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

Soheila Khaghani1*, Ahmad Shamsizadeh1, 2, Roya Nikfar2, Ali Hesami3

1Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. 2Abuzar Children’s Teaching Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. 3Department of Immunology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Received: September 2013, Accepted: June 2014

**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 trimethoprime-sulfamethpxazole (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...
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 \textit{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 \textit{Shigella} isolates by a slide agglutination test.

Serotypes of \textit{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 shiglosis. According to the results of the serological tests, 87 (49.8%) of the isolates were identified as \textit{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 \textit{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 \textit{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 1. Distribution of serotypes of \textit{S. flexneri} serotypes isolated from children , Ahvaz , 2008-2010 |
|---------------------------------------------------------------|
| \textit{S. flexneri} 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  |

| Table 2. Resistance pattern of \textit{Shigella flexneri} isolated in Ahvaz , 2008-2010 |
|-----------------------------------------------|
| Antibiotic              | No. of resistant strains | %    |
| Trimethoprim-Sulfamethoxazole | 74                  | 85   |
| Ampicillin              | 76                  | 87.5 |
| Nalidixic acid         | 9                   | 10   |
| Gentamicin              | 11                  | 12.6 |
| Ceftriaxone             | 16                  | 18.4 |
| Chloramphenicol        | 9                   | 10   |
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 3. Antimicrobial Resistant pattern of **Shigella flexneri** serotypes from children, Ahvaz,2008-2010

| Antibiotic                     | I (73.5%) | II (84%) | III (100%) | IV (100%) | VI (71.4%) |
|-------------------------------|-----------|----------|------------|-----------|------------|
| Trimethoprim- Sulfamethoxazole| 14        | 42       | 1          | 3         | 5          |
| 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%)  |

### 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.

ACKNOWLEDGEMENT

This study was supported by Grants No: 90101 from Tropical and Infection Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

REFERENCES

1. Von Seidlein L, Kim DR, Ali M, Lee, H, Wang XY, Thiem VD et al. A multicenter study of *Shigella* diarrhea in six Asian countries: disease burden, clinical manifestation, and microbiology. *PLoS Med* 2006;3:e353.
2. Bhattacharya D, Sugunan AP, Bhattacharjee H,Thamizhmani R, Sayi DS, Thanasekaran K, et al. *Shigella dysenteriae* 1 and *Shigella flexneri*. *Health Place* 2008; 14: 96–105.
3. Bhattacharya D, Sugunan AP, Bhattacharjee H, Thanasekaran K, et al. Antimicrobial resistance in *Shigella* rapid increase & widening of spectrum in Andaman Islands,India. *Indian J Med Res*. 2012;135:365.
4. Maurelli AT, Lampel KA, Hui YH, Pierson MD, Gorham JR (2001) Foodborne disease handbook. Vol. 1. Bacterial pathogens. 2nd ed. New York: Dekker. 323-43.
5. Liu B, Knirel YA, Feng L, Perеполов AV, Сенченкова SN, Wang Q, et al.Structure and genetics of *Shigella* O antigens. *FEMS Microbiol Rev* 2008; 32:627-653
6. Simmons DA, Romanowska E. Structure and biology of *Shigella flexneri* O antigens. *J Med Microbiol Rev* 1987; 23 :289-302.
7. Clemens J, Kotloff K, Kay B. (1999). Generic protocol to estimate the burden of *Shigella* diarrhoea and dysenteric mortality. WHO/VandB/99.26. World Health Organization, Geneva, Switzerland.
8. Shrestha CD, Malli S, Maharjan L. Multi drug resistant *Shigella* species in Nepal, a retrospective study conducted at National Public Health Laboratory (NPHL), 1999 to 2002. *J Nepal Health Res Coun* 2002;4:51-55.
9. Wilson G, Easow JM, Mukhopadhyay C, Shivananda PG. Isolation & antimicrobial susceptibility of *Shigella* from patients with acute gastroenteritis in western Nepal. *Indian J Med Res* 2006; 123:145-150.
10. Ghosh AR, Sehgal SC. *Shigella* infections among children in Andaman -an archipelago of tropical islands in Bay of Bengal. *Epidemiol Infect* 1998; 121:43-48.
11. Ashkenazi S, May-Zahar M, Diniari G, Gabbay U, Zilberberg R, Samra Z. Recent trends in the epidemiology of *Shigella* species in Israel. *Clin Infect Dis* 1993; 17: 897-9.
12. Rasolofo-Razanamparany V, Cassel-Beraud AM, Roux J, Sansonetti PJ, Phalipon A. Predominance of serotype-specific mucosal antibody response in *Shigella flexneri* infected humans living in an area of endemicity. *Infect Immun* 2001; 69(9):5230-4.
13. Oaks EV, Turbyfill KR. Ross Turbyfill. Development and evaluation of a *Shigella flexneri* 2a and *S. sonnei* bivalent invasin complex (Invaplex) vaccine. *Vaccine* 2006; 24:2290-301.
14. National Committee for Clinical Laboratory Standards. (2000). Performance Standards for Antimicrobial Disk Susceptibility Tests:Approved Standard M2-A7. NCCLS, Villanova, PA, USA.
15. Carlin NI, Rahman M, Sack A, Zaman A, Kay B, Lindberg AA. Use of monoclonal antibodies to type *Shigella flexneri* in Bangladesh. *J Clin Microbiol* 1989;27:1163–1166.
16. Haider K, Huq MI, Talukder KA, Ahmad QS. Electropherotyping of plasmid deoxyribonucleic acid (DNA) of different serotypes of *Shigella flexneri*. *J Med Microbiol* 1999; 48: 323-43.
17. Mutanda LN, Kibriya AKMG, Mansur MN, Huq M I. Antibiotic-resistance and pattern of *Shigella flexneri* serotypes in Dacca. *Bangladesh Med J* 1980; 9:1–7.
18. Mutanda LN, Kibriya AKMG, Mansur M N. Pattern of *Shigella flexneri* serotypes and drug-resistance in Dacca. *Indian J Med Res* 1981; 73:8–12
19. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, et al. Global burden of Shigella infections: implications for vaccine development and implementation of control strategies. *Bull WHO* 1999;77: 651-666.

20. Nandy S, Mitra U, Rajendran K, Dutta P, Dutta S. Subtypes prevalence, plasmid profiles and growing fluoroquinolone resistant in *shigella* from Kolkata,India(2001-2007): a hospital- based study. *Trop Med Int Health* 2010; 15:499-507.

21. Kaisar A, Talukder, Dilip K, Dutta, Ashrafus Safa, M. Ansaruzzaman, Ferdaus Hassan, Khorsheed Alam, K. M. N. Islam, N. I. A. Carlin, G. B. et al. Altering trends in the dominance of *Shigella flexneri* serotypes and emergence of serologically atypical *S. flexneri* strains in Dhaka, Bangladesh. *J Clin Microbiol* 2001;39:3757-3759.

22. Savadkoohi R, Ahmadpour-Kacho M. Prevalence of *Shigella* Species and Their Antimicrobial Resistance Patterns at Amirkola Children’s Hospital, North of Iran. *Iran J Ped* 2007; 17:118-122.

23. Peirano G, Santos Souza F D, Prazeres Rodrigues DD. Frequency of serovars and antimicrobial resistance in *Shigella* spp. from Brazil. *Mem Inst Oswaldo Cruz Rio de Janeiro* 2006;101(3): 245-250.

24. Zafar A, Hasan R, Nizami SQ, von Seidlein L, Soofi S, Ahsan T, et al. Frequency of isolation of various subtypes and antimicrobial resistance of *shigella* from urban slums of Karachi, Pakistan. *Int J Infect Dis* 2009; 13: 668-672.

25. Ahmed SF, Riddle MS, Wierzbta TF, Messih IA, Monteville MR, Sanders JW, et al. Epidemiology and genetic characterization of *Shigella flexneri* strains isolated from three paediatric populations in Egypt (2000–2004). *Epidemiol Infect* 2006; 134(6):1237–1248.

26. World Health Organization. Guidelines for the Control of Shigelllosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva: WHO; 2005. Available from: http://whqlibdoc.who.int/publications/2005/9241592330.pdf, accessed on September 22, 2010

27. Naheed A, Kalluri P, Talukder KA, Faruque AS, Khatun F, Nair GB, et al. Fluoroquinolone-resistant *Shigella dysenteriae* type 1 in northeastern Bangladesh. *Lancet Infect Dis* 2004; 4: 607-608.

28. Hirose K, Terajima J, Izumiy H, Tamura K, Arakawa E, Takai N, et al. Antimicrobial susceptibility of *Shigella sonnei* isolates in Japan and molecular analysis of *S. sonnei* isolates with reduced susceptibility to fluoroquinolones. *Antimicrob Agents Chemother* 2005; 49 :1203-1205

29. Roy S, Bhattacharya D, Thanasekaran K, Ghosh. AR, Manimunda SP, Bharadwaj AP, et al. Emergence of fluoroquinolone resistance in *Shigella* isolated from Andaman & Nicobar Islands, India. *Indian J Med Res* 2010; 131: 720-722

30. Ayazi P .Prevalence of clinical symptoms and antimicrobial sensitivity of *shigella* in children. *J Qazvin Univ Med Sciences*. 2001;16:46-50. (In Persian)

31. Pazhani GP, Ramamurthy T, Mitra U, Bhattacharya SK, Niyog SK. Species diversity and antimicrobial resistance of *Shigella* spp isolated between 2001 and 2004 from hospitalized children with diarrhoea in Kolkata (Calcutta), *Epidemiol Infect* 2005;133:1089– 1095.

32. Zafar A, Sabir N, Bhatta ZA. Frequency of isolation of *shigella* serogroups/serotypes and their antimicrobial susceptibility pattern in children from slum areas in Karachi. *J Pak Med Assoc.* 2005;55:184–188.

33. Sojtan-Dallal MM, Ranjbar R, Poursefane MR. The study of antimicrobial resistance among *Shigella flexneri* strains isolated in Tehran, Iran. *J Pediatr Infect Dis* 2011; 6:125–129.

34. Yang H, Chen G, Zhu Y, Liu Y, Cheng J, Hu L, et al. Surveillance of antimicrobial susceptibility patterns among *Shigella* specie isolated in China during the 7-years period of 2005-2011 *Ann Lab Med* 2013; 33:115- 5

35. Tanej M, Mewara A,Kumara A, Verma G , Sharma M.Cephalosporin-resistant *shigella flexneri* over 9 years(2001-2009) in India. *J Antimicrob Chemother*2012;7:1347-1353

36. Pullukcu H, Aydemir S, Sipahi OR, Yamazhan T. Species Distribution and antibacterial resistance patterns of 439 *Shigella* spp. strains isolated from stool cultures between 1999-2006. *ANKEM Derg* 2007;21:137-141.

37. Sivapalasingam S, Nelson JM, Joyce K, Hoekstra M, Angulo FJ, Mintz ED. High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the national antimicrobial resistance monitoring system from 1999 to 2002. *Antimicrob Agents Chemother* 2006;50:49-54