, 35/36), and convulsions (25%, 9/36) were the most common clinical symptoms in patients suffering from shigellosis.

Table 1. Distribution of *Shigella* Species by Age Groups[a]

Age Group, y	*S. flexneri*	*S. sonnei*	*S. boydii*	*S. dysenteriae*	Total
< 2	1 (10)	3 (13.6)	1 (33.3)	0	5 (13.9)
2 - 5	5 (50)	13 (59.1)	1 (33.3)	1 (100)	20 (55.5)
5 - 10	4 (40)	6 (27.3)	1 (33.3)	0	11 (30.5)
Total	10 (100)	22 (100)	3 (100)	1 (100)	36 (100)

[a]Data are presented as No. (%).

Table 2. Prevalence of Antibiotic Resistance Among *Shigella* species

Species	Antibiotic					Total Number of Strains, No.
	Resistant Strains, No. (%)					
	Amoxicillin	Ceftazidime	Cefotaxime	Co-trimoxazole	Tetracycline	
S. flexneri	9 (90)	3 (30)	3 (30)	9 (90)	7 (70)	10
S. sonnei	18 (81.8)	5 (22.7)	4 (18.2)	18 (81.8)	13 (59)	22
S. boydii	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	1 (33.3)	3
S. dysenteriae	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1
Total	30 (83.3)	11 (30.5)	10 (27.8)	30 (83.3)	22 (61.1)	36

5. Discussion

In endemic regions of developing countries, shigellosis is predominantly a pediatric disease, with the urban poor being affected the most (9). In this study, the prevalence of *Shigella* spp. was 3.8%, which was lower compared with reports of other similar studies in southern and northern parts of Iran (9, 10). This may be due to the socioeconomic development of these regions compared to Tehran. However, the incidence rate of shigellosis in other developing countries such as India (5%), Ghana (5%), and Cameroon (4.5%) is still higher than those in our findings (11-13). The highest prevalence of shigellosis (55.6%) was seen in patients between two and five years of age. This finding was in agreement with the results of other studies (14-16).

The distribution of *Shigella* species varies geographically both within countries and between countries. Shigellosis is predominantly caused by *S. sonnei* in industrialized countries, whereas *S. flexneri* prevails in the developing world (17, 18). In this study, *S. sonnei* (61.1%, n = 22) was the most common, followed by *S. flexneri* (27.8%, n=10), *S. boydii* (8.3%, n = 3), and *S. dysenteriae* (2.8%, n = 1). This finding differs from those of studies conducted in other developing countries, where *S. flexneri* was the most frequently isolated species (10, 11, 13). Previous studies from Tehran and Shiraz showed similar results (18, 19). However, the predominant species in other regions of Iran differ from those identified in our study (9, 12, 14). For instance, Jomezadeh et al. recently reported a higher prevalence of *S. flexneri* among hospitalized children with diarrhea in Abadan (9). This may suggest the possible replacement of *S. flexneri* by *S. sonnei* in some areas of Iran as the standard of living improved, as inferred from observations obtained from developed countries (17, 18).

Antimicrobial resistance in human pathogens has become a major public health problem. Because shigellosis is highly contagious, awareness of the prevalence of the disease and the antimicrobial susceptibility of the strains is crucial to ensuring proper clinical treatment and patient management. *Shigella* spp. still accounts for a significant proportion of bacillary dysentery in many developing countries (16). In this study, most of the *Shigella* isolates were resistant to ampicillin, tetracycline, and co-trimoxazole, which is in accordance with previous findings from our country (9, 17-19). These results are also in agreement with those of other studies from India (20), Chile (21), and Nepal (22). Co-trimoxazole is a common drug used as an empirical therapy in treatment of diarrheal diseases. The extensive use of this drug has led to the emergence of resistant *Shigella* strains. As for ampicillin and tetracycline, these antibiotics are inexpensive, broad-spectrum, and are used widely for prophylaxis and treating bacterial infections (6). Some studies have showed different resistance patterns by species. In our survey, *S. sonnei* had a higher rate of susceptibility than *S. flexneri* for most of the antibiotics. Overall, *S. flexneri* was more frequently resistant to most of the antibiotics than *S. sonnei*. One possible explanation is that infections due to *S. sonnei* are milder than infections due to the other *S. flexneri*, making exposure to selective pressure from antibiotics less likely (15, 23, 24). Fortunately, no resistance was found against ciprofloxacin and imipenem in our study. This finding is similar to results of other studies from Iran, in which all of the *Shigella* isolates showed susceptibility to these antibiotics (6, 18, 19). The World Health Organization (WHO) currently recommends ciprofloxacin for all patients with bloody diarrhea, irrespective of age, with ceftriaxone as an alternative agent in adults and children (25).

In conclusion, this study showed that *S. sonnei* is currently the predominant species in Tehran, Iran. The antimicrobial resistance patterns suggest widespread resistance of *Shigella* to amoxicillin, co-tromoxazole, and tetracycline. Because resistance to antibiotics changes constantly, these strains should be under surveillance in order to monitor local susceptibility and subsequently formulate policies for the use of antimicrobial drugs.

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Footnote

Authors' Contribution: Amir Talebreza and Mojtaba Memariani designed the research. Mojtaba Memariani

has written the manuscript. Parvaneh Eghbali Shamsabad and Hamed Memariani performed the microbiological and molecular studies. Mohammad Hasan Shirazi and Maryam Bakhtiari advised the research.

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