





Multidrug-resistant *Shigella* infection in pediatric patients with diarrhea from central Iran

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Background: *Shigella* spp. are primary pathogens of diarrhea in children worldwide. Emergence of resistance to fluoroquinolones and third-generation cephalosporins is crucial in the management of pediatric shigellosis. We determined the prevalence and the antibiotic resistance patterns of *Shigella* species isolated from pediatric patients in central Iran.

Materials and methods: Pediatric diarrhea samples (n=230) were cultured on MacConkey and XLD agar media and in GN broth. Genus-specific PCR for *ipaH* was also used for detection directly from fecal specimens. Antibiotic resistance and the frequency of ESBL and AmpC genes were determined.

Results: Out of the 230 samples, 19 (8.2%) cases of *Shigella* spp. were identified using culture. Twenty-six samples were positive by PCR (11.3%), *S. flexneri* (4/19; 21%) and *S. sonnei* (15/19; 78.9%) being the most detected. The highest antibiotic resistance rates were found for cotrimoxazole (19/19; 100%), ampicillin (16/19; 84.2%), cefixime (13/19; 68.4%) and ceftriaxone (12/19; 63.1%). Ten cases showed phenotypic ESBL presence and all these strains were positive for *bla*_{TEM}, *bla*_{CTX-M-1}, and *bla*_{CTX-M-15}. Three strains were AmpC positive, all of which harbored *bla*_{CMY-2} and two contained *bla*_{CIT}. Of the 19 *Shigella* isolates 5 (26.3%), 2 (10.5%), and 1 (5.2%) were phenotypically resistant to nalidixic acid, ciprofloxacin, and norfloxacin, respectively. Class 1 integron was found in 18 (94.7%) isolates whereas class 2 integron was found in 19 (100%) strains.

Conclusion: We found a considerable presence of *Shigella* species with elevated antibiotic resistance levels. In particular, the resistance to third-generation cephalosporins (ESBL) and ciprofloxacin must be taken seriously.

Keywords: *Shigella*, dysentery, antibiotic resistance, MDR, integrons, Iran

Introduction

Shigella species belong to the Enterobacteriaceae and are Gram-negative rods, known as important causative agents of diarrhea and dysentery. *Shigella* spp. pose major public health problems in developing countries.¹ In 2013, the annual and global number of deaths amongst children under 5 years due to shigellosis was estimated to be between 28,000 and 48,000.² The most prevalent species causing those infections are *Shigella flexneri* and *Shigella sonnei*.³ Due to resistance to trimethoprim-sulfamethoxazole, ampicillin, sulfonamides, and tetracycline World Health Organization (WHO) recommends fluoroquinolone (ciprofloxacin) for first-line treatment of all patients with bloody diarrhea, regardless of age. Ceftriaxone is proposed as second-line therapy or alternative antimicrobial agent in adults and children.⁴ The increase of multidrug resistance (MDR) in *Shigella* spp., mostly due to third-generation cephalosporins (TGC), azithromycin and fluoroquinolones, is

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a major issue in developing countries.⁵ The elevated resistance levels are often the consequence of the horizontal transfer of complex resistance determinants including plasmids, integrons, and transposons.⁶

Although studies have found different frequencies and different *Shigella* species from north and south Iran,^{7–9} most reported phenotypic criteria such as frequency and antibiotic resistance profiles. In addition, Iran is a huge country with different climates in different geographical regions. In Central Iran, the prevalence of diarrhea and dysentery among children is especially high in the summer. Still, laboratory diagnosis of *Shigella* spp. is not common and most patients are treated empirically with TGCs.

Therefore, the main objective of the present study was to determine the frequency of *Shigella* spp. in children with diarrhea in Central Iran. In addition, we investigated their phenotypic antimicrobial resistance levels and resistance gene content.

Materials and methods

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Arak University of Medical Sciences under approval numbers ARAKMU.REC. 93–176-30. A questionnaire and a consent form were provided to the parents, guardians and/or patients. The two main inclusion criteria were as follows: 1) completion of the consent form; and 2) direct observation of over five white blood cells per high-power microscope field (HPF) in a diarrhea sample.⁷ None of the patients had taken antibiotics in the week before hospitalization.

Sample collection

This descriptive cross-sectional study included samples from 230 pediatric patients with infectious diarrhea who were referred to the Educational-Therapeutic Centers affiliated with the Arak University of Medical Sciences (Valiasr, Amirkabir, Amiralmomenin) from May to September 2015.

Phenotypic investigation

First, 500 μ L of the liquid stool samples were enriched in 5 mL of Gram-negative (GN) broth (Merck, Hamburg, Germany) and after 6 h at 37°C the enriched samples were streaked on xylose lysine deoxycholate (XLD) and MacConkey agar. In addition, stool samples were directly

cultured on MacConkey agar, XLD (Merck) and incubated at 35–37°C for 18–24 h. Individual isolates were surveyed using previously defined standard biochemical and serological tests. All isolates were confirmed to be *Shigella* spp. applying API 20E test strips (bioMérieux, Craponne, France). Serogrouping of the *Shigella* spp. isolates was performed using slide agglutination with specific antisera (SSI, Copenhagen, Denmark).⁸ The confirmed *Shigella* spp. isolates were kept in brain heart infusion broth with 20% glycerol at –80°C. *S. sonnei* PTCC 1777 and *S. flexneri* PTCC 1234 were used as controls in each assay (obtained from the Iranian Research Organization for Science and Technology). *S. boydi* and *S. dysenteriae* control strains were obtained from the microbiology department of Arak University of Medical Sciences.

Investigating *Shigella* antibiotic resistance by disk diffusion

Antibiotic resistance of *Shigella* spp. isolates was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines.⁹ The antibiotic discs used contained cotrimoxazole (25 μ g), cefoxitin (30 μ g), ampicillin (10 μ g), ceftizoxime (30 μ g), ceftazidime (30 μ g), ceftriaxone (30 μ g), cefixime (5 μ g), cefotaxime (30 μ g), tetracycline (30 μ g), gentamicin (10 μ g), nalidixic acid (30 μ g), chloramphenicol (30 μ g), ciprofloxacin (5 μ g), azithromycin (15 μ g) and imipenem (10 μ g) (Mast Diagnostics, Bootle, UK).

Detection of extended spectrum β -lactamase (ESBL) and AmpC by phenotypic methods

Shigella spp. isolates were subjected to double-disk synergy testing, combination disk diffusion methods, and AmpC disk testing and phenyl boronic acid assays for identification of ESBL and AmpC resistance according to the 2016 CLSI guidelines.⁹ The MICs of 19 of the isolates to ciprofloxacin were determined by the E-test (Liofilchem, Roseto degli Abruzzi, Italy).

Genotypic investigations

DNA extraction

DNA extraction was performed directly from the stool samples and the reference *S. sonnei* and *S. flexneri* isolates using the QIAamp DNA Stool Minikit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The amount and purity of extracted DNA were measured using a NanoDrop device (Thermo Fisher Scientific, Waltham, MA, USA).

Genotypic identification

The *ipaH* gene was used as a genetic marker for confirming the *Shigella* genus by PCR.¹⁰ PCR assays were performed to detect the *stx* gene for Shiga toxin-producing isolates and the ESBL genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M-1, 2, 8, 14, 15}) for the ESBL-producing *Shigella* spp. isolates. In addition, AmpC genes (*bla*_{CMY-2}, *bla*_{CIT}, *bla*_{ACC}, *bla*_{FOX}, *bla*_{MOX}, and *bla*_{DHA}) were also detected by PCR.^{11–13} PCR was carried out to amplify plasmid-mediated quinolones resistance (PMQR) targets for *qnr* determinants including *qnrA*, *qnrB*, and *qnrS*. Mutations in *gyrA* and *parC* among the fluoroquinolone-resistant *Shigella* spp. isolates were identified with the use of DNA sequencing as well.^{11,13}

Integron detection

To detect and distinguish class 1, 2 and 3 integrons, PCR analysis was performed as described before.^{3,10,11}

Results

Out of the 230 pediatric infectious diarrhea samples, 19 (8.2%) cases of shigellosis were identified using the culture method. All culture-positive samples were identified as positive using the PCR method and an additional seven samples which were culture-negative were identified as positive using the PCR method with specific primers 26 (11.3%). Of 230 patients included in the study, 10 (52.6%) male and 9 (47.3%) female patients were infected by *Shigella* spp., giving an infection ratio of males to females of 1.1:1. The average age of the patients was 6 years and 4 months. The youngest of these patients was a boy aged one year and 10 months, and the oldest was a 16-year-old boy. The majority of *Shigella* spp. infections (7 cases, 36.8%) were observed in pediatric patients between 5 and 10 years of age. Clinical symptoms of pediatric patients are given in Table 1.

Phenotypic and genotypic investigation

Of the 19 cultured *Shigella* spp. isolates, 15 (78.9%) were identified as *S. sonnei* and 4 (21%) as *S. flexneri*. No Shiga toxin-producing isolate was found.¹⁴

Phenotypic and genotypic antibiotic resistance determination

Using the CLSI 2016 guidelines, the highest resistance rates in *Shigella* spp. were found against cotrimoxazole (19/19; 100%), ampicillin (16/19; 84.2%), cefixime (13/19; 68.4%), ceftriaxone (12/19; 63.1%), and cefotaxime

(12/19; 63.1%). All *Shigella* isolates were susceptible to azithromycin and imipenem (Table 2). Ten *Shigella* spp. isolates were ESBL positive. All isolates contained *bla*_{TEM}, *bla*_{CTX-M-1}, and *bla*_{CTX-M-15} resistance genes. In addition, all AmpC positive isolates (3/19; 15.8%) were *bla*_{CMY-2} positive and were resistant to ciprofloxacin and nalidixic acid; only 2 (66.6%) were *bla*_{CIT} type positive. (Table 2). Of the 19 *Shigella* isolates studied, 5 (26.3%), 2 (10.5%), and 1 (5.2%) showed resistance to nalidixic acid, ciprofloxacin, and norfloxacin, respectively. Among the PMQR determinants, *qnrS* was positive only in 60% (3/5) of the isolates. In addition, no isolates harbored *qnrA* or *qnrB* genes. The majority of the *Shigella* spp. isolates (89.4%) were multidrug resistant (Table 3). All the isolates harboring PMQR had the same mutations in *gyrA* at amino acid 83 (replacement of serine with leucine) and in *parC* at amino acid 80 (replacement of serine with isoleucine; GenBank accession no. HM068910). Out of 5 (26.3%) fluoroquinolone-resistant isolates, 4 (80%) were *S. sonnei* and 1 (20%) was identified as *S. flexneri*. Also, 3 of the fluoroquinolone-resistant isolates (two *S. sonnei* and one *S. flexneri*) were resistant to the TGC and fourth-GC and presented AmpC producer. Class 1 integrons were found in a total of 18 (94.7%) strains: *S. sonnei* (n=14) and *S. flexneri* (n=4), whereas class 2 integrons were found in all strains: *S. sonnei* (n=15) and *S. flexneri* (n=4); Class 3 integrons were not found (Table 4).

Discussion

In total, an 8.2% prevalence of Shigellosis was defined using cultures and 11.3% using PCR. The sensitivity of the PCR method is higher than those of the culture methods.^{15,16} However, our result was almost similar to studies conducted in Kerman, Iran and the North of Ethiopia, which had reported frequencies of 9% and 13.3%, respectively.^{17,18} Our result was different from studies conducted in India which had reported frequencies close to 2%.¹⁹ These differences in frequency of Shigellosis may be related to age, economic development level, geography, climate as well as many other environmental conditions. Facilities such as general water supply systems and sewage systems, closely related to the level of sanitation and individual hygiene, contamination of food, and improper medical health-care levels may be missing or of poor quality.

S. sonnei is more generally found in industrialized countries, whereas *S. flexneri* seems overrepresented in the developing world.¹ Our results show that *S. sonnei*

Table 1 Classification of age groups and clinical symptoms of pediatric patients infected by *Shigella*spp

Age grouping	Gender male/female	Shigella spp		Mucus in the stool		Blood in the stool		Abdominal pain/Tenesmus		Fever		Vomiting	
		S. flexneri	S. sonnei	S. flexneri	S. sonnei	S. flexneri	S. sonnei	S. flexneri	S. sonnei	S. flexneri	S. sonnei	S. flexneri	S. sonnei
1-2	Male Female	1 0	2 1	1 0	2 1	1 0	2 1	1 0	2 1	0 0	2 0	0 0	0 0
Total:		1/4, 25%	3/15, 20%	1/1, 100%	3/3, 100%	1/1, 100%	3/3, 100%	1/1, 100%	3/3, 100%	0%	2/3, 66.6%	0%	0%
3-4	Male Female	0 0	2 2	0 0	2 2	0 0	1 2	0 0	1 2	0 0	0 2	0 0	0 0
Total:		0%	4/15, 26.6%	0%	4/4, 100%	0%	3/4, 75%	0%	3/4, 75%	0%	2/4, 50%	0%	0%
5-10	Male Female	1 1	2 3	1 1	2 3	1 1	2 3	1 1	2 3	1 0	0 3	0 0	0 1
Total:		2/4, 50%	5/15, 33.3%	2/2, 100%	5/5, 100%	2/2, 100%	5/5, 100%	2/2, 100%	5/5, 100%	1/2, 50%	3/5, 60%	0%	1/5, 20%
10>	Male Female	0 1	2 1	0 1	2 1	0 1	2 1	0 1	2 1	0 1	2 0	0 0	1 0
Total:		1/4, 25%	3/15, 20%	1/1, 100%	3/3, 100%	1/1, 100%	3/3, 100%	1/1, 100%	3/3, 100%	1/1, 100%	2/3, 66.6%	0%	1/3, 33.3%
Final total		4/19, 21%	15/19, 78.9%	4/4, 100%	15/15, 100%	4/4, 100%	14/15, 93.3%	4/4, 100%	14/15, 93.3%	2/4, 50%	9/15, 60%	0%	2/15, 13.3%

Table 2 Frequency of antibiotic resistance among *Shigella* isolates

Antibiotic	<i>Shigella</i> spp., n=19	<i>Shigella sonnei</i> , n=15	<i>Shigella flexneri</i> , n=4
Cotrimoxazole	19 (100%)	15 (100%)	4 (100%)
Ampicillin	16 (84.2%)	12 (80%)	4 (100%)
Cefixime	13 (68.4%)	11 (73.3%)	2 (50%)
Cefotaxime	12 (63.1%)	11 (73.3%)	1 (25%)
Ceftriaxone	12 (63.1%)	11 (73.3%)	1 (25%)
Ceftazidime	6 (31.5%)	6 (40%)	0%
Ceftizoxime	4 (21%)	4 (26.6%)	0%
Cefoxitin	3 (15.7%)	2 (13.3%)	1 (25%)
Tetracycline	7 (36.8%)	4 (26.6%)	3 (75%)
Gentamicin	7 (36.8%)	6 (40%)	1 (25%)
Chloramphenicol	2 (10.5%)	0(0%)	2 (50%)
Nalidixic acid	5 (26.3%)	4 (26.6%)	1 (25%)
Ciprofloxacin	2 (10.5%)	2(13.3%)	0%
Norfloxacin	1 (5.2%)	1 (6.6%)	0%
Azithromycin	0%	0%	0%
Imipenem	0%	0%	0%
ESBL	10 (52.6%)	9 (90%)	1 (10%)
AmpC	3 (15.8%)	2 (66.6%)	1 (33.3%)
MDR	17 (89.4%)	13 (86.6%)	4 (100%)

was the major species in this study and comprised 78.9% of all *Shigella* spp., followed by *S. flexneri* with 21%. This finding is similar to recently reported data from Iran (Shiraz)²⁰ as well as other countries including Thailand.²⁰ However, this pattern is different from other studies in Iran (Abadan), south of Iran²¹ and countries such as India²² where the most common species was *S. flexneri*. During recent decades, most cases of shigellosis in Iran were caused by *S. flexneri*, although recent studies have shown an increasing number of infections caused by *S. sonnei*.^{20,23} Socioeconomic conditions and general hygiene affect the frequency of *Shigella* spp. serogroups.²⁴

In this study, no shiga toxin-producing isolate was found. In similar studies, prevalence of *stx* gene was reported to be 1.4% in *S. dysenteriae* in Tehran (Iran), 3 (1.4%) of *S. flexneri* isolates in Canada and 21% *S. flexneri* and *S. dysenteriae* in France.^{25–27}

Because shigellosis is highly contagious, information about the frequency of the disease and the antimicrobial susceptibility of the strains is important to ensuring suitable clinical treatment and patient management.²⁸ Mild symptoms are self-limiting, but antibiotics are advised to reduce the duration of diarrhea in case of severe dysentery

cases. The antibiotic resistance properties of *Shigella* spp are various, and regionally distinct.²⁹ We provide the first report regarding the prevalence of shigellosis and associated resistance patterns to a panel of 16 antibiotics in the center of Iran. The appearance of multidrug-resistant (MDR) strains of *Shigella* spp is a growing concern across the globe.¹¹

Cotrimoxazole is a drug often used for empirical therapy of diarrheal diseases; extensive use of this drug has led to the advent of resistant *Shigella* spp. strains.³⁰ In this study, *Shigella* spp. showed a high rate of resistance to cotrimoxazole (100%). Previous reports in Iran have reported a 92.2% to 94% resistance level to cotrimoxazole (2000–2017).^{30,31} A high-level resistance to cotrimoxazole has also been reported from Turkey (95%).³²

Based on the reports from our region, resistance to ampicillin ranged from 12–20% to 87%.^{33,34} We here document 84.2%, and a previous study reported 57% from Iran.³⁵ These results strongly suggest that cotrimoxazole and ampicillin can no longer be empirically used for the treatment of severe diarrhea and dysentery in central Iran.

A previous Iranian study from Tabriz found that 4.2% of *Shigella* spp. isolates were resistant to ciprofloxacin.¹¹ Ciprofloxacin-resistant *S. flexneri* was also recognized from parts of India (56.2%).¹⁹ In the present study, 5 (26.3%) of *Shigella* isolates were resistant to nalidixic acid, a marked increase in resistance to nalidixic acid. In two reports from Tehran and Tabriz, Iran, 17.4% and 31% of *Shigella* spp. isolates were resistant to nalidixic acid, respectively.^{11,36} In China, Pu et al³⁷ found that 5 (33.3%) of 15 fluoroquinolone-resistant isolates contained the *qnrS* gene. The findings of the current study regarding other PMQR genes (*qnrA* and *qnrB*) are in accordance with the findings of Taneja et al,³⁸ who explained that none of the *Shigella* spp. isolates they found were positive for *qnrA* and *qnrB* genes.

In this study, high resistance (21–68.4%) to TGC was observed in the *Shigella* spp. isolates. Previous studies from Iran have also indicated the resistance of *Shigella* spp. to cephalosporins at the range of 7.3–57.7% between 2008 and 2018.¹¹ This indicated that the rate of resistance to cephalosporins is growing among *Shigella* spp. in Iran, similar to what is happening in other countries.³⁹ This finding is alarming and contrary to previous studies from China, the Middle East, and Southeast Asia which have reported lower level resistance to cephalosporins (2.0–15.1%).^{11,12}

In the present investigation, *bla*_{TEM}, *bla*_{CTX-M-1}, and *bla*_{CTX-M-15} were the most common ESBL genes, and *bla*_{CMY-2} and *bla*_{CIT} were the most common AmpC genes.

Table 3 Frequency of antibiotic resistance genes among *Shigella* isolates

Resistance	Target gene	Frequency of resistance genes in <i>Shigella</i> spp.	Frequency of resistance genes in <i>Shigella sonnei</i>	Frequency of resistance genes in <i>Shigella flexneri</i>
Shiga toxin sulfonamide, n=19	<i>Stx</i>	0%	0%	0%
	<i>Sul1</i>	0%	0%	0%
	<i>Sul2</i>	19 (100%)	15 (100%)	4 (100%)
ESBL+, n=10	<i>bla</i> _{TEM}	10 (100%)	9 (100%)	1 (100%)
	<i>bla</i> _{CTX-M-1}	10 (100%)	9 (100%)	1 (100%)
	<i>bla</i> _{CTX-M-15}	10 (100%)	9 (100%)	1 (100%)
	<i>bla</i> _{CTX-M-14}	9 (90%)	9 (100%)	0%
	<i>bla</i> _{SHV}	1 (10%)	1 (11.1%)	0%
	<i>bla</i> _{CTX-M-2}	1 (10%)	1 (11.1%)	0%
	<i>bla</i> _{CTX-M-8}	0%	0%	0%
AmpC+, n=3	<i>bla</i> _{CMY-2}	3 (100%)	2 (100%)	1 (100%)
	<i>bla</i> _{CIT}	2 (66.6%)	2 (100%)	0%
	<i>bla</i> _{ACC}	1 (33.3%)	1 (100%)	0%
	<i>bla</i> _{FOX}	0%	0%	0%
	<i>bla</i> _{MOX}	0%	0%	0%
	<i>bla</i> _{DHA}	0%	0%	0%
Fluoroquinolone, n=5	<i>gyrA</i>	5 (100%)	4 (100%)	1 (100%)
	<i>parC</i>	5 (100%)	4 (100%)	1 (100%)
	<i>qnrS</i>	3 (60%)	2 (50%)	1 (100%)
	<i>qnrA</i>	0%	0%	0%
	<i>qnrB</i>	0%	0%	0%
Integrase, n=19	<i>Int1</i>	18 (94.7%)	14 (93.3%)	4 (100%)
	<i>Int2</i>	19 (100%)	15 (100%)	4 (100%)
	<i>Int3</i>	0%	0%	0%
Quaternary ammonium compounds, n=19	<i>qac</i>	9 (47.3%)	8 (53.3%)	1 (25%)

The current report shows that resistance to β -lactams mediated by *bla*_{CMY} enzymes in Iran has a similar pattern as in other parts of the world. In recent years, various ESBL-producing *Shigella* were also reported from Argentina (*bla*_{CTX-M-2}), Korea and China (*bla*_{CTX-M-14}), and Vietnam (*bla*_{CTX-M-15} and *bla*_{CTX-M-24}).^{40–42} *bla*_{CMY-2} type AmpC β -lactamase producers were predominant. However, in several other studies, the *bla*_{CMY-2}-type AmpC β -lactamases were the most prevalent cephalosporinases in Iran (Tehran) and Taiwan.^{43,44}

In this study, class 1 integrons were found in 18 (94.7%) strains, whereas class 2 integrons were found in 19 (100%) of the strains. In Brazil, it was found that class 1 integrons were achieved in only two strains, whereas class 2 integrons were achieved in 56 (90.3%) of the strains.⁴⁵ Of the three classes of integrons linked to antimicrobial resistance, the class I integron is the most frequently found in Gram-negative

bacteria.⁴⁶ The class II integron is the most predominant integron in *S. sonnei*.⁴⁷ The prevalence of integrons in the Enterobacteriaceae family has been varied and played an important role in the development of drug-resistant bacteria. Therefore, a high prevalence of antibiotic resistance is probably related to a high prevalence of class I and II integrons. In epidemiologic studies, particularly of infectious diseases caused by bacteria, typing of the isolates is a useful method for source tracing and understanding the pathogenesis and eventual disease prevention. One limitation of the present study was that typing of *Shigella* isolates was not performed.

Conclusion

The recent emergence of *S. sonnei* in developing countries reinforces the need for effective epidemiological surveillance systems.⁴⁸ The wide distribution of MDR *Shigella* spp.

Table 4 Phenotypic and genotypic antibiotic resistance rates in *Shigella* spp

Antibiotic	<i>Shigella</i> spp, n=19	<i>Shigella sonnei</i> , n=15	<i>Shigella flexneri</i> , n=4	Resistance	Target gene	Frequency of resistance genes	Resistance	Target gene	Frequency of resistance genes
Cotrimoxazole	19 (100%)	15 (100%)	4 (100%)	Sulfonamide	<i>SulI</i>	0 (0%)			
					<i>Sul2</i>	19 (100%)			
Ampicillin	16 (84.2%)	12 (80%)	4 (100%)						
Cefixime	13 (68.4%)	11 (73.3%)	2 (50%)	ESBL+	<i>bla_{TEM}</i>	10 (100%)	AmpC+	<i>bla_{CMY2}</i>	3 (100%)
Cefotaxime	12 (63.1%)	11 (73.3%)	1 (25%)		<i>bla_{CTX-M1}</i>	10 (100%)		<i>bla_{CIT}</i>	2 (66.6%)
Ceftriaxone	12 (63.1%)	11 (73.3%)	1 (25%)		<i>bla_{CTX-M15}</i>	10 (100%)		<i>bla_{AAC}</i>	1 (33.3%)
Ceftazidime	6 (31.5%)	6 (40%)	0%		<i>bla_{CTX-M14}</i>	9 (90%)		<i>bla_{FOX}</i>	0%
Ceftizoxime	4 (21%)	4 (26.6%)	0%		<i>bla_{SHV}</i>	1 (10%)		<i>bla_{MOX}</i>	0%
Cefoxitin	3 (15.7%)	2 (13.3%)	1 (25%)		<i>bla_{CTX-M2}</i>	1 (10%)		<i>bla_{DHA}</i>	0%
					<i>bla_{CTX-M8}</i>	0%			
Tetracycline	7 (36.8%)	4 (26.6%)	3 (75%)						
Gentamicin	7 (36.8%)	6 (40%)	1 (25%)						
Chloramphenicol	2 (10.5%)	0(0%)	2 (50%)						
Nalidixic acid	5 (26.3%)	4 (26.6%)	1 (25%)	Fluoroquinolone	<i>gyrA</i>	5 (100%)			
Ciprofloxacin	2 (10.5%)	2(13.3%)	0%		<i>parC</i>	5 (100%)			
Norfloxacin	1 (5.2%)	1 (6.6%)	0%		<i>qnrS</i>	3 (60%)			
					<i>qnrA</i>	0%			
					<i>qnrB</i>	0%			
Azitromycine	0%	0%	0%						
Imipenem	0%	0%	0%						

(Continued)

Table 4 (Continued)

Antibiotic	Shigella spp, n=19	Shigella sonnei, n=15	Shigella flexneri, n=4	Resistance	Target gene	Frequency of resistance genes	Resistance	Target gene	Frequency of resistance genes
		14 (93.3%)	3 (75%)	Integrase	Int1	17 (94.7%)			
		15 (100%)	4 (100%)		Int2	19 (100%)			
		0%	0%		Int3	0%			
		8 (53.3%)	1 (25%)	Quaternary ammonium compounds	qac	9 (47.3%)			
MDR	17 (89.4%)	13 (86.6%)	4 (100%)						

isolates and the continuing emergence of ESBL-producing and ciprofloxacin-resistant isolates were shown in the study. The high prevalence of ESBL-producing genes in *Shigella* spp. isolates in pediatric patients in our study can be a major challenge for dysentery treatment. We found that the frequency of ESBL producing *Shigella* spp. isolates was higher than those in many other countries. Our results enhance concerns about the dissemination of ESBL among the strains of endemic *S. sonni* throughout the country, because it is now the most frequently isolated *Shigella* species in Iran. Thus, to prevent outbreaks due to these resistant isolates, the surveillance of the antimicrobial resistance of *Shigella* spp. isolates should be continuously considered, and empiric antibiotic therapy should be adapted appropriately.

Abbreviation list

ESBL, third generation cephalosporins; MDR, multidrug resistance; TGC, third generation cephalosporins; GN, Gram-negative; XLD, xylose lysine deoxycholate; CLSI, Clinical and Laboratory Standards Institute; PMQR, plasmid mediated quinolones resistance.

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Disclosure

AvB is an employee of bioMerieux, a company designing, developing and selling infectious disease tests. The company had no influence on the design and execution, either of the study or in the writing of the manuscript. The authors report no other conflicts of interest in this work.

References

1. Taneja N, Mewara A. Shigellosis: epidemiology in India. *Indian J Med Res.* 2016;143(5):565. doi:10.4103/0971-5916.187104
2. Williams PCM, Berkley JA. Guidelines for the treatment of severe acute malnutrition: a systematic review of the evidence for antimicrobial therapy. *Paediatr Int Child Health.* 2018;38(sup1):S32–S49. doi:10.1080/20469047.2017.1409453
3. Chang C-Y, Lu P-L, Lin -C-C, Lee T-M, Tsai M-Y, Chang -L-L. Integron types, gene cassettes, antimicrobial resistance genes and plasmids of *Shigella sonnei* isolates from outbreaks and sporadic cases in Taiwan. *J Med Microbiol.* 2011;60(2):197–204. doi:10.1099/jmm.0.022517-0

4. World Health Organization. *Guidelines for the Control of Shigellosis, Including Epidemics Due to Shigella Dysenteriae Type 1*, World Health Organization. Available from: <http://www.who.int/iris/handle/10665/43252>. Accessed May 29, 2019.
5. Li YL, Tewari D, Yealy CC, Fardig D, M'ikanatha NM. Surveillance for travel and domestically acquired multidrug-resistant human Shigella infections—Pennsylvania, 2006–2014. *Health Secur*. 2016;14(3):143–151. doi:10.1089/hs.2016.0026
6. Barrantes K, Achi R. The importance of integrons for development and propagation of resistance in Shigella: the case of Latin America. *Braz J Microbiol*. 2016;47(4):800–806. doi:10.1016/j.bjm.2016.07.019
7. Mazaheri M, Haji Rezaei M, Aalinezhad M, Sharif MR, Akhavan T. Clinical and Laboratory Characteristics of Pediatric Campylobacter spp. Acute Gastroenteritis, Arch Pediatr Infect Dis. 2016;4(4):e35730. doi:10.5812/pedinfect.35730.
8. Garcia L, Isenberg H. *Clinical Microbiology Procedures Handbook*. Washington, DC: American Society for Microbiology; 2010.
9. Clinical, Institute LS. *Performance Standards for Antimicrobial Testing: Twenty-Sixth Informational Supplement M100-S26*. Wayne, PA, USA: CLSI; 2016.
10. Alizadeh-Hesar M, Bakhshi B, Najar-Peerayeh S. Clonal dissemination of a single Shigella sonnei strain among Iranian children during fall 2012 in Tehran, IR Iran. *Infect*. 2015;34:260–266. doi:10.1016/j.meegid.2015.06.024
11. Zamanlou S, Ahangarzadeh Rezaee M, Aghazadeh M, Ghotaslou R, Babaie F, Khalili Y. Characterization of integrons, extended-spectrum β -lactamases, AmpC cephalosporinase, quinolone resistance, and molecular typing of Shigella spp. *Infect Dis*. 2018;50(8):616–624. doi: 10.1080/23744235.2018.1455222.
12. Taneja N, Mewara A, Kumar A, Verma G, Sharma M. Cephalosporin-resistant Shigella flexneri over 9 years (2001–09) in India. *J Antimicrob Chemother*. 2012;67(6):1347–1353. doi:10.1093/jac/dks061
13. Bhattacharya D, Bhattacharya H, Thamizhmani R, et al. Shigellosis in Bay of Bengal Islands, India: clinical and seasonal patterns, surveillance of antibiotic susceptibility patterns, and molecular characterization of multidrug-resistant Shigella strains isolated during a 6-year period from 2006 to 2011. *Eur J Clin Microbiol Infect Dis*. 2014;33(2):157–170. doi:10.1007/s10096-013-1937-2
14. Toma C, Lu Y, Higa N, et al. Multiplex PCR assay for identification of human diarrheagenic Escherichia coli. *J Clin Microbiol*. 2003;41(6):2669–2671. doi:10.1128/jcm.41.6.2669-2671.2003
15. Mamuye Y, Metaferia G, Birhanu A, Desta K, Fantaw S. Isolation and antibiotic susceptibility patterns of Shigella and Salmonella among under 5 children with acute diarrhoea: a cross-sectional study at selected public health facilities in Addis Ababa, Ethiopia. *Clin Microb*. 2015. doi:10.4172/2327-5073.1000186
16. Ranjbar R, Bolandian M, Behzadi P. Virulotyping of Shigella spp. isolated from pediatric patients in Tehran, Iran. *Acta Microbiol Immunol Hung*. 2017;64(1):71–80. doi:10.1556/030.64.2017.007
17. Nave HH, Mansouri S, Sadeghi A, Moradi M. Molecular diagnosis and anti-microbial resistance patterns among Shigella spp. isolated from patients with diarrhea. *Gastroenterol Hepatol Bed Bench*. 2016;9(3):205.
18. Kahsay AG, Teklemariam Z. Prevalence of Shigella among diarrheic children under-5 years of age attending at Mekelle health center, north Ethiopia. *BMC Res Notes*. 2015;8(1):788. doi:10.1186/s13104-015-1752-1
19. Aggarwal P, Uppal B, Ghosh R, et al. Multi drug resistance and extended spectrum beta lactamases in clinical isolates of Shigella: a study from New Delhi, India. *Travel Med Infect Dis*. 2016;14(4):407–413. doi:10.1016/j.tmaid.2016.05.006
20. Farshad S, Sheikhi R, Japoni A, Basiri E, Alborzi A. Characterization of Shigella strains in Iran by plasmid profile analysis and PCR amplification of ipa genes. *J Clin Microbiol*. 2006;44(8):2879–2883. doi:10.1128/JCM.00310-06
21. Jomezadeh N, Babamoradi S, Kalantar E, Javaherizadeh H. Isolation and antibiotic susceptibility of Shigella species from stool samples among hospitalized children in Abadan, Iran. *Gastroenterol Hepatol Bed Bench*. 2014;7(4):218.
22. Sangeetha A, Parija SC, Mandal J, Krishnamurthy S. Clinical and microbiological profiles of shigellosis in children. *J Health Popul Nutr*. 2014;32(4):580.
23. Ranjbar R, Dallal MMS, Talebi M, Pourshafie MR. Increased isolation and characterization of Shigella sonnei obtained from hospitalized children in Tehran, Iran. *J Health Popul Nutr*. 2008;26(4):426.
24. Zhang J, Jin H, Hu J, et al. Antimicrobial resistance of Shigella spp. from humans in Shanghai, China, 2004–2011. *Diagn Microbiol Infect Dis*. 2014;78(3):282–286. doi:10.1016/j.diagmicrobio.2013.11.023
25. Yaghoubi S, Ranjbar R, Dallal MMS, Fard SY, Shirazi MH, Mahmoudi M. Profiling of virulence-associated factors in Shigella species isolated from acute pediatric diarrheal samples in Tehran, Iran. *Osong Public Health Res Perspect*. 2017;8(3):220. doi:10.24171/j.phrp.2017.8.3.09
26. Bekal S, Pilon PA, Cloutier N, Doualla-Bell F, Longtin J. Identification of Shigella flexneri isolates carrying the Shiga toxin 1-producing gene in Quebec, Canada, linked to travel to Haiti. *Can J Microbiol*. 2015;61(12):995–996. doi:10.1139/cjm-2015-0538
27. Gray MD, Lacher DW, Leonard SR, et al. Prevalence of Shiga toxin-producing Shigella species isolated from French travellers returning from the Caribbean: an emerging pathogen with international implications. *Clin Microbiol Infect Dis*. 2015;21(8):765.e9–e14. doi:10.1016/j.cmi.2015.05.006
28. Singh -K-KB, Ojha SC, Deris ZZ, Rahman RA. A 9-year study of shigellosis in Northeast Malaysia: antimicrobial susceptibility and shifting species dominance. *J Public Health (Bangkok)*. 2011;19(3):231–236. doi:10.1007/s10389-010-0384-0
29. Williams PC, Berkley JA. Guidelines for the treatment of dysentery (shigellosis): a systematic review of the evidence. *Paediatr Int Child Health*. 2018;38(sup1):S50–S65. doi:10.1080/20469047.2017.1409454
30. Pourakbari B, Mamishi S, Mashoori N, et al. Frequency and antimicrobial susceptibility of Shigella species isolated in children medical center hospital, Tehran, Iran, 2001–2006. *Braz J Infect Dis*. 2010;14(2):153–157.
31. Nikfar R, Shamsizadeh A, Darbor M, Khaghani S, Moghaddam M. A Study of prevalence of Shigella species and antimicrobial resistance patterns in paediatric medical center, Ahvaz, Iran. *Iran J Microbiol*. 2017;9(5):277.
32. Kacmaz B, Unaldi O, Sultan N, Durmaz R. Drug resistance profiles and clonality of sporadic Shigella sonnei isolates in Ankara, Turkey. *Braz J Microbiol*. 2014;45(3):845–849.
33. Ashkenazi S, Levy I, Kazaronovski V, Samra Z. Growing antimicrobial resistance of Shigella isolates. *J Antimicrob Chemother*. 2003;51(2):427–429.
34. Akcali A, Levent B, Akbaş E, Esen B. Typing of Shigella sonnei strains isolated in some provinces of Turkey using antimicrobial resistance and pulsed field gel electrophoresis methods. *Mikrobiyol Bul*. 2008;42(4):563–572.
35. Jafari F, Hamidian M, Rezadehbashi M, et al. Prevalence and antimicrobial resistance of diarrheagenic Escherichia coli and Shigella species associated with acute diarrhea in Tehran, Iran. *Can J Infect Dis Med Microbiol*. 2009;20(3):e56–e62.
36. Ranjbar R, Behnood V, Memariani H, Najafi A, Moghbeli M, Mamma C. Molecular characterisation of quinolone-resistant Shigella strains isolated in Tehran, Iran. *J Glob Antimicrob Resist*. 2016;5:26–30. doi:10.1016/j.jgar.2016.01.010
37. Pu X-Y, Pan J-C, Wang H-Q, Zhang W, Huang Z-C, Gu Y-M. Characterization of fluoroquinolone-resistant Shigella flexneri in Hangzhou area of China. *J Antimicrob Chemother*. 2009;63(5):917–920. doi:10.1093/jac/dkp087

38. Taneja N, Kumar A, Appannanavar S, Verma G, Sharma M. Plasmid-mediated quinolone resistance in *Shigella* isolates over a decade in India. *J Glob Antimicrob Resist*. 2014;2(1):59–60. doi:10.1016/j.jgar.2013.10.006
39. Varghese S, Aggarwal A. Extended spectrum beta-lactamase production in *Shigella* isolates-A matter of concern. *Indian J Med Microbiol*. 2011;29(1):76. doi:10.4103/0255-0857.76534
40. Pai H, Choi E-H, Lee H-J, Hong JY, Jacoby GA. Identification of CTX-M-14 extended-spectrum β -lactamase in clinical isolates of *Shigella sonnei*, *Escherichia coli*, and *Klebsiella pneumoniae* in Korea. *J Clin Microbiol*. 2001;39(10):3747–3749. doi:10.1128/JCM.39.10.3747-3749.2001
41. Radice M, Gonzealez C, Power P, Vidal M, Gutkind G. Third-generation cephalosporin resistance in *Shigella sonnei*, Argentina. *Emerg Infect Dis*. 2001;7(3):442. doi:10.3201/eid0703.017313
42. Nhu N, Vinh H, Nga T, Stabler R, Duy P. The sudden dominance of blaCTX-M harbouring plasmids in *Shigella* spp. Circulating in Southern Vietnam. *PLoS Negl Trop Dis*. 2010;4(6):e702. doi:10.1371/journal.pntd.0000702.
43. Tajbakhsh M, García Migura L, Rahbar M, et al. Antimicrobial-resistant *Shigella* infections from Iran: an overlooked problem? *J Antimicrob Chemother*. 2012;67(5):1128–1133. doi:10.1093/jac/dks023
44. Huang I-F, Chiu C-H, Wang M-H, Wu C-Y, Hsieh K-S, Chiou CC. Outbreak of dysentery associated with ceftriaxone-resistant *Shigella sonnei*: first report of plasmid-mediated CMY-2-type AmpC β -lactamase resistance in *S. sonnei*. *J Clin Microbiol*. 2005;43(6):2608–2612. doi:10.1128/JCM.43.6.2608-2612.2005
45. Peirano G, Agersø Y, Aarestrup FM, Dos Prazeres Rodrigues D. Occurrence of integrons and resistance genes among sulphonamide-resistant *Shigella* spp. from Brazil. *J Antimicrob Chemother*. 2005;55(3):301–305. doi:10.1093/jac/dki012
46. Kang HY, Jeong YS, Oh JY, et al. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J Antimicrob Chemother*. 2005;55(5):639–644. doi:10.1093/jac/dki076
47. Pan J-C, Ye R, Meng D-M, Zhang W, Wang H-Q, Liu K-Z. Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. *J Antimicrob Chemother*. 2006;58(2):288–296. doi:10.1093/jac/dk1228
48. The HC, Thanh DP, Holt KE, Thomson NR, Baker S. The genomic signatures of *Shigella* evolution, adaptation and geographical spread. *Nat Rev Microbiol*. 2016;14(4):235. doi:10.1038/nrmicro.2016.10

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