

Shigella flexneri: a three-year antimicrobial resistance monitoring of isolates in a Children Hospital, Ahvaz, Iran

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ABSTRACT

Background and Objectives: Shigellosis is an acute gastroenteritis that is one of the most common causes of morbidity and mortality in children with diarrhea in developing countries. The purpose of this study was to describe the distribution of *Shigella* serogroups and serotypes and their antibacterial drug resistance profiles.

Materials and Methods: Fecal samples of all children suffering from shigellosis who had been admitted to Abuzar Children's Hospital in Ahvaz, southwestern Iran, from September 2008 to August 2010 were examined. Antibiotics susceptibility testing was performed according to the Kirby Bauer disk diffusion method.

Results: *Shigella flexneri* was the predominant serogroup and being identified in 87 isolates (49.8%). The most common *S. flexneri* serotypes were type 2 (57.5%) and type 1 (21.8%). High rates of resistance were observed to trimethoprim-sulfamethoxazole (85%) and ampicillin (87.5%).

Conclusion: *S. flexneri* and its serotypes was the most frequently isolated *Shigella* species from southwest of Iran, Ahvaz. Identification of predominant *S. flexneri* serotypes in developing countries can help in prioritizing strategies such as development of effective vaccines.

Keywords: *Shigella flexneri*, children, antimicrobial resistance

INTRODUCTION

Shigellosis, an acute invasive enteric infection is recognized as a major public health problem worldwide (1). It is caused by microorganisms belonging to the genus *Shigella*, one of the most important causes of gastroenteritis-induced deaths in 3-5 million children aged less than five years in developing countries (2). The majority of cases and deaths occur among children

less than five years of age (1). Increasing antimicrobial resistance in *Shigella* spp. has been reported worldwide (3). Serological typing of *Shigella* has long been used to characterize isolates for epidemiological and diagnostic purposes (4). These organisms, belonging to the *Enterobacteriaceae* family, include four serogroups: *S. flexneri*, *S. sonnei*, *S. boydii*, and *S. dysenteriae*. Each serogroup contains multiple serotypes based on the structure of the O-antigen component of the lipopolysaccharide present on the outer membrane of the cell wall (5,6). Until recently, at least 47 serotypes of *Shigella* were recognized; of which 15 belong to *S. flexneri* (7). In the tropics, most infections are caused by *S. flexneri* whereas infections primarily due to *Shigella sonnei* are less common (8,9). Changing trend in the epidemiology

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Table 1. Distribution of serotypes of *S. flexneri* serotypes isolated from children , Ahvaz , 2008-2010

<i>S. flexneri</i> type	No of isolates	%
I	19	21.8
II	50	57.5
III	1	1.1
IV	3	3.4
V	0	0
VI	7	8.1
Nontypable	7	8.1

of shigellosis and antimicrobial resistance pattern of *Shigella* strains has been noticed throughout the world over the last two decades (10, 11). For the development of vaccine-based control strategies it is essential to know the prevalent serotypes and their geographic distribution, since immunity against shigellosis is serotype-specific (12, 13).

The aim of this study was to describe the distribution of *S. flexneri* serotypes and their antibacterial drug susceptibility profiles among children in Ahvaz, southwest of Iran in the three-year period 2008-2010.

MATERIALS AND METHODS

This study was performed in the microbiology laboratory of the Children Hospital of Ahvaz, Iran, between September 2008 and August 2010. Stool specimens were collected from children younger than 12 years who had diarrhea. Stool samples were collected in stool vials prior to the administration of antibiotics. The samples were immediately brought to laboratory and inoculated on plates of MacConkey agar (Merck, Germany), XLD agar (Merck, Germany) and Selenite-F broth. The plates were incubated at 37°C for 24 hours and then suspected colonies were examined by conventional biochemical tests. (14). Specific antisera (Baharafshan, Iran) were used for serogrouping of *Shigella* isolates by a slide

agglutination test.

Serotypes of *S. flexneri* were serologically confirmed by type-specific monovalent antisera (Mast, UK). Antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method, against ampicillin (10 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), gentamicin (10 µg) and trimethoprim - sulfamethoxazole (25 µg) (Padtan-Teb, Iran).

RESULTS

Out of 4380 children with diarrhea, 175(4%) were confirmed as cases of shigellosis. According to the results of the serological tests, 87 (49.8%) of the isolates were identified as *S. flexneri*. Of all cases with shigellosis, 97 (55.4%) were male. The age distribution of these patients ranged from 5 months to 12 years, with a mean age of 6 years. Serotyping of the 87 *shigella flexneri* isolates yielded 19 (21.8%) type 1, 50 (57.5%) type 2, 1 (1%) type 3, 3 (3.4%) type 4 and 7 (8.1%) type 6. Seven isolates were untypable (Table 1).

Several *S. flexneri* isolates were resistant to trimethoprim-sulfamethoxazole (85%) and ampicillin (87.5%). Resistance to chloramphenicol was less frequent (10%) (Table 2).

A high rate of resistant to ceftriaxone was found in type 6 (57%). None of the type 3 and 4 were

Table 2. Resistance pattern of *Shigella flexneri* isolated in Ahvaz , 2008-2010

Antibiotic	No. of resistant strains	%
Trimethoprim-Sulfamethoxazole	74	85
Ampicillin	76	87.5
Nalidixic acid	9	10
Gentamycin	11	12.6
Ceftriaxone	16	18.4
Chloramphenicol	9	10

Table 3. Antimicrobial Resistant pattern of *Shigella flexneri* serotypes from children, Ahvaz, 2008-2010

Antibiotic	serotypes				
	I	II	III	IV	VI
Trimethoprim- Sulfamethoxazole	14 (73.5%)	42(84%)	1(100%)	3(100%)	5(71.4%)
Ampicillin	18(94.7%)	43 (86%)	1(100%)	3(100%)	5(71.4%)
Nalidixic acid	5(26.3%)	4(8%)	0	0	1 (14.2%)
Gentamycin	3(15.8%)	5(10%)	0	0	1 (14.2%)
Ceftriaxone	2(10.5%)	7(14%)	0	0	4(57%)
Chloramphenicol.	1(5.2%)	6(12%)	0	0	1 (14.2%)

resistant to nalidixic acid, gentamicin, ceftriaxone and chloramphenicol (Table 3).

The majority of *S.flexneri* isolates (72.5%) were resistant to (trimethoprim- sulfamethoxazole and ampicillin) (Table 4).

DISCUSSION

Shigellosis is a frequent cause of diarrhea in the more impoverished areas of Asia (1). Effective antimicrobial therapy can reduce both severity and duration of illness and can prevent potential complications. Over the past decades, *Shigella* spp. has become progressively more resistant to most widely-used and inexpensive antimicrobials. High rate of resistance to many of the first-line antimicrobial agents among the strains of *S. flexneri* have been reported from many parts of the world in recent years (8-10). It is important to determine the prevalence of various serotypes of *S. flexneri* and antimicrobial susceptibility in different communities worldwide. However, we believe that there are no published data on the incidence of antimicrobial resistance amongst *S. flexneri* serotypes in Iran. This was the first study reporting the prevalence of *S. flexneri* serotypes in Ahvaz, southwestern, Iran. Of 87 *S. flexneri* isolates,

type 2 was the most prevalent serotypes followed by type 1, 6, 4, 3 respectively. This finding is similar to other published studies in Bangladesh (15-18). These are the most common serotypes occurring in developing countries (19, 20). *S. flexneri* serotype 1 was the second most prevalent serotype in our study as Talukder study in Bangladesh (21).

The most common antibacterial resistance was observed for trimethoprim-sulfamethoxazole and ampicillin. Similar patterns of resistance were reported from children with acute diarrhea from different parts of world such as North of Iran, Brazil, Pakistan and Egypt (22-25).

Although fluoroquinolones are represented as the drugs of choice for shigellosis by World Health Organization (26), increase of fluoroquinolone resistance among *Shigella* spp. has now been documented in many countries (27-29). We observed little overt resistance to nalidixic acid (10%) similar to Ayazi study in Iran (30). While in some other countries this proportion is 56% or 39% (31, 32). In another study from Iran no resistance to nalidixic acid reported among *S. flexneri* isolates (33). These results suggesting that use of nalidixic acid in treatment of shigellosis can be in doubt. A gradually increase in multidrug resistance was noted against

Table 4. Multiple drug resistance patterns among *Shigella flexneri* isolated from children, Ahvaz, 2008-2010

Resistance types	Resistant isolates N (%)
Am, SXT	58(72.5%)
Am, SXT, NA	6(7.5%)
Am, SXT, Gm	6(7.5%)
Am, SXT, CRO	5(6.25%)
Am, SXT, C	2(2.5%)
Am, SXT, C, CRO	2(2.25%)
Am, SXT, Gm, CRO	1(1.25%)
Am, SXT, Gm, CRO, NA	4(5%)

Am, ampicillin; C, chloramphenicol; Gm, gentamicin; CRO, ceftriaxone; SXT, trimethoprim/sulfamethoxazole

commonly used antibiotics such as ampicillin and, co-trimoxazole (72.5%). Ceftriaxone resistance rate was 18.75% for *S. flexneri* isolates in our study and it was observed most frequently in type 6 (57%). Similar results were observed in a study which was conducted by Yang *et al.* (34). Taneja and colleagues in a nine-year study on *S. flexneri* isolates, found a 16.8% rate of resistance /intermediately susceptible to ceftriaxone/cefepime(35). Whereas the ceftriaxone resistance has been reported between 1.7% and 5.4% in various studies from Turkey (36).

In our study, at least 25% of *S. flexneri* were resistant to three commonly used antibiotics Such as, ampicillin, trimethoprim-sulfamethoxazole and nalidixic acid, which is comparable with recent reports (37). The findings thus clearly demonstrate that Shigelle spp. are becoming increasingly resistant to the commonly used antimicrobials. All these observations emphasize the need for surveillance of *Shigella* species in endemic regions to obtain a more detailed knowledge of epidemiology of shigellosis. Our data suggest that ampicillin and cotrimoxazole should not be used in treating infections caused by *S. flexneri* in our region and use of these drugs should be restricted. Our findings reinforce also the need for further investigation on ongoing trends in antibiotic resistance, to help clinicians in providing appropriate and effective empiric therapies.

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