ORIGINAL ARTICLE

Frequency and antimicrobial susceptibility of *Shigella* species isolated in Children Medical Center Hospital, Tehran, Iran, 2001-2006

ABSTRACT

Appropriate antimicrobial treatment of shigellosis depends on identifying its changing resistance pattern over time. We evaluated 15,255 stool culture submitted from July 2001 to June 2006 to the Laboratory of Children Medical Center Hospital. Specimen culture, bacterial identification, and disk diffusion susceptibility testing were performed according to National Committee for Clinical Laboratory Standards guidelines. From 15,255 stool samples, 682 (4.5%) were positive for *Shigella* species. The most common species of *Shigella* were *S. flexneri* (48%) and *S. sonnei* (45%); other results were *S. dysenteriae* (5%) and *S. boydii* (2%). The rate of Sensitivity to ceftriaxone (95%), ceftizoxime (94%), and nalidixic acid (84%) were among our isolates. Resistance to co-trimoxazole and ampicillin was 87% and 86%, respectively. *S. flexneri* was more multiresistant than other species (47.9%). Our isolates are overall most sensitive to ceftriaxone, ceftazidime, and nalidixic acid (> 84%). They were most resistant to co-trimoxazole and ampicillin (> 86%). Because resistance varies according to specific location, continuous local monitoring of resistance patterns is necessary for the appropriate selection of empirical antimicrobial therapy.

Keywords: *Shigella*, antimicrobial susceptibility, dysentery.

[Braz J Infect Dis 2010;14(2):153-157]

Authors
Babak Pourakbari[1,2]
Setareh Mamishi, MD[2]
Negar Mashoori[3]
Nastaran Mahboobi[3]
Mohammad H Ashtiani[4]
Shahla Afsharpaiman[5]
Masomeh Abedini[6]

[1]Pediatrics Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Infectious Disease, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[3]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[4]Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[5]Department of Pediatrics, School of Medicine, Sanandaj University of Medical Sciences, Tehran, Iran.
[6]Department of Pediatrics, School of Medicine, Baghiatallah University of Medical Sciences, Tehran, Iran.

Submitted on: 05/23/2009
Approved on: 08/18/2009

Correspondence to:
Setareh Mamishi, MD
Department of Pediatrics – Infectious Disease
Children Medical Center Hospital
Medical Sciences/ University of Tehran
No. 62, Gharib St., Keshavarz Blvd.
Tehran – Iran
Phone: 98 021 6428996
Fax: 98 021 6428996
E-mail: smamishi@sina.tums.ac.ir

We declare no conflict of interest.

INTRODUCTION

Dysentery caused by *Shigella* species is called shigellosis, which is one of the most common causes of dysentery in children. It is responsible for increased cases of morbidity and mortality in developing countries. Global studies suggest there are 164.7 million episodes of shigellosis per year, of which 163.2 million were in developing countries, and 1.5 million in developed countries. These episodes result in 1.1 million deaths, of which two-thirds occur in children under 5 years of age.[1]

The epidemiology and antibiotic susceptibility of *Shigella* species changes over time. Treatment of shigellosis by appropriate antimicrobial agents has proven to be effective in shortening the duration of fever, diarrhea and toxemia, and apparently in reducing the risk of lethal complications as well. Also, the excretion of pathogen in stool is shortened significantly, reducing the spread of infection.[2] Appropriate antibiotic treatment of shigellosis depends on identifying resistance patterns. So, updated knowledge of *Shigella* susceptibility is necessary for appropriate empirical antibiotic treatment. In this study, we assessed the frequency of *Shigella* species and their antimicrobial resistance at Children's Medical Center (CMC) Hospital between July 2001 and June 2006. The aims of this study were to analyze antimicrobial resistance of *Shigella* isolates to suggest timely recommendations for empirical antibiotic therapy.

MATERIAL AND METHODS

Source of specimens

We evaluated 15,255 stool culture submitted from July 2001 to June 2006 to the Laboratory of CMC Hospital. Only one *Shigella* isolate per patient per diarrheal episode was included in the analysis. All the included patients lived in Tehran at the time of study and they were not hospitalized or received any antibiotic during the month before presentation with shigellosis. In addition to being a referral tertiary care centre, CMC Hospital is one of the educational hospitals of Tehran University of Medical Sciences.

Microbial examination

Stool specimens were cultured on *Salmonella-Shigella* (SS) Agar and Eosin methylene blue (EMB) agar and incubated at 37° C for 1 to 5 days. Biochemical tests were done by standard methods on grown bacteria to identify *Shigella* species, which grouped serologically by slide agglutination with specific antisera. Antibiotic susceptibility to ceftazidime, tobramycin, ceftizoxime, ceftriaxone, chloramphenicol, kanamycin, nalidixic acid, cephalothin, co-trimoxazole, ampicillin, gentamicin and amikacin was determined in all specimens by disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS).[3] To verify that susceptibility test results were accurate, we used *E. coli* ATCC 25922 as control strain according NCCLS guideline.[3] The antimicrobial agents selected for analysis were those commonly included in the treatment of shigellosis in Iran. Results were interpreted according to NCCLS guidelines as either sensitive, intermediate, or resistant.[4] In our study, we considered both intermediate and resistant as resistant. Isolates were considered multiresistant if they were resistant to ampicillin and co-trimoxazole.[5] Antimicrobial susceptibility results were rounded down if < 0.5 and were presented as whole numbers if ≥ 0.5.

Table 1. Frequency of Shigella species isolated from stool cultures of patients at Children Medical Center Hospital, Tehran, Iran

***Shigella* species**	**Frequency**	**Percent**
S. flexneri	190	47.9
S. sonnei	179	45.1
S. dysenteriae	20	5.0
S. boydii	8	2.0
Total	397	100.0

Statistical analysis

The significance of differences in proportions of antimicrobial resistance of *Shigella* species was determined by the chi-square test or the Fisher Exact test (when the expected value in > 20% of the cells was < 5). P-value < 0.05 was considered statistically significant. Statistical calculations were performed with SPSS statistical software (version 13.0; SPSS Inc., Chicago, IL, USA).

RESULTS

From 15,255 stool samples, 682 (4.5%) were positive for *Shigella* species. The species of 397 out of 682 *Shigella* isolates were determined. The most common species of *Shigella* was *S. flexneri* (48%) and *S. sonnei* (45%), and other results were *S. dysenteriae* (5%) and *S. boydii* (2%) (Table 1). *Shigella* isolates were totally most sensitive to ceftriaxone (95.4%), ceftizoxime (94%), and nalidixic acid (83.5%); and most resistant to co-trimoxazole (87%) and ampicillin (86%). All results are shown in Table 2. *S. flexneri* was most sensitive to Ceftriaxone (94%), Ceftizoxime (90%), and Nalidixic acid (89%); and most resistant to Ampicillin (96%) and Co-trimoxazole (90%). Comparing the results of susceptibility by year over the study period, it was found increase of rates of resistance to cefazolin, cefixime, tobramycin, amikacin in *S. flexneri* isolates (p-value < 0.05). *S. sonnei* was most sensitive to ceftizoxime (98%), ceftriaxone (97%), and chloramphenicol (93%); and most resistant to co-trimoxazole (92%) and ampicillin (73%). These isolates comparing the results of susceptibility by year showed increase of rates of resistance

Table 2. In vitro antimicrobial susceptibility of Shigella species isolated from stool cultures at Children Medical Center Hospital, Tehran, Iran

Antibiotics	**Sensitive**		**Resistant**	
	n	**valid percent**	**n**	**valid percent**
Chloramphenicol	308	67%	134	30%
Nalidixic acid	543	84%	107	16%
Tobramycin	486	80%	112	20%
Gentamicin	537	82%	121	18%
Amikacin	530	82%	115	18%
Kanamicin	235	40%	358	60%
Ampicillin	94	14%	577	86%
Co-trimoxazole	83	12%	581	88%
Cephalotine	374	58%	276	42%
Ceftizoxime	586	94%	40	6%
Ceftazidime	312	73%	118	27%
Ceftriaxone	596	95%	29	5%

Table 3. In vitro antimicrobial susceptibility of various species of Shigella isolated from stool cultures at Children Medical Center Hospital, Tehran, Iran

Antibiotics	*S. flexneri*		*S. sonnei*		*S. dysenteriae*		*S. boydii*	
	n	valid percent	n	Valid percent	n	valid percent	n	valid percent
Chloramphenicol	37	32%	136	93%	14	82%	6	75%
Nalidixic acid	162	89%	137	80%	6	38%	6	75%
Tobramycin	138	79%	129	79%	16	80%	7	100%
Gentamicin	152	82%	139	81%	9	45%	8	100%
Amikacin	151	84%	136	81%	7	37%	8	100%
Kanamicin	64	42%	64	39%	-	-	2	40%
Ampicillin	7	4%	47	27%	18	95%	3	38%
Co-trimoxazole	19	10%	13	8%	18	100%	2	25%
Cephalotine	96	53%	112	66%	19	100%	2	25%
Ceftizoxime	147	90%	167	98%	4	100%	8	100%
Ceftazidime	72	64%	88	87%	16	94%	4	100%
Ceftriaxone	161	94%	158	97%	19	100%	8	100%

Table 4. Frequency of multiresistant Shigella species isolated from stool cultures at Children Medical Center Hospital, Tehran, Iran*

Shigella species	Frequency	Percent
S. flexneri	158	83%
S. sonnei	106	59%
S. dysenteriae	12	60%
S. boydii	4	50%

to kanamycin, cefalotin, ampicillin, gentamycin, amikacin, ceftriaxone, ceftazidime, tobramycin (p-value < 0.05). *S. dysenteriae* was completely sensitive to ceftriaxone, ceftizoxime, cephalotine, co-trimoxazole (100%), and it was most resistant to kanamycin (100%), amikacin (63%), and nalidixic acid (63%). *S. boydii* was totally sensitive to gentamicin, amikacin, ceftriaxone, ceftizoxime, ceftazidime, and tobramycin (100%); and most resistant to co-trimoxazole (75%), cephalotine (75%), ampicillin (63%), and kanamycin (60%) (Table 3). In *S. dysenteriae* and *S. boydii* isolates were not seen any valuable significance in rate of resistance when comparing the results by the years. Among 682 isolates, 500 isolates were multiresistant (74%). *S. flexneri* was more multiresistant than other species (Table 4).

DISCUSSION

From the epidemiologic perspective, *Shigella* is a pathogen that persists as a major public health problem in developing countries, causing treatment center visits, hospitalizations, and deaths, although it also remains an intermittent cause of morbidity and mortality in high risk groups in industrialized countries.

S. sonnei is the predominant *Shigella* species isolated in developed countries,[2,6,7] whereas in developing countries and low socio-economic conditions, *S. flexneri* predominates.[1,2,6,8-10] In our study, *S. flexneri* (48%) was the most common, followed by *S. sonnei* (45%). Other species were rare (*S. dysenteriae* and *S. boydii* 5% and 2%, respectively).

Our isolates are overall most sensitive to ceftriaxone, ceftazidime and nalidixic acid (> 84%). They were most resistant to co-trimoxazole and ampicillin (> 86%) which was compatible with other studies.[1,2,5]

Antibiotic susceptibility results are to some extent different among different species. *S. flexneri*, the most common species, was most sensitive to ceftriaxone, ceftizoxime, and nalidixic acid; and *S. sonnei* was most sensitive to ceftizoxime, ceftriaxone, and chloramphenicol. *S. flexneri* was more sensitive to nalidixic acid than *S. sonnei* (p-value < 0.05) and *S. sonnei* was more sensitive to cephalosporines (ceftizoxime, ceftriaxone, ceftazidime, cephalotine) than *S. flexneri* (p-value < 0.05). It shows that for an empiric therapy, when the suspected species is *S. flexneri*, nalidixic acid will be more effective and its resistance trend over the study period had not valuable significance, while third cephalosporines will be more effective than nalidixic acid in treatment of shigellosis due to *S. sonnei*. *S. flexneri* and *S. sonnei* were most resistant to ampicillin and co-trimoxazole. *S. flexneri* was more resistant to ampicillin than *S. sonnei* (p-value < 0.0001). Other reports from all over the world also indicate

high resistance rates (58.3% to 85%) for ampicillin. Studies from Greece[12] and Oregon[13] did not report species specific sensitivity, and results from Israel[7] have shown similar high resistance rates for both *S. sonnei* and *S. flexneri* (87% and 71%, respectively).[2,12-14] The high rate of resistant to ampicillin and co-trimoxazole, which was reported in this study, was similar to those reported by the Centers for Disease Control and Prevention, NARMS data from 2001 and other studies.[5,11] In Malaysia, the co-trimoxazole resistance rate of *S. sonnei* was relatively low (37.1%).[1] In one study, *S. flexneri* was much more resistant to chloramphenicol compared with *S. sonnei*.[14] This is also in accordance with the other reports. One study from Oregon reported a rate of 72% resistance of *S. flexneri* to chloramphenicol, whereas the resistance of *S. sonnei* was only 1%.[13] In our study, all *Shigella* species were sensitive to chloramphenicol, except *S. flexneri*, which was resistant to it (68%). Ampicillin and chloramphenicol were the inexpensive and broad-spectrum antimicrobial agents commonly used in developing countries. Increase of resistance to ampicillin or chloramphenicol is often related with extensive use of these antimicrobial agents.[1]

This information is important because *S. flexneri* and *S. sonnei* are the most common species and it should be taken into account for treatment. Co-Trimoxazole is a common drug used as an empiric therapy in treatment of shigellosis and other diarrheal diseases of bacterial origins. The extensive use of this drug has contributed to the emergence of resistant *S. sonnei* and sustained the resistant trait.[1,15,16]

In addition to co-Trimoxazole and ampicillin, ongoing resistance of *Shigella* species to tetracycline, chloramphenicol, and most recently to fluoroquinolones has been shown. Oral aminoglycosides and first and second generation cephalosporins are clinically ineffective, despite their in vitro activity.[5,17-21] In our study *Shigella* species were sensitive to aminoglycosides, except kanamycin. Kanamycin is a member of aminoglycosides, which resistance to it was reported before by Lee *et al*. (2001) and Jeong *et al*. (2003).[22,23] They had reported the emergence of kanamycin-resistant *S. sonnei* isolates in Korea.[1] Although resistance to fluoroquinolones has been rarely reported, nearly all *Shigella* isolates are susceptible to these agents. Indeed, quinolones, which are also efficacious against other causes of bacterial gastroenteritis, are often recommended as empirical therapy in areas with high resistance to *Shigella* spp. They are, however, not approved for children because of the potential risk of damage to growing cartilage.[2,6,24] Another suitable antibiotic for treatment in children with severe shigellosis, especially in those who are hospitalized, is parenteral ceftriaxone, which is effective and usually recommended.[2] In this study, *Shigella* species (esp. *S. dysenteriae* and *S. boydii*) had high sensitivity to ceftriaxone.

In milder cases in children, choosing the optimal oral therapy is more problematic and should be based on local epidemiological data. Nalidixic acid or extended spectrum cephalosporins are usually adequate.[2] In our study, *Shigella* species (esp. *S. dysenteriae* and *S. boydii*) were highly sensitive to third generation cephalosporins (ceftiaxone, ceftizoxime, ceftazidime). Despite some studies that has reported resistance to nalidixic acid (esp. about *S. flexneri* and *S. dysenteriae*),[8,9,25] *Shigella* species were also sensitive to nalidixic acid, and *S. flexneri* isolates were even more sensitive to it than other species. This data makes nalidixic acid still one of the most appropriate antibiotics for treatment of shigellosis due to *Shigella* species' sensitivity to it, its cost and its easy accessibility in Iran. *S. dysenteriae* causes one of the most severe forms of epidemic severe dysenteries.[26] It was most sensitive to ceftriaxone, ceftizoxime, cephalotine, and co-trimoxazole; and most resistant to kanamycin, amikacin and nalidixic acid.

Multiresistance to the antimicrobial agents used in treatment of shigellosis has been reported in many parts of the world.[1,22,23,27,28] In our study, *S. flexneri* was more multiresistant than other species, which is very important due to its frequency. Ceftriaxone and ciprofloxacin have been shown to be highly effective for shigellosis.[5,29-31] Ceftriaxone requires parenteral administration, whereas ciprofloxacin is avoided in children.

The increasing relative prevalence of *Shigella* species and the emergence of new resistant strains pose a clear public health problem. As resistance to antimicrobial agents changes constantly, it is important to keep these strains under surveillance in order to monitor the local susceptibility and subsequently formulate policies for the rational use of antimicrobial agents.

REFERENCES

1. Hoe CH, Yasin RM, Koh YT, Thong KL. Antimicrobial susceptibility and pulsed-field gel electrophoresis of *Shigella* sonnei strains in Malaysia (1997–2000). Applied Microbiology J. 2005; 99:133-40.
2. Ashkenazi SH, Levy I, Kazaronovski V, Samra Z. Growing antimicrobial resistance of *Shigella* isolates. Antimicrobial Chemotherapy J. 2003; 51:427-9.
3. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. NCCLS, Villanova, PA, USA. 2000.
4. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS, Wayne, PA, 2000.
5. Jain SK, Gupta A, Glanz B, Dick J, Siberry GK. Antimicrobial-Resistant *Shigella* sonnei: Limited Antimicrobial Treatment Options for Children and Challenges of Interpreting In Vitro Azithromycin Susceptibility. Pediatr Infect Dis J. 2005; 24:494-7.
6. Ashkenazi, S. (1999). *Shigella* spp. In Antimicrobial Therapy and Vaccines (Yu, V. L., Merigan, T. C. & Borriere, S. L., Eds), pp. 382-7. Williams & Wilkins, Baltimore, MD, USA.
7. Green MS, Block C, Cohen D, Slater P. Four decades of shigellosis in Israel—the epidemiology of a growing public health problem. Review of Infectious Diseases. 1991; 13:248-53.

8. DuPont, HL (1988). Shigellosis. Infectious Disease Clinics of North America 2, 599-605.
9. Pazhani GP, Ramamrthy T, Mitra U, Bhattacharya SK, Niyogi SK. Species diversity and antimicrobial resistance of *Shigella* spp. isolated between 2001 and 2004 from hospitalized children with diarrhea in Kolkata (Calcutta), India. Epidemiology and Infection. 2005; 133:1089-95.
10. Khan A, Huq S, Malek MA *et al*. *Shigella* serotypes among hospitalized patients in urban Bangladesh and their antimicrobial resistance. Epidemiology and Infection. 2004; 132:773-7
11. Centers for Disease Control and Prevention. NARMS 2001 Annual Report: antimicrobial resistance of Salmonella, *Shigella*, and *E. coli* O157 isolates, 2001. Available at: http://www.cdc.gov/narms/annual/2001/table/0104.htm. Accessed June 15, 2004.
12. Maraki S, Georgiladakis A, Tselentis Y, Samonis GA. 5 year study of the bacterial pathogens associated with acute diarrhea on the island of Crete, Greece, and their resistance to antibiotics. Eur J Epidemiol. 2003; 18:85-90.
13. Replogle ML, Fleming DW, Cieslak PR. Emergence of antimicrobial- resistant shigellosis in Oregon. Clin Infect Dis. 2000; 30:515-9.
14. Ozmert EN, Göktürk B, Yurdakök K, Yalçin SS, Gür D. *Shigella* Antibiotic Resistance in Central Turkey: Comparison of the Years 1987-1994 and 1995-2002. Pediatric Gastroenterology and Nutrition J. 2005; 40:359-62.
15. Shahid NS, Rahaman MM, Haider K, Banu H, Rahman N. Changing pattern of resistant *Shigella* bacillus (*Shigella* dysenteriae type 1) and *Shigella* flexneri in Bangladesh. J Infect Dis. 1985; 152:1114-9.
16. Wharton M, Spiegel RA, Horan JM *et al.* A large outbreak of antibiotic-resistant shigellosis at a mass gathering. J Infect Dis. 1990; 162:1324-8.
17. Islam MR, Alam AN, Hossain MS, Mahalanabis D, Hye HK. Double-blind comparison of oral gentamicin and nalidixic acid in the treatment of acute shigellosis in children. J Trop Pediatr. 1994; 40:320-5.
18. Landa L. Cephradine in the treatment of intestinal infections caused by *Shigella* or Salmonella organisms. Curr Ther Res Clin Exp. 1972; 14:496-502.
19. Orenstein WA, Ross L, Overturf GD, Wilkins J, Redfield DR, Underman A. Antibiotic treatment of acute shigellosis: failure of cefamandole compared with trimethoprim/sulfamethoxazole and ampicillin. Am J Med Sci. 1981; 282:27-33.
20. Ostrower VG. Comparison of cefaclor and ampicillin in the treatment of shigellosis. Postgrad Med J. 1979;55(suppl 4):82- 4.
21. Parry HE. Oral kanamycin in the treatment of *Shigella* and Salmonella infections. Postgrad Med J. 1967; suppl:7-13.
22. Lee JC, Oh JY, Kim KS *et al*. Antimicrobial resistance of *Shigella* sonnei in Korea during the last two decades. APMIS. 2001;109:228-34.
23. Jeong YS, Lee JC, Kang HY *et al.* Epidemiology of nalidixic acid resistance and TEM-1- and TEM-52-mediated ampicillin resistance of *Shigella* sonnei isolates obtained in Korea between 1980 and 2000. Antimicrob Agents Chemother. 2003; 47:3719-23
24. Bennish ML, Salam MA. Rethinking options for the treatment of shigellosis. Journal of Antimicrobial Chemotherapy. 1992; 30:243-7.
25. Vasilev V, Japheth R, Yishai R, Andorn N. Antimicrobial resistance of *Shigella* flexneri serotypes in Israel during a period of three years: 2000-2002. Epidemiology and Infection. 2004, 132(6):1049-54.
26. Prado V, Lagos R, Nataro JP *et al.* Population-based study of the incidence of *Shigella* diarrhea and causative serotypes in Santiago, Chile. Pediatr Infect Dis J. 1999; 18: 500-5.
27. Ling J, Kam KM, Lam AW, French GL. Susceptibilities of Hong Kong isolates of multiply resistant *Shigella* spp. to 25 antimicrobial agents, including ampicillin plus sulbactam and new 4-quinolones. Antimicrob Agents Chemother. 1988; 32:20-3.
28. Ashkenazi S, May-Zahav M, Sulkes J, Zilberberg R, Samra Z. Increasing antimicrobial resistance of *Shigella* isolates in Israel during the period 1984 to 1992.Antimicrob Agents Chemother. 1995; 39:819-23.
29. Varsano I, Eidlitz-Marcus T, Nussinovitch M, Elian I. Comparative efficacy of ceftriaxone and ampicillin for treatment of severe shigellosis in children. J Pediatr. 1991; 118:627-632.
30. Leibovitz E, Janco J, Piglansky L *et al*. Oral ciprofloxacin *vs*. intramuscular ceftriaxone as empiric treatment of acute invasive diarrhea in children. Pediatr Infect Dis J. 2000; 19:1060-7.
31. Eidlitz-Marcus T, Cohen YH, Nussinovitch M, Elian I, Varsano I. Comparative efficacy of two- and five-day courses of ceftriaxone for treatment of severe shigellosis in children. J Pediatr. 1993; 123:822-4.