

First investigation of the presence of *SPATE* genes in *Shigella* species isolated from children with diarrhea infection in Ahvaz, southwest Iran

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Background: *SPATE* (serine protease autotransporters of enterobacteriaceae) genes are considered as a group of the main virulence factors of *Shigella* species. This study aimed to investigate for the first time the distribution of *SPATE* genes among *Shigella* spp. isolated from children with diarrhea infection in Ahvaz, Iran.

Methodology: In this study, a total of 74 *Shigella* isolates were collected between August 2016 and June 2017 from feces of children with diarrhea and identified by biochemical and molecular methods for *Shigella* species. The frequency distribution of the *SPATE* genes, including *pic*, *pet*, *sat*, *sigA* and *sepA*, was evaluated using PCR. The genetic relationship of all isolates was evaluated by enterobacterial repetitive intergenic consensus-PCR.

Results: The most common species of *Shigella* was *S. flexneri*, followed by *S. sonnei* and *S. boydii*. In total, 95.94% of *Shigella* isolates had at least one of the *SPATE* genes. The presence of *pic*, *pet*, *sat*, *sigA* and *sepA* genes was confirmed among 35.13%, 27%, 47.29%, 58.1% and 39.18% of *Shigella* isolates, respectively. Of these *SPATE* genes, the *sat* and *sigA* genes were recognized as the most common autotransporters among *S. flexneri* and *S. sonnei* isolates, respectively. Also, either *S. flexneri* or *S. sonnei* isolates belonging to a same clone type had similar *SPATE* genes profile.

Conclusion: Our results revealed that the high distribution of *SPATE* genes among *Shigella* isolates in our region. Hence, this study highlights a need for epidemiological programs to monitor the distribution of *SPATE* genes locally for prevention from further dissemination of the *Shigella* isolates harboring them.

Keywords: *SPATE* genes, *Shigella* spp., PCR, antibiotic resistance

Introduction

Shigellosis is one of the main agents of diarrheal infections in developing countries especially among children <5 years of age. *Shigella* spp. isolates are the causative agents of 5–15% of all diarrheal infections worldwide¹ that have involved approximately 80–165 million cases, with over 600,000 deaths occurring each year.² The infection dose of *Shigella* spp. is as low as 10–100 organisms and over 80% of infections are due to person-to-person transmission.³ Shigellosis infection is associated with the colonization of four *Shigella* spp. – *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* – that among them *S. flexneri* and *S. sonnei* are frequently isolated from Iran⁴ and other developing countries.⁵ Many reports have also demonstrated a relatively high prevalence of shigellosis among children with diarrheal infections.^{6–8}

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The first step for the successful colonization of *Shigella* spp. in the intestine is the disruption of mucus integrity that can be facilitated by several virulence factors produced by *Shigella* spp. such as SPATE (serine protease autotransporters of enterobacteriaceae) genes.⁹ The SPATEs were first identified as autotransporters secreted by diarrheagenic *E. coli* and *Shigella*spp.; however, these genes are now detected in other members of the Enterobacteriaceae family. In *Shigella*spp., the SPATE genes were classified phylogenetically into two main classes, including the class I SPATEs that comprised of *Shigella* IgA-like protease homologue (*sigA*), secreted autotransporter toxin (*sat*) and plasmid-encoded toxin (*pet*) which are directly cytotoxic for epithelial cells, as well as the class 2 SPATEs, including *Shigella* extracellular protein A (*sepA*) and protease involved in intestinal colonization (*pic*), which facilitate the intestinal inflammation and colonization. Overall, *Shigella* isolates harboring these genes are able to induce the inflammation and extensive mucosal damages in intestinal infections, especially when these isolates encode more than one the SPATE gene.¹⁰ Hence, the understanding of the distribution of SPATE genes in *Shigella* isolates in the epidemiologic and local researches can be useful gene targets for other researchers who are designing new antimicrobial therapies against them.

The aim of this study was to investigate for the first time the presence of the genes encoding SPATEs among clinical isolates of *Shigella*spp. from children with diarrheal infection in Ahvaz, southwest, Iran.

Materials and methods

The collection of samples

We collected 3,254 stool culture of children <16 years of age with diarrheal infections referred to Golestan and Abuzar Hospitals during a period of 10 months from August 2016 to June 2017. The study design was approved by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran (IR.AJUMS.REC.1395.427). These samples were inoculated into Gram-negative (GN) Broth tubes as an enrichment medium as a part of the routine hospital laboratory procedure and then immediately transferred to the Laboratory of Microbiology Department of Medicine School of Ahvaz, Iran. From each patient, only one *Shigella* isolate in the diarrheal phase was included in this study. The GN Broth tubes were incubated at 37°C for 4–6 hrs and then streaked on Xylose Lysine

Deoxycholate Agar and Eosin Methylene Blue Agar (Merck-Germany). All plates were incubated at 37°C for 24–48 hrs. The suspected colonies were biochemically identified as *Shigella*spp. using Lysine Decarboxylase Agar, Sulfide-indole-motility, Triple-sugar iron Agar, Urea Agar and Simmons citrate Agar (Merck-Germany).¹¹

Antimicrobial susceptibility testing

Determination of antimicrobial susceptibility was performed by Kirby-Bauer-disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute.¹³ The antibiotic discs used were ceftriaxone (CRO; 30 µg), ciprofloxacin (CIP; 5 µg), trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 µg), nalidixic acid (NA;30 µg), amikacin (AK; 30 µg), gentamycin (GM; 10 µg), ceftazidime (CAZ; 30 µg) and tetracycline (TE; 30 µg). All of these plates were incubated for an overnight at 35°C and the clear zones of bacterial growth inhibition were measured with an accuracy of ±0.1 mm.¹²

DNA extraction

The whole genome DNA was extracted using boiling method as described previously.¹³

Multiplex PCR assay for molecular identification of *Shigella* spp

The Tetraplex PCR assay was setup using whole genome DNA of the reference *Shigella* strains, including *S. flexneri* ATCC 12022, *S. sonnei* ATCC 9290, *S. boydii* ATCC 9207 and *S. dysenteriae* PTCC 1188 as the positive control strains along with water as the negative control. A multiplex PCR reaction was prepared in the final volume of 25 µL, including 1 U of AmpliTaq DNA polymerase, 2 µL of genomic DNA, 1X PCR buffer, 1.5 mM MgCl₂, 200 µM dNTPs and distilled water up to the final volume of 25 µL. In this study, all primers were synthesized by Cinna gene Company, Iran. The primer concentrations were as follows: 0.2 Pmol/µL of primers SflexDF1 and SflexDR1; 0.35 Pmol/µL of primers SsonDF1 and SsonDR1; 0.15 Pmol/µL of primers SdysDF1 and SdysDR1; and 0.4 Pmol/µL of primers SboyDF1 and SboyDR1. The sequences and sizes of the primers are shown in Table 1.^{14,15} The amplification process was performed in Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle of initial denaturation at 94°C for 5 mins, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C, with a final

Table 1 Primers used for identifying each species with their sequences sizes

Name	Primer sequence (5' to 3')	Length	Target gene	Target identity	Ref
SflexDFI SflexDRI	F- TTT ATG GCT TCT TTG TCG GC R-CTG CGT GAT CCG ACC ATG	570 bp	<i>rfc</i>	<i>S. flexneri</i>	14
SsonDFI SsonDRI	F-TCT GAA TAT GCC CTC TAC GCT R-GAC AGA GCC CGA AGA ACC G	430 bp	<i>wbgZ</i>	<i>S. sonnei</i>	14
SdysDFI SdysDRI	F-TCT CAA TAA TAG GGA ACA CAG C R-CAT AAA TCA CCA GCA AGG TT	211 bp	<i>rfpB</i>	<i>S. dysenteriae</i>	14
SboyDFI SboyDRI	F- GAGCA CGGAA ACAGA GAGCGCC R- GGTGC GTTCT TCCGG TGTC TG	240bp	Hypothetical protein	<i>S. boydii</i>	15
SgenDFI SgenDRI	F- TGC CCA GTT TCT TCA TAC GC R- GAA AGTAGCTCC CGAAATGC	870 bp	<i>invc</i>	<i>Shigella genus</i>	14

extension for 7 mins at 72°C.¹⁵ All PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide and their images were visualized by Gel Documentation (Vilber Company, Germany).

Amplification of SPATE genes

The amplifications of SPATEs including the *pet*, *sat*, *pic*, *sigA* and *sepA* genes were performed on the whole genome, as previously described.¹⁰ All reactions were established using the primers and PCR conditions described in Table 2. The PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide and their images were visualized by Gel Documentation (Vilber Company, Germany).

ERIC-PCR typing and analysis

The genetic relationship of *S. flexneri* and *S. sonnei* isolates was evaluated by the enterobacterial repetitive intergenic

consensus-PCR (ERIC-PCR) using primers of ERIC-F (5'-ATGTAAGCTCCTGGGGATTAC-3') and ERIC-R (5'-AAGTAAGTGACTGGGGTGA GCG-3').¹⁶ The PCR reaction was performed in the final volume of 20 µ as follows: 1U Taq DNA polymerase, 1.5 mM MgCl₂, 200 µM dNTPs, 0.20 µM of each primer, 10x PCR buffer, 3 µL of template DNA and distilled water up to a final volume of 20 µL. The amplification process was performed in Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle of initial denaturation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 90 s, with a cycle of final extension at 72°C for 10 mins. The amplified products were resolved on agarose gel 1.5%, stained with ethidium bromide 0.5 µg/mL. The data analyses were performed using the Gel Compare II software version 6.6 (Applied Math, Sint-Martens-Latem, Belgium). The

Table 2 Primer used for the amplifications of qnr determinants and SPATE genes

Gene	Primer sequence (5' to 3')	bp	Condition PCR	Ref
<i>pic</i>	F- ACTGGATCTTAAGGCTCAGGAT R-GACTTAATGTCACTGTTTCAGCG	572	94°C, 15 mins; 94°C, 30 s; 58°C, 45 s; 72°C, 45 s (35 cycles)	11
<i>pet</i>	F- GGCACAGAATAAAGGGGTGTTT R-CCTCTTGTTTCCACGACATAC	302		
<i>sepA</i>	F- GCAGTGGAAATATGATGCGGC R-TTGTTTCAGATCGGAGAAGAACG	794		
<i>sigA</i>	F- CCGACTTCTCACTTTCTCCCG R-CCATCCAGCTGCATAGTGTGTTG	430		
<i>sat</i>	F- TCAGAAGCTCAGCGAATCATTG R-CCATTATCACCAGTAAAACGCACC	930		

similarity pattern was calculated using the Unweighted-Pair Group Method/the Dice similarity coefficient with a position tolerance of 1.5%. Isolates with more than 90% similarity were considered as clonal type.

Statistical analysis

The descriptive statistic tests were performed in SPSS version 16.00.

Results

Identification of *Shigella* spp. and determination of antibiotic susceptibility

In our study, of 3,254 stool samples, 74 (2.27%) were positive for *Shigella* species using bacterial culture. Of those, 736 samples (22.6%) belong to children <2 years of age, 435 (13.4%) samples in the age group between 2 and 5 years, 1,095 (33.6%) samples in the age group between 6 and 10 years and 988 (30.36%) samples in the age group between 11 and 15 years. According to PCR results, the most common *Shigella* species was *S. flexneri* (36 isolates; 48.6%), followed by *S. sonnei* (33 isolates; 46.6%) and *S. boydii* (five isolates; 6.7%). The distribution of *Shigella* spp. isolates in different age groups is shown in Table 3. According to our study findings, both *S. sonnei* and *S. flexneri* were more prevalent in the age group of 6–10 years of age than other groups.

The results of antibiotic susceptibility assay demonstrated that the majority of these isolates were resistant to trimethoprim/sulfamethoxazole (87.8%) and tetracycline (67.6%), followed by ceftriaxone (51.5%), nalidixic acid (28.4%) and ceftazidime (20.3%). However, the rates of resistance

to gentamycin (2.7%), amikacin (2.7%) and ciprofloxacin (6.7%) were low. The frequency rates of antibiotic resistance of all strains are shown in Table 4. According to these results, all *S. boydii* strains were resistant to TMP-SXT.

Frequency of class 1 and 2 SPATEs

We screened the presence of class 1 and 2 SPATEs among *Shigella* spp. (Table 5). Moreover, among 36 *S. flexneri* isolates, 12 (33.33%) harbored only class 1 and 24 (66.66%) harbored both of class 1 and 2 SPATE genes while any strain did not harbor only class 2 SPATEs. On the other hand, of 33 *S. sonnei* isolates, 11 (33.33%) harbored only class 1, 2 (6.06%) harbored only class 2 and 19 (57.57%) harbored both of class 1 and 2 SPATE genes, while only one *S. sonnei* strain harbored neither of class 1 nor class 2 SPATEs. Also, of five *S. boydii* strains, only one strain harbored the *sigA* gene, one harbored the *sepA* gene and one harbored both the *sigA* and *sepA* genes.

Of 36 *S. flexneri* isolates, the genes encoding *pet*, *sat*, *sigA*, *sepA* and *pic* were detected among 7 (19.44%), 27 (75%), 25 (69.44%), 13 (36.11%) and 14 (38.88%) isolates, respectively. Also, of 33 *S. sonnei* isolates, the genes encoding *pet*, *sat*, *sigA*, *sepA* and *pic* were detected among as well as 13 (39.39%), 8 (24.24%), 17 (51.51%), 14 (42.42%) and 12 (36.36%), respectively.

Genetic diversity of *S. flexneri* and *S. sonnei* isolates

In our study, 36 *S. flexneri* isolates were categorized into 7 clone types and 8 single types using ERIC-PCR (Figure 1A).

Table 3 Distribution of *Shigella* spp. isolates in different age groups

Age groups	<24 months N=736 (%)	2–5 years N=435(%)	6–10 years N=1095(%)	11 to <15 years N=988(%)
<i>S. flexneri</i> N=36	0	5 (13.8)	19 (52.7)	12 (33.33)
<i>S. sonnei</i> N=33	0	1 (3.03)	22 (66.6)	10 (30.3)
<i>S. boydii</i> N=5	0	0	4 (80)	1 (20)

Table 4 The distribution pattern of antibiotic resistance based on species

Antibiotic	AK n (%)	GM n (%)	CIP n (%)	NA n (%)	CAZ n (%)	CRO n (%)	TMP –SXT n (%)	TET n=(%)
<i>S. flexneri</i> N=36	1 (2.7)	1 (2.7)	4 (11.11)	16 (44.44)	9 (25)	20(55.5)	32(88.80)	27(75)
<i>S. sonnei</i> N=33	1 (3.03)	1 (3.03)	1 (3.03)	4 (12.12)	5 (27.27)	16(48.4)	21(63.60)	28(84.84)
<i>S. boydii</i> N=5	0	0	0	1 (20)	1 (20)	2(40)	5(100)	3(60)

Abbreviations: AK, amikacin; GM, gentamycin; CIP, ciprofloxacin; NA, nalidixic acid; CAZ, ceftazidime; TMP-SMX, trimethoprim/sulfamethoxazole; CRO, ceftriaxone; TET, tetracycline.

Table 5 Distribution of the SPATE genes in *Shigella* isolates

Species	Strain	Class 1			Class 2		
		Pet	sat	sigA	sepA	pic	
<i>S. flexneri</i>	SF01	-	+	+	-	-	
	SF02	+	+	+	-	+	
	SF03	-	+	-	+	+	
	SF04	-	+	+	+	+	
	SF05	-	+	+	+	-	
	SF06	+	-	-	-	-	
	SF07	-	+	-	-	-	
	SF08	-	+	+	-	+	
	SF09	-	+	+	-	+	
	SF10	-	-	+	-	-	
	SF11	+	+	+	+	-	
	SF12	-	+	-	+	-	
	SF13	-	+	-	+	+	
	SF14	-	+	+	-	+	
	SF15	-	+	-	-	+	
	SF16	+	-	-	-	-	
	SF17	-	+	+	-	-	
	SF18	-	+	+	+	-	
	SF19	-	+	+	-	+	
	SF20	-	+	-	-	-	
	SF21	-	+	+	-	+	
	SF22	-	+	+	+	-	
	SF23	+	-	+	-	-	
	SF24	-	-	+	-	-	
	SF25	-	+	+	-	+	
	SF26	-	+	-	+	-	
	SF27	-	+	+	+	-	
	SF28	-	-	+	-	-	
	SF29	+	-	+	-	+	
	SF30	-	+	+	+	+	
	SF31	-	+	+	-	-	
	SF32	-	-	+	-	-	
	SF33	-	+	-	-	-	
	SF34	-	+	+	+	-	
	SF35	+	-	+	+	+	
	SF36	-	+	-	-	-	
Total		7	27	25	13	14	
<i>S. sonnei</i>	SN01	-	-	-	+	-	
	SN02	+	+	-	-	+	
	SN03	+	-	+	+	+	
	SN04	-	-	+	+	+	
	SN05	+	-	+	-	-	
	SN06	-	-	-	-	-	
	SN07	+	-	-	+	+	
	SN08	+	-	-	+	-	
	SN09	-	+	-	-	+	
	SN10	+	-	-	-	-	
	<i>S. sonnei</i>	SN11	+	+	+	+	-
		SN12	-	-	+	+	+
		SN13	+	-	-	+	-
		SN14	-	+	-	-	-
		SN15	-	-	+	-	+

(Continued)

Table 5 (Continued).

Species	Strain	Class 1			Class 2		
		Pet	sat	sigA	sepA	pic	
<i>S. flexneri</i>	SN16	-	-	+	-	-	
	SN17	-	-	+	+	-	
	SN18	-	-	-	+	-	
	SN19	-	-	+	-	-	
	SN20	-	-	+	-	-	
	SN21	+	-	+	-	-	
	SN22	-	-	+	+	+	
	SN23	-	+	-	+	-	
	SN24	-	-	+	-	-	
	SN25	-	+	-	-	+	
	SN26	+	-	-	+	+	
	SN27	+	-	+	-	+	
	SN28	+	-	+	-	-	
	SN29	-	-	+	-	-	
	SN30	-	+	+	+	+	
	SN31	-	+	-	+	-	
	SN32	-	-	-	-	-	
	SN33	+	-	-	-	-	
	Total		13	8	17	14	12
	<i>S. boydii</i>	SB01	-	-	+	-	-
		SB02	-	-	-	+	-
		SB03	-	-	+	+	-
		SB04	-	-	-	-	-
		SB05	-	-	-	-	-
		Total		0	0	2	2

Abbreviations: SF, *S. flexneri*; SS, *S. sonnei*; SB, *S. boydii*.

Also, 33 *S. sonnei* isolates were clustered into 10 clone types and 3 single types (Figure 1B). According to the results demonstrated in Figure 1 and Table 5, either *S. flexneri* or *S. sonnei* isolates belonging to a same clone type had similar SPATE genes profile.

Discussion

The local epidemiology of *Shigella* spp. is changing over time.¹⁷ In our study, the prevalence rate of *Shigella* spp. in diarrheal infection was 2.27% and *S. flexneri* was recognized as the dominant species. In consistent with our results, in a previous study in our region by Jomezadeh et al¹⁸ during June 2011–May 2013, *S. flexneri* was the most common *Shigella* spp. among the pediatric age group. However, the frequency rate of *Shigella* spp. in their study was higher than our study (5.1% vs 2.27%) that may reflect the increasing hygiene levels in our region than that reported by Jomezadeh et al. Nevertheless, the frequency rate of *Shigella* spp. in our study was higher than

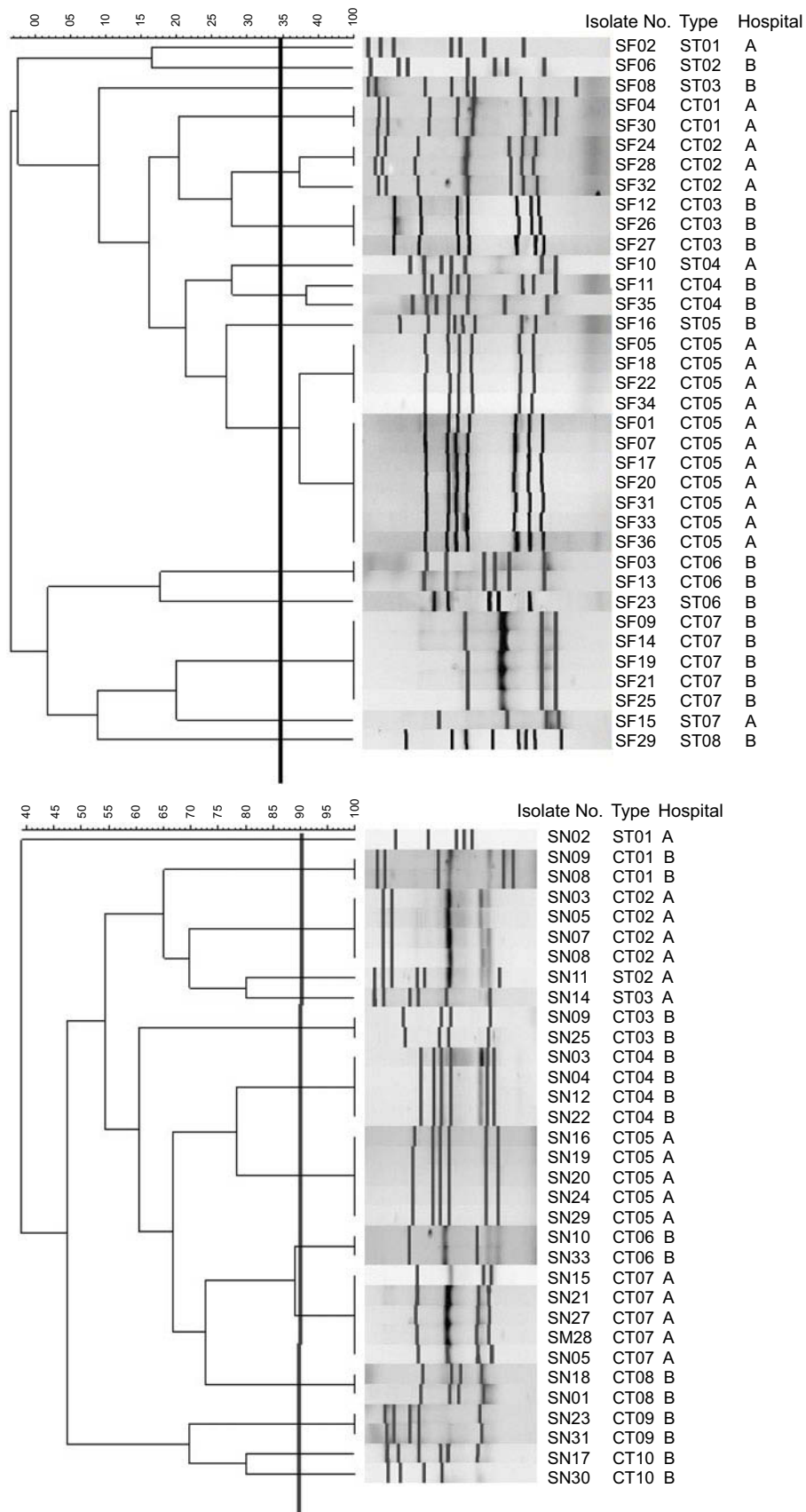


Figure 1 Dendrograms of 36 *S. flexneri* isolates (A) and 33 *S. sonnei* (B) isolates by ERIC-PCR fingerprinting. **Abbreviations:** SF, *S. flexneri*; SN, *S. sonnei*; CT, clonal type; ST, single type; A, Abuzar Hospital; B, Golestan Hospital.

those reported in other reports,^{19,20} but was comparable to a previous study in Iran.¹⁷

According to data obtained from most researches conducted in Iran, *S. flexneri* was diagnosed as the main agent of shigellosis^{17,18,21,22} whereas in a few studies, *S. sonnei* has been found as the dominant species.^{4,23} In our study, the frequency of *S. flexneri* (36 isolates) was relatively similar to *S. sonnei* (33 isolates), indicating that *S. flexneri* may be replaced locally with *S. sonnei*. We also found a few *S. boydii* isolates which was in accordance with some other studies conducted in Iran,^{4,17,18,20,21} however, in the research conducted by Mashoof et al in Tabriz,²⁴ *S. sonnei* had the lowest frequency. *S. boydii* was first detected in India and is still relatively prevalent in Indian subcontinent.²⁵ However, many researchers in other regions of the world have reported the lower frequencies of *S. boydii* than India subcontinent.^{26–30}

In this study, most isolates were resistant to TMP-SXT and tetracycline (more than 67%) that are in agreement with other studies reported in Iran and some other countries.^{17,18,21,25,31} In our study, *S. flexneri* isolates were more resistant to TMP-SXT than *S. sonnei* (88.9% vs 63.6%; $p>0.05$). TMP-SXT and tetracycline were previously used as two first-line antibiotics for the treatment of bacterial diarrheas such as shigellosis.³² However, because of the increasing prevalence of tetracycline or TMP-SXT-resistant *Shigella* strains, the use of these antibiotics is only limited to some regions where the resistance rates still are low.³⁰

In this study, the resistance to gentamicin and amikacin was much low, as reported in other studies by Orrett et al³⁰ in Trinidad and Peirano et al³³ in Brazil. Overall, *Shigellaspp.* isolates are usually susceptible to aminoglycoside agents in vitro, but these agents are ineffective in vivo,^{14,34} hence do not recommend for shigellosis treatment.

The results of our study implied that the resistance rate to ciprofloxacin was much low. In contrast to our results, Jomezadeh et al,¹⁸ Hosseini Naveh et al,²¹ Özmert et al³⁵ and Ghosh et al²⁵ documented a relatively high prevalence of resistance to ciprofloxacin and nalidixic acid among *Shigella* isolates. It is suggested that the cross-resistance to nalidixic acid or/and the excessive use of these antibiotics may have been contributed to the high prevalence of fluoroquinolone-resistant isolates.³⁴

The SPATE genes as an expanding family of secreted autotransporters in GN bacteria have various functions such as protease or mucinase that directly or indirectly can be toxic for the intestine cells.³⁶ There is limited available information

on the distribution of the SPATE genes in *Shigella* isolates, so that more research is needed to fully understand the distribution of these genes among *Shigellaspp.* isolates. In this current study, the distribution of five SPATE genes was determined by PCR. According to our data, most *S. flexneri* and *S. sonnei* isolates harbored two or more than two SPATE genes, as described in other studies by Hosseini Naveh et al³⁷ and Boisen et al¹⁰ Moreover, Hosseini Naveh et al³⁷ in Iran showed that 11 of 31 *S. flexneri* isolates and 6 of 7 *S. boydii* isolates were positive for four SPATE genes. According to the evidences obtained from the study of Boisen et al,¹⁰ the numbers of SPATE genes in the bacterial pathogens had the direct relationship with the virulence of the organisms and subsequently the severity of tissue damages. Also, these researchers showed that *Shigella* isolates commonly had encoded more than one SPATE gene rather than diarrheal *E. coli* pathotypes (EAEC, EIAC, ETEC, DAEC and EPEC), therefore, clinically, *Shigellaspp.* are more virulent than *E. Coli* pathotypes.¹⁰

In this study, the *sat* gene was recognized as the most common SPATE gene among *S. flexneri* isolates. In agreement with our study, Hosseini Naveh et al, Fan et al and Ruiz et al found the high rates of this gene among *S. flexneri* rather than other *Shigella* species.^{37,38,39} The *sat* gene was first studied in uropathogenic *E. coli* strains;³⁶ however, recently several studies showed the presence of this gene in the Enteroaggregative *Escherichia Coli* clinical strains.^{40,41} The in vitro experiments indicated that *sat* can induce the cytoskeletal derangement in intestinal epithelium directly although cleaving spectrin proteins.⁴²

We also showed a high prevalence of the *sigA* gene among both *S. flexneri* (24 of 36) and *S. sonnei* (17 of 31) isolates. In consistent with our results, Hosseini Naveh et al³⁷ showed the presence of the *sigA* gene among all *S. sonnei* isolates while in contrast to our results, they detected a relatively low frequency of this gene in *S. flexneri* isolates. Also, Fan et al³⁸ showed a high frequency of the *sigA* gene in the majority of *S. flexneri* serotypes. The *sigA* gene was initially reported as a part of chromosomal pathogenicity island in a *S. flexneri* clinical strain.³⁵ Later, studies on the action mechanism of this gene by Al-Hasani et al⁴³ revealed that the *sigA* gene induced the fluid accumulation in intestine and cleaved intracellular fodrin (an analog of spectrin) in situ that resulted in the derangement of the cytoskeleton during the pathogenesis of shigellosis.⁴³ In addition *Shigella* isolates, some other studies indicated the high distribution of the *sigA* gene in Enteroinvasive *Escherichia coli* (EIEC)^{10,44} than other pathotypes of

E. coli.^{40,41} Moreover, Boisen et al¹⁰ found that six of ten EIEC isolates were positive for the amplification of the *sigA* gene as only the SPATE gene, suggesting that *sigA* may play an important role in the pathogenesis of EIEC, as well as *Shigella* isolates.

We indicated that class 2 SPATE genes (*sepA* and *pic*) were relatively common among *Shigella* isolates: 35.13% of the isolates were positive for the *pic* gene and 39.18% were positive for the *sepA* gene. Moreover, the frequency rates of the class 2 SPATE genes were relatively similar in *S. flexneri* and *S. sonnei* strains. In agreement with our results, Fan et al³⁸ and Boisen et al¹⁰ showed the distribution of the class 2 SPATEs in different serotypes of *S. flexneri*, as well as Hosseini Naveh et al³⁷ in Iran that reported the presence of the class 2 SPATE genes in both *S. flexneri* and *S. boydii* isolates.

pic initially was reported in a clinical strain of *S. flexneri* 2a, mediates the serum resistance through the degradation of one of the components of the complement system along with a mucinase activity, resulting in the successful intestinal colonization of *Shigella* isolates.

sepA also was originally described in *S. flexneri* 2a, induces the intestinal inflammation together with the fluid accumulation and mucosal atrophy by an unknown mechanism.³⁶

In our study, *S. boydii* isolates had the low rates of SPATE genes (*sepA* and *sigA*) that is in contrast to findings of Hosseini Naveh et al³⁷ in Iran who reported the high frequencies of the SPATE genes except to the *sat* gene among *S. boydii* isolates.

Molecular typing methods are used to establish the clonal relationships between isolates and confirm the clinical or epidemiological data, as well as the spread of an infectious agent in community.⁴⁵ ERIC-PCR is a rapid and low-cost method that has differentiated closely related strains of bacteria from each other.⁴⁶ In this present study, *S. flexneri* and *S. sonnei* isolates were clustered in 7 clonal types and 10 clonal types, respectively. Also, either *S. flexneri* or *S. sonnei* isolates belonging to a same clone type had similar SPATE genes profile, indicating that ERIC-PCR has high discriminatory power.

Conclusion

In this current study, we showed that the frequency rates of *S. flexneri* and *S. sonnei* were relatively similar. Also, we indicated the high distribution of the SPATE genes among *Shigella* isolates especially in *S. flexneri*. Hence, this study

highlights a need for the epidemiological programs to monitor the distribution of SPATE genes locally for prevention from further dissemination of the *Shigella* isolates harboring them.

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Disclosure

The authors report no conflict of interests in this work.

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