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Investigating the Prevalence of *Shigella* Species and Their Antibiotic Resistance Pattern in Children With Acute Diarrhea Referred to Selected Hospitals in Tehran, Iran

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Abstract

Introduction: Shigellosis is a major health problem, especially in developing countries and in children under 5 years of age. The prevalence of *Shigella* species in a region can be considered as an indicator for hygiene level of that region. Due to the lack of an efficient vaccine, antibiotic therapy is the main strategy to combat the disease. In this study, the prevalence of the *Shigella* species and their antibiotic resistant pattern has been investigated.

Materials and Methods: A total of 300 diarrheal stool samples were collected from 4 different hospitals in Tehran during a period of 6 months June to November 2016. Bacterial identification and species discrimination was performed using biochemical and serotyping tests. Antibiotic resistance patterns of isolates were obtained using Bauer-Kirby method.

Results: 8.7% of all diarrheal cases were caused by *Shigella* species (5% by *Shigella sonnei* and 3.7% by *Shigella flexneri*). Antibiogram test revealed that the isolates were more sensitive or intermediate to ciprofloxacin (92.3%), while most of the isolates were resistant to tetracycline.

Conclusions: The prevalence of *Shigella* species has changed in Tehran. Since antibiotics are the treatment of choice to combat these pathogens, also, because of the emergence of the antibiotic resistance *Shigella* strains, there is a need for regularly updated regional antibiotic sensitivity patterns of the pathogen to guide therapy.

Keywords: *Shigella* species, Antibiotic Sensitivity Pattern, Prevalence, Diarrhea

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Introduction

Caused by *Shigella* species, shigellosis is an infectious disease which is endemic in many developing countries.[1] Annually, there are about 80 million cases of bloody diarrhea and 700 000 deaths due to this infection.[2] Children under 5 years old, elderly, and immunocompromised people are at a higher risk to this infection and develop more severe symptoms.[3,4] The majority of the disease cases are seen in developing countries and it is due to the poor hygiene standards, poor water quality, etc.[5]

Four different species of *Shigella* genus can develop the disease, *Shigella sonnei, Shigella dysenteriae, Shigella flexneri*, and *Shigella boydii*. The incidence of the species is so different in various countries: while *S. flexneri* is the prevalence serogroup in developing countries, the most cases of the infection in developed countries are caused by *S. sonnei*.[6,7] Since it is transmitted via fecal-oral rout in a low dose (1-500 organisms) and also via direct spread by person to person contact, the control of the infection is a great challenge.[8,9]

Shigella infection can cause different symptoms from a mild self-limiting diarrhea to severe dysentery associated with blood and mucus excretion, high fever, and cramps.[10,11] Since there is no approved vaccine, despite all attempts,[12-14] antibiotic therapy is the main strategy to combat the disease. Appropriate antibiotic therapy shortens the disease symptoms and prevents the life-threatening effects, also, it decreases the bacterial shedding in feces and, therefore, decreases the bacterial spread.[15] However, during recent decades, the indiscriminate use of antibiotics has led to the resistance of *Shigella* spp. to common antibiotics.[16,17] Due to the self-limiting nature of the

infection, the high risk of the emergence of drug resistance strains, and the costs of drugs, some physicians avoid the antibiotic administration.[18] However, there is a convincingly logic in favor of empirical treatment of shigellosis in suspected patients. If the disease won't be treated, a child may be quite sick for several weeks or more and chronic diarrhea may be created. During prolonged illness, the risks for developing or worsening the disease, malnutrition, permanent disposal of microorganisms, and subsequent infection of family members will be increased. However, the resistance of the bacteria to common antibiotics has limited the use of these antimicrobial agents. Although it was thought that *Shigella* is sensitive to ceftriaxone, azithromycin, and quinolones, there are increasing number of reports which show the increase in multiple drug-resistancy of the bacterial strains in different parts of the world. In Iran, more than 90% of *Shigella* species are resistant to one or more antibiotics and about 87% of them are resistant to more than one antibiotic.[19] Since shigellosis is the worldwide health problem and it is endemic in some regions of Iran,[20] and also because of the importance of the determination of antibiotic resistant pattern of the bacteria in the selection of the proper treatment regimen, in the present study, the prevalence of *Shigella* species and also the antibiotic resistance pattern of them were investigated.

Materials and Methods

This is a hospital-based descriptive-analytic study based on stool samples of patients with diarrhea and fever diagnosed with colitis in four educational hospitals.

Sample Collection

Three hundred diarrhea samples were collected from four hospitals in Tehran, including Sina, Shariati, Imam Khomeini, and children medical center hospitals in a time period from June to November 2016. Sampling performed before antibiotic therapy and stool specimens were transported in Cary-Blair transport medium and carried to the microbiology laboratory for future analysis.

Bacterial Culture and Identification

Samples were firstly cultured on Xylose Lysine Deoxycholate (XLD) agar (Merck, Germany), Hekton enteric agar (Merck, Germany), and S-S agar (Merck, Germany) for 24 hours at 37°C. Then, they were transferred to Muller-Hinton agar (Sigma, USA) for biochemical tests. Identification was performed by different biochemical tests, including Triple Sugar Iron (TSI) (Merck, Germany), lysine decarboxylase, SIM (Merck, Germany), MR-VP (methyl red, Voges–Proskauer), citrate utilization, and urea hydrolysis tests.

Species Discrimination

For discrimination of different *Shigella* species, ornithine decarboxylase, ONPG (o-nitrophenyle –β-D-galactosidase), manitole fermentation, and finally, serotyping tests was performed. Serotyping was done via the slide agglutination method using antisera (Bahar Afshan, Iran) against each species.

Antibiotic Resistance Investigation

Antibiotic resistance test was carried out using Bauer-Kirby method according to the US Clinical and Laboratory Standards Institute (CLSI). To do this, firstly, a bacterial suspension equivalent to 0.5 McFarland Standard was prepared for all isolates. Using a sterile swab, the suspension was spread on a Muller-Hinton agar medium. After 15 minutes, antibiotic discs were placed on the media. The studied antibiotics were: ampicillin (AMP; 10 mg), tetracycline (TCN; 30 mg), ciprofloxacin (CIP; 5 mg), nalidixic acid (NAL; 30 mg), gentamycin (GEN; 25 mg), sulfamethoxazole/trimethoprim (SXT; 25 mg), ceftriaxone (CAX, 30 mg), ciprofoxacine (CIP; 30 mg), and chloramphenicol (CAM: 30 mg), all purchased from Mast Company, England.

Statistical Analysis

The data in each experiment was a representative of three independent experiments. Statistical analysis was carried out using the SPSS software (version 24.0).

Ethical Considerations

Ethical approval was obtained from the research ethics committee of selected hospitals.

Results

Bacterial Identification

Isolates which pose the following biochemical characteristics were considered *Shigella*: lactose negative, gas production negative, non-motile, MR positive, lysine decarboxylation negative, citrate negative, and finally, urea hydrolysis negative. According to these tests, out of 300 samples, 26 samples (8.7%) were detected as *Shigella*. For species discrimination, serological typing through specific antisera against isolates was performed. Based on serotyping, it was shown that out of 26 isolates, 15 isolates (57.7%) belonged to *S. sonnei*, and 11 isolates (42.3%) belonged to *S. flexneri* (Figure 1).

Antibiogram Test

As shown in Table 1, the result of Bauer-Kirby method demonstrated that most of *Shigella* isolates were resistant to tetracycline (88.46%), followed by ampicillin,

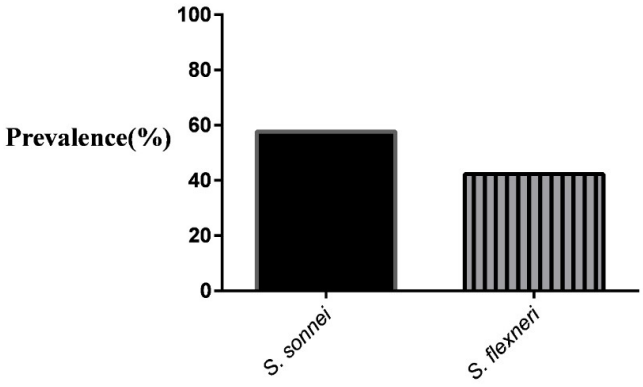

Figure 1. The Prevalence of *Shigella* Species Isolated From the Studied Samples.

Table 1. Antimicrobial Susceptibility of *Shigella* Strains Isolated From Children

Antimicrobial Agent	Susceptible No. %	Intermediate No. %	Resistant No. %
Ampicillin	9 (34.61)	1 (3.80)	16 (61.53)
Tetracycline	3 (11.53)	0 (0)	23 (88.46)
Co-trimoxazole	8 (30.76)	2 (7.69)	16 (61.53)
Ceftriaxone	21 (80.76)	0 (0)	5 (19.23)
Chloramphenicol	7 (36.92)	3 (11.53)	16 (61.53)
Gentamicin	19 (73.07)	3 (11.53)	4 (15.38)
Ciprofloxacin	20 (76.92)	4 (15.38)	2 (7.69)
Nalidixic acid	14 (53.84)	5 (19.23)	7 (26.92)

chloramphenicol, and Sulfamethoxazole/trimethoprim (61.53%), while 92.30% and 85% of the isolates were sensitive or intermediate to ciprofloxacin and gentamicin, respectively.

Discussion

Shigellosis is a worldwide health problem, especially in developing countries. It is estimated that the annual morbidity rate of the infection is 80 million, which most of cases are children under 5 years old.[2] Etiologically, *Shigella* species are the leading cause of acute diarrhea among hospitalized children (27% of all cases).[21] Different studies have shown that the prevalence of the diarrhea committed by *Shigella* is almost high in Iran. However, geographically the prevalence is so variable. For example, the bacterial diarrhea caused by *Shigella* in different cities has been as follow: Zanjan 19.6 %,[22] Ahvaz 14.1 %,[23] Kerman 31.6%,[12] Shahr-e-Kord 7.8%,[24] and Kashan 7.8%.[25] In Tehran, in a study by Afshari et al in summer and autumn of 2012, it was shown that 5.3% of diarrheal cases are caused by *Shigella*,[21] and Dolatshahi et al, in another study in a period of 10 months from April to January 2015, showed that this rate was 5.16%.[22] In the present study the prevalence of the *Shigella* in bacterial diarrhea cases was 8.7%, which the differences are not statistically significant. The prevalence of *Shigella* species pattern is different among developing and developed countries, while *S. sonnei* is the most common species in developed countries, the predominant *Shigella* species in developing countries is *S. flexneri*. The present study shows that the most prevalent *Shigella* strains in Tehran are belonged to *S. sonnei* and it is in agreement with Rahbar et al,[26] Talebreza et al,[27] Jafari et al,[28] Tajbakhsh et al,[29] Ranjbar et al,[30] Eftekhari et al,[31] and Farshad et al[32] reports. It is while in the not-too-distant past, *S. flexneri* was the most common species in Tehran[33,34] ($P<0.05$), which indicates the improvement of hygiene level in this city. Antibiotic therapy is the choice strategy for the treatment of patient with acute *Shigella* infection.[35] It can reduce the symptoms of disease, decrease the number of carriers, and prevent the spread of the infection. However, the irregular use of antibiotics has arisen antibiotic resistance of the bacteria and, therefore, the treatment of the infection has been complicated.[15,32] Beta lactams, quinolones, and macrolides are tree classes of antibiotics which are recommended by World Health Organization (WHO) to treat the disease. The second generation of fluoroquinolones (ciprofloxacin) is now recommended by the organization as the first choice for dysentery patients resistant to third-generation cephalosporins and nalidixic acid.[2] However, in recent years, resistance to quinolones has been reported and it is required to update the sensitivity pattern of the species.[36-38] In the present study, the highest resistance to antibiotics was observed for tetracycline, ampicillin, and chloramphenicol, while the most sensitivity was observed for gentamicin and ciprofloxacin. In a study conducted by Hossein-Pour et al, the highest antibiotic resistance to ampicillin was reported to be 89.6 percent and all cases were reported sensitive to the third generation cephalosporins and nalidixic acid, while the sensitivity to gentamicin, amikacin, and chloramphenicol was 89.8%, 82.5%, and 69%, respectively.[35] Also in a study by Jamshidi et al, the highest resistance was observed for ampicillin and the highest sensitivity was seen to ciprofloxacin.[22] In a study by Saadati et al., the prevalence of nalidixic acid resistant strains in Kerman was reported 9.7%.[18] Ayazi et al, in Qazvin[36] and Kurdi Daryan et al,[37] in Isfahan reported that the prevalence of Nalidixic Acid resistant *Shigella* were 2% and 4%, respectively. In a survey by Rahbar et al, it was observed that the most strains were resistant to ampicillin and trimethoprime/sulphamethoxazole (88.5% and 98%, respectively), whereas resistance to Chloramphenicol and Ciprofloxacin was observed to be 2.5% and 1% respectively.[26] The results of the present study revealed that the prevalence of nalidixic acid-resistant *Shigella* strains has increased to 26.82%. In agreement to some other studies, the results of the present study show that the *Shigella* strains have the most susceptibility to ciprofloxacin.[39-41] Various reasons can lead to the dissemination of *Shigella* MDR strains, including easy access to antibiotics and their irregular use.[42,43] Increasing *Shigella* resistance to antibiotics is a serious warning that should be prevented by implementing necessary measures.

Conclusions

Shigella's common species pattern has changed from *S. flexneri* to *S. sonnei* during the recent years in Tehran. The results of this study show that the antibiotic resistance pattern among these species is also changing, therefore, regular examination of the prevalence of different species and their drug resistance pattern is necessary to make the right decisions of the proper treatment. Indeed, increasing Shigella resistance to antibiotics is a serious warning that should be prevented by implementing necessary measures.

Authors' Contributions

All authors contributed equally to this research..

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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