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Genetic Characterization of Atypical *Shigella flexneri* Isolated in Korea

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Three types of serotypically atypical *Shigella flexneri* isolates were collected between 2007 and 2008 from Korean patients at the Korea National Institute of Health (NIH). These atypical isolates were characterized and compared with serologically typical *S. flexneri*. The first grouping of 11 atypical isolates displayed agglutination only with polyB antiserum and exhibited no reaction with any typing or grouping sera (PolyB:un). The second group of 3 isolates displayed reactions with typing sera IV, but also did not bind with any grouping sera (IV:un). The third group of 14 isolates exhibited a plural agglutination pattern, reacting with typing sera II, and two grouping sera (II:(3)4,7(8)). Amongst these atypical isolates, isolates belonging to IV:un and II:(3)4,7(8) exhibited greater antibiotic resistance, in particular to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole, than typical *S. flexneri* strains. Furthermore, all II:(3)4,7(8) strains harbored integrons. This study suggests that these multiple antibiotic-resistant atypical *S. flexneri* are new subserotypes of *S. flexneri* that await further serological classification.

Keywords: Atypical, *Shigella*, integron

A total of 50 serotypes based on the O-antigenic structure are recognized within the *Shigella* genus. There are four subtypes or species: *Shigella dysenteriae* (subtype A), *S. flexneri* (subtype B), *S. boydii* (subtype C), and *S. sonnei* (subtype D). Among these, *S. flexneri* is characterized by 14 serotypes. The serotypes of *S. flexneri* (with the exception of serotype 6) have some degree of antigenic relatedness attributable to a common repeating tetrasaccharide unit, to which the α-D-glucopyranosyl and *O*-acetyl groups are added, providing the basis for their type (I to VI) and group [(3)4, 6, and 7(8)] antigenic factors [3, 9, 12]. Recently, isolation of uncommon serotypes and subserotypes of *S. flexneri* have been reported. An atypical serotype of *S. flexneri*, 1c, was first identified in Bangladesh and proved to be a novel O-antigen modification gene [18, 22]. Another atypical serotype of *S. flexneri*, designated 4c, not included in the typing scheme of *Shigella,* was isolated in Russia and Bangladesh [19]. All these atypical serotypes have atypical agglutination patterns with commercially available antisera.

Determinants of antibiotic resistance in *Shigella* isolates are frequently borne within mobile genetic elements, including the R plasmids, transposons, integrons, and genomic islands, on the bacterial genome [8, 13]. Mobile genetic elements may facilitate the dissemination of resistance determinants among species, even genera. Amongst these, integrons are gene-capture systems that harbor antibiotic resistance genes and may provide a flexible approach for the adaptation of bacteria to the pressures caused by antibiotics. Integrons have class 1 and class 2 types. Class 2 integrons are known to carry the three conserved resistance gene cassettes, *dfrA1* (trimethoprim-sulfamethoxazole), *sat1*, and *aadA1* (resistance to streptomycin). The class 1 type integron contains two gene cassettes; esterase/lipase (*estX*) and aminoglycoside adenyltransferase (*aadA1*) [1, 15]. In addition OXA-type β-lactamases confer resistance to amoxicillin, and cephalothin, and are characterized by their high hydrolytic activity against oxacillin and cloxacillin [10].

In the Republic of Korea (hereinafter "Korea"), the annual incidence of shigellosis was estimated to be approximately 10 cases per year before 1997, but thereafter exploded to between 1,000 and 2,500 cases annually during the period of 1998–2000 [4]. Serologically, atypical *S. flexneri* isolates were first identified in 2007 in Korea, with the identified numbers of isolates and types of atypical characteristics increasing in 2008. In the present study, atypical *S. flexneri* isolates collected in Korea in 2007 and 2008 are analyzed for their atypical traits and antibiotics resistance patterns in relation to integrons.

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Materials and Methods

Sample Collection

Over a 2-year span, between 2007 and 2008, 129 *S. flexneri* isolates, based on general biochemical tests with the API 20E (bioMerieux S.A., Lyons, France) and agglutination with species specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan), were collected. From the year 2007, 9 atypical isolates were collected from 42 of these isolates of *S. flexneri* (9/42, 21.4%), while in 2008, 19 atypical isolates were collected from the aggregate of 87 isolates (19/87, 21.8%). In total, 28 serotypically atypical *S. flexneri* strains were isolated between 2007 and 2008 from shigellosis patients.

Serological Test

Serotyping of the *S. flexneri* isolates was performed using a commercially available antisera kit (Denka Seiken Co., Ltd., Tokyo, Japan) specific for all type- and group-factor antigens. Bacteria were subcultured on tryptic soy broth (TSB) Difco agar plates (Becton Dickinson and Company Inc., U.S.A.), and after about 18 h of incubation, serological reactions were performed by the slide agglutination test in accordance with the manufacturer's instructions.

Biochemical Characterization

S. flexneri was identified using the API-20E System Analytical Profile Index (bioMerieux S.A., Lyons, France). One well-isolated colony from each culture was tested. After the results for all biochemicals were obtained, the identification of the organism was made using the 7 digit number generated, alongside the analytical profile index.

Antimicrobial Susceptibility

The antimicrobial susceptibility of the *S. flexneri* isolates was determined using the disk diffusion test according to the methodology of the Clinical and Laboratory Standards Institute (CLSI). Antibiotics tested were AM (ampicillin), AN (amikacin), SM (streptomycin), CF (cephalothin), CZ (cefazolin), FEP (cefepime), CTT (cefotetan), CTX (cefotaxime), CIP (ciprofloxacin), C (chloramphenicol), GM (gentamicin), IPM (imipenem), NA (nalidixic acid), TE (tetracycline), TIC (ticarcillin), and SXT (trimethoprim-sulfamethoxazole). The *Escherichia coli* ATCC 25922 strain was used as a control for bacterial growth.

DNA Manipulation

Chromosomal DNA was purified using the GenomicPrep Cell and Tissue DNA isolation kit (Amersham Biosciences Corp., The Netherlands). All PCRs were performed with the Expanded High Fidelity Polymerase System (Roche) or *Taq* polymerase (Takara, Japan) according to the manufacturer's instructions. The PCR fragment was purified and the PCR products were sequenced. The detection of class 1 and class 2 integrons and β-lactamase encoding genes utilized well-documented methods [1, 17]. The detection of *ipa* genes (*ipaBCD* and *ipaH*) for confirmation of *Shigella* spp. similarly followed an established method [7] (see supplementary data in Table. S1). MLST (multilocus sequence typing) was performed in full accordance with a known approach [5].

Pulsed-Field Gel Electrophoresis (PFGE) and Data Analysis

Bacteria were grown overnight at 37°C on tryptic soy agar (TSA) (Oxoid Ltd., Basingstoke, U.K.). The growth was then harvested and the cells were suspended in a TE buffer (100 mM Tris and 100 mM EDTA, pH 7.5), partially embedded in low melting point agarose (FMC Bio Products Inc., Rockland, U.S.A.), and digested overnight with 10 U of Proteinase K (Invitrogen) at 55°C. Briefly, the DNA was digested with the enzyme *Not*I (New England Biolabs Inc., U.S.A.), and then electrophoresis was performed using the Gene Path system (Bio-Rad Laboratories Inc., U.S.A.) in a 1% agarose gel in 0.5× TBE (Tris Borate–EDTA) buffer at 14°C with a linear ramp time of 2.16 to 35.07 s over a period of 18 h, a 120° angle, and a gradient of 6.0 V/cm. After PFGE, the gels were stained with ethidium bromide and photographed under UV

Table 1. Agglutination reactions of atypical and typical strains of *S. flexneri*.

Strain	PolyB	Typing sera						Grouping sera			Antigenic formula
		I	II	III	IV	V	VI	(3)4	6	7(8)	
Atypical	+	-	-	-	-	-	-	-	-	-	PolyB:un
	+	-	-	-	+	-	-	-	-	-	IV:un
	+	-	+	-	-	-	-	+	-	+	II:(3)4,7(8)
Typical	+	-	-	-	-	-	-	+	-	-	Y (-:(3)4)
	+	-	-	-	+	-	-	+	-	-	4a (IV:(3)4)
	+	-	+	-	-	-	-	+	-	-	2a (II:(3)4)
	+	+	-	-	-	-	-	+	-	-	1a (I:(3)4)
	+	+	-	-	-	-	-	+	+	-	1b (I:(3)4,6)
	+	-	+	-	-	-	-	-	-	+	2b (II: 7(8))
	+	-	-	+	-	-	-	+/-	+	+	3a III:(3)4,6,7(8)
	+	-	-	+	-	-	-	+/-	+	-	3b (III:(3)4,6)
	+	-	-	-	+	-	-	-	+	-	4b (IV:6)
	+	-	-	-	-	+	-	+	-	-	5a (V:(3)4)
	+	-	-	-	-	+	-	-	-	+	5b (V:7(8))
	+	-	-	-	-	-	+	+/-	-	-	6 (VI:(3)4)
	+	-	-	-	-	-	-	-	-	+	X (-:7(8))

transillumination. The gel images were also digitized for computer-aided analysis. The Molecular Analyst software package (Bio-Rad Laboratories Inc., U.S.A.) was used for the analysis. Calculation of the similarity matrix was performed with the Dice algorithm after defining each band size between the sizes of 145 and 582 kb. Percent similarities were identified on a dendrogram, derived with the unweighted pair group method, using arithmetic averages and based on Dice coefficients [20].

RESULTS

Serological Typing

Three types of serologically atypical traits were identified. The first atypical characteristic was observed in 11 isolates that displayed no reaction to typing or grouping sera, but only reacted strongly with PolyB antisera (noted hereinafter as "PolyB:un"). The second type of strain, observed in 3 isolates, displayed a reaction with only one of the typing sera (IV) and did not bind with any of the grouping sera (noted hereinafter as "IV:un"). The third and final type of atypical *S. flexneri* strain, observed in 14 isolates, exhibited a plural agglutination pattern, reacting with one typing sera (II) and binding with two grouping sera (noted hereinafter as "II:(3)4,7(8)").

Table 2. Serotypes of isolated atypical and typical strains of *S. flexneri* in 2007 and 2008.

Antigenic formula	Atypical strains		Typical strains	
	2007	2008	2007	2008
PolyB:un	9	2	-	-
IV:un	-	3	-	-
II:(3)4,7(8)	-	14	-	-
Y (-:(3)4)	-	-	1	2
4a (IV:(3)4)	-	-	-	3
2a (II:(3)4)	-	-	5	23
1a (I:(3)4)	-	-	-	25
1b (I:(3)4,6)	-	-	3	-
2b (II:7(8))	-	-	9	8
3a III:(3)4,6,7(8)	-	-	12	2
3b (III:(3)4,6)	-	-	-	-
4b (IV:6)	-	-	-	-
5a (V:(3)4)	-	-	-	-
5b (V:7(8))	-	-	-	-
6 (VI:(3)4)	-	-	3	5
X (-:7(8))	-	-	-	-
Total	9	19	33	68
	129			

Biochemical Characterization and Detection of Virulence Genes

On the basis of biochemical tests (API 20E), all atypical *S. flexneri* were found to ferment glucose and mannitol identically to typical *S. flexneri*. One distinction of these atypical *S. flexneri* biotypes (PolyB:un) was that they were able to degrade gelatin and produce indole within 24 h (see supplementary data in Table S1). The results of PCR detection showed the presence of the *ipaBCD* gene and the *ipaH* gene in all atypical and typical strains. These data establish that all atypical isolates contain the *Shigella* virulence plasmid [2].

Antibiotic Susceptibility

Tests on the antibiotic susceptibility of the isolates to an array of 16 antibiotics were performed by disk diffusion testing and the results compared with the CLSI reference range. The number of strains resistant to antibiotics was converted to a percentage and each isolated atypical strain was then compared with typical strains, including the 11 strains of PolyB:un to the 3 strains of Y, the 14 strains of II:(3)4,7(8) to the 28 strains of 2a, and the 3 strains of IV:un to the 3 strains of 4a isolated in same period. All strains were found to be susceptible to cefepime (FEP), cefotetan (CTT), cefotaxime (CTX), ciprofloxacin (CIP), gentamicin (GM), and imipenem (IPM). Furthermore, strains were selectively resistant to ampicillin (AM), streptomycin (SM), chloramphenicol (C), tetracycline (TE), ticarcillin (TIC), and trimethoprim-sulfamethoxazole (SXT). Finally, the strains showed intermediate susceptibility to amikacin (AN), cephalothin (CF), cefazolin (CZ), and nalidixic acid (NA). As shown in Table 3, the II:(3)4,7(8) and IV:un strains showed a higher resistance pattern to AM, SM, and

Table 3. Antimicrobial resistance of atypical and typical strains.

strains	No. of isolates	No. of resistant isolates (%)						
		AM	SM	CF	C	TE	TIC	SXT
PolyB:un	11	9 (81.8)	9 (81.8)	7 (63.6)	6 (54.5)	9 (81.8)	9 (81.8)	8 (72.7)
II(3)4,7(8)	14	14 (100)	14 (100)	0	12 (85.7)	14 (100)	14 (100)	12 (85.7)
IV:un	3	3 (100)	3 (100)	0	1 (33.3)	3 (100)	3 (100)	3 (100)
Y	3	2 (66.7)	2 (66.7)	1 (33.3)	0	3 (100)	2 (66.7)	3 (100)
2a	28	25 (89.3)	25 (89.3)	2 (7.1)	19 (67.9)	27 (96.4)	26 (92.9)	17 (60.7)
4a	5	2 (40)	3 (60)	0	0	3 (60)	2 (40)	2 (40)

Table 4. Antibiotic resistance genes of atypical and typical strains.

Antigenic formula	Tested Strains	Integron Class 1	Integron Class 2 (Types 1, 2)	bla_{OXA}	bla_{TEM}
PolyB:un	11	0	8 (Type 1)	8	0
Y (-:(3)4)	3	0	1 (Type 1)	0	1
IV:un	3	0	3 (Type 1)	3	0
4a (IV:(3)4)	3	0	0	2	0
II:(3)4,7(8)	14	10	14 (Type 2)	14	0
2a (II:(3)4)	6	0	3 (Type 1)	6	0

SXT than the typical strains (2a and 4a). PolyB:un strains showed a lower resistance when compared with Y (-:(3)4) in TE and SXT.

Integron and Antibiotic Resistance of Atypical Isolates

Atypical strains, especially the II:(3)4,7(8) and IV:un strains, showed resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. Therefore, it was prudent to test for whether these atypical strains harbored the integron gene cassette. PCR and DNA sequencing results confirmed the presence of integron cassettes in these strains. The II:(3)4,7(8) isolates were found to have a class 1 integron (10 out of 14 strains) and a class 2 integron (14 out of 14 strains). The class 1 type integron was 1,955 bp and contained two gene cassettes; esterase/lipase (*estX*) and aminoglycoside adenyltransferase (*aadA1*), which confer resistance to streptomycin and spectinomycin. IV:un strains had only class 2 integrons (3 out of 3 strains). All PolyB:un isolates contained class 2 integrons. Typical strains (2a, Y) also harbored type 2 integrons, but only half of these strains had class 2 integrons, and no integrons were present in 3 strains of 4a (Table 4).

The class 2 integrons were of two types. One was the classic type (Type 1, 2,158 bp) and carried the three conserved resistance gene cassettes, *dfrA1* (trimethoprim-sulfamethoxazole), *sat1*, and *aadA1* (resistance to streptomycin). The other type was shorter (Type 2, 1,313 bp) and carried only two gene cassettes, *dfrA1* and *sat1*. The shorter type of class 2 integron was only present in the II:(3)4,7(8) strain.

All isolates also showed a high frequency of resistance to ampicillin, especially the II:(3)4,7(8) and IV:un strains, which exhibited 100% ampicillin resistance. Therefore, further testing for the presence of β-lactamase genes, through PCR analysis, was deemed appropriate. Many different β-lactamases have been described; OXA-, TEM-, SHV-, and CTX-M-type β-lactamases are predominant in Gram-negative bacteria [10]. Atypical strains harbored more OXA-type β-lactamase genes than typical strains (Table. 4). All tested strains were negative for SHV- and CTX-M β-lactamases. Only one typical strain (Y) had TEM-type β-lactamase. These results indicate that the majority of atypical strains contain OXA-type β-lactamase, and that they are resistant to ampicillin.

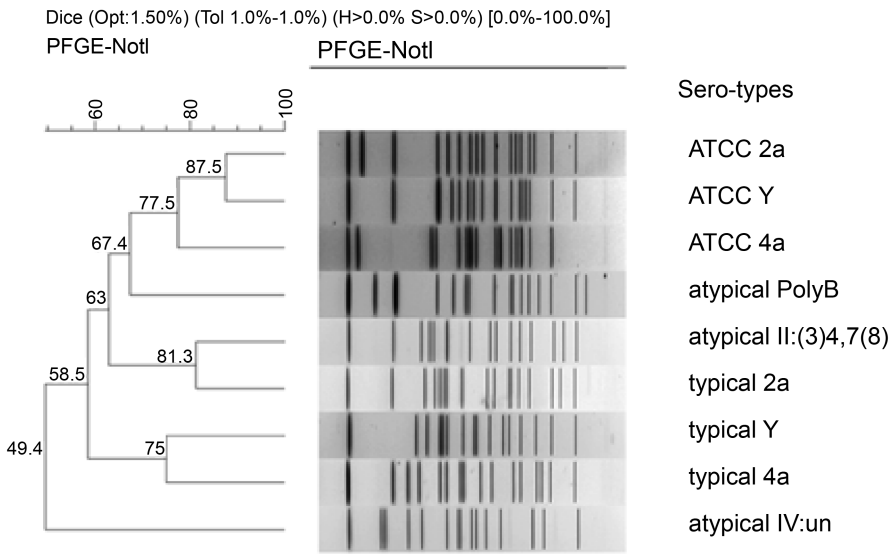

Fig. 1. Representative PFGE *Not*I patterns of atypical *S. flexneri* and reference stains.
Serotypes 2a (ATCC 12022), 4a (ATCC 12023), and Y (ATCC 12027) reference strains and typical serotype strains were compared with the atypical strains.

PFGE and MLST Analyses

PFGE analysis of *Not*I-digested chromosomal DNA of the atypical, typical, and ATCC *S. flexneri* strains yielded reproducible DNA fragments ranging in size from approximately 20 to 1,050 kb (Fig. 1). As shown in the dendrogram, similarities ranged from 49.4% to 87.5%. PFGE patterns of atypical and typical strains showed either indistinguishable or similar PFGE patterns among strains (IV:un strains showed similarities of less than 50% to other strains). These PFGE patterns were associated with similar resistance phenotypes, with the predominance of class 2 integrons in most strains of *S. flexneri*, and with $bla_{\text{OXA-30}}$ in all strains (Table 4). These data suggest a clonal relatedness of strains of the same species, especially those isolated from the same locality. Similar limited diversity in strains of *Shigella* spp. has been reported previously in another country [6, 21].

Two major sequence type complexes (ST complex), by an MLST method, have been identified among *S. flexneri* serotypes 1–5, X, and Y [5]. The majority of these serotypes of *S. flexneri* belong to the ST complex 245 (representative sequence type ST245, which contains an allele profile of 6, 61, 6, 11, 13, 3, 50 in the order of *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*). A novel sequence type, ST630, was reported from a serotype Y isolate, which contained an allele profile of 6, 61, 6, 11, 6, 95, 7. Two isolates, each of atypical strains, were subjected to MLST analysis and the isolates belonging to the PolyB:un and II:(3)4,7(8) strains found to contain ST245, which showed that these atypical strains are closely related to the majority of *S. flexneri* ST complexes. However, two isolates of atypical IV:un harbored ST630, which indicates that they are a newly emerged ST group amongst the *S. flexneri* [5]. These MLST results also confirm PFGE results indicating that the IV:un group is distant from others.

DISCUSSION

In this study, three types of serologically atypical *S. flexneri* isolates were identified from Korean patients during the period between 2007 and 2008. Utilizing currently available standard serological methods, it is not possible to classify any of the latter isolates as typical serotypes in nature. Their antigenic formula can be presented as PolyB:un, IV:un, and II:(3)4,7(8). Among these three types of atypical *S. flexneri*, II:(3)4,7(8) isolates were collected in the highest numbers in Korea during the year of 2008.

Antimicrobial resistance patterns are valuable as a guide to empirical therapy, as a typing method, and as an indicator of the dissemination of antimicrobial resistance determinants [6]. By analyzing trends in the resistance patterns of the various atypical strains, we found that the II:(3)4,7(8) and IV:un strains were more resistant than typical *S. flexneri* serotype strains to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. The II:(3)4,7(8) and IV:un strains have integron gene cassettes. Specifically, II:(3)4,7(8) strains have class 1 and 2 integrons, whereas typical 2a strains do not harbor the class 1 integron. The IV:un strains harbor class 2 integrons as do many (but not all) 4a strains. PolyB:un strains also harbor class 2 integrons indistinctively.

It can be surmised that the prevalence of a high resistance to streptomycin and trimethoprim-sulfamethoxazole can be explained by the presence of class 1 and 2 integrons on the atypical strains. Interestingly, of the three atypical strains, the PolyB:un and IV:un strains were isolated from East Asian workers who had migrated to Korea, whereas the II:(3)4,7(8) strains were isolated from nonmigrant Korean patients. Antimicrobial resistance patterns, and their correlation with integrons among *S. sonnei* isolates, have been recently documented in Korea [11, 14]. The genetic relatedness and dissemination of these integrons among *Shigella* spp. should be monitored for the preparedness of shigellosis in Korea. Although other mechanisms of resistance are possible, from these results, ampicillin, streptomycin, and trimethoprim-sulfamethoxazole resistances are most likely attributable to expression from the genes contained in the corresponding integrons. The overall susceptibility patterns of the test strains focus on the fact that the strains were frequently exposed to expanded broad-spectrum antibiotics. Since the antimicrobial susceptibilities of *S. flexneri* isolates are readily changed by antibiotic selective pressures, an appropriate antimicrobial therapy is necessary to prevent the emergence of resistant strains and the dissemination of resistance genes. Ampicillin, streptomycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole have been the most commonly prescribed antimicrobial agents to treat shigellosis during the last decade in Korea. This may account for the emergence of resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole resistant strains.

Moreover, all atypical strains have pathogenic genes (the *ipaBCD* gene and *ipaH* gene). Thus, these pathogenic and multiple antimicrobial-resistant *Shigella* could prove to be a serious public health problem in developing countries. Atypical strains, or newer subserotypes, are also being isolated from different parts of the world [19, 23]. There have been reports of a serotype 4c with an atypical strain IV:un, which should also be considered as a novel serotype [12, 16]. The genetic background of serotype 1c has also been reported [18]. The atypical strains presented in this study should also be analyzed for the identification of new O-antigen modification genes.

In conclusion, the present study mandates local monitoring of antimicrobial resistance and its molecular characterization for the therapy of atypical *S. flexneri* infections. This study suggests that multiple antibiotic-resistant atypical *S. flexneri*

are new subserotypes of *S. flexneri* that await further serological classification.

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Table S1. Primers used to amplify the target genes.

Primer	Primer sequence (5' to 3')	PCR product length (bp)	Reference
5'-CS	GGCATCCAAGCAGCAAG	Variable	Ahmed *et al*. [1]
3'-CS	AAGCAGACTTGACCTGA		
IntI2-F	GATCCTGCCATCATTGAGTA	Variable	Ahmed *et al*. [1]
IntI2-R	AGGGGAAGCCGAAGTTTCC		
TEM-F	ATAAAATTCTTGAAGACGAAA	1,080	Ahmed *et al*. [1]
TEM-R	GACAGTTACCAATGCTTAATC		
SHV-F	TTATCTCCCTGTTAGCCACC	795	Ahmed *et al*. [1]
SHV-R	GATTTGCTGATTTCGCTCGG		
OXA-F	TCAACTTTCAAGATCGCA	591	Ahmed *et al*. [1]
OXA-R	GTGTGTTTAGAATGGTGA		
CTX-M-F	CGCTTTGCGATGTGCAG	550	Ahmed *et al*. [1]
CTX-M-R	ACCGCGATATCGTTGGT		
ipaH-f	GTTCCTTGACCGCCTTTCCGTTACCGT	619	Farshad *et al*. [7]
ipaH-b	GCCGGTCAGCCACCCTCTGAGAGTAC		
ipaBCD-f	GCTATAGCAGTGACATGG	612	Farshad *et al*. [7]
ipaBCD-b	ACGAGTTCGAAGCACTC		

5'-CS, 3'-CS; class 1 integron
IntI2 –F, -R; class 2 integron
TEM-F, -R; bla_{TEM}
SHV-F, -R; bla_{SHV}
OXA-F, -R; bla_{OXA}
CTX-M-F, -R; $bla_{CTX\text{-}M}$
ipaH-f, -b; *ipaH*
ipaBCD-f, -b; *ipaBCD*

Table S2. Biochemical profiles (API 20E) of atypical and typical strains of *S. flexneri*.

Antigenic structure	Reactions[a]									
	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	GEL
PolyB	-	-	-	-	-	-	-	-	-	+
IV:un	-	-	-	+	-	-	-	-	-	-
II:(3)4,7(8)	-	-	-	-	-	-	-	-	-	-
Y (-:(3)4)	-	-	-	+	-	-	-	-	-	-
4a(IV:(3)4)	-	-	-	-	-	-	-	-	-	-
2a (II:(3)4)	-	-	-	-	-	-	-	-	-	-

Antigenic structure	Reactions[a]									
	GUL	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	VP
PolyB	+	+	-	-	-	-	-	-	-	-
IV:un	+	+	-	-	-	-	-	-	d	-
II:(3)4,7(8)	+	+	-	-	-	-	-	-	-	-
Y (-:(3)4)	+	+	-	-	-	-	-	-	+	-
4a (IV:(3)4)	+	+	-	-	-	-	-	-	d	-
2a (II:(3)4)	+	+	-	-	-	-	d	-	d	-

[a]ONPG, Ortho nitrophenyl-β D-galactopyranosidase; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; CIT, citrate utilization; H_2S, H_2S production; URE, urease; TDA, tryptophane deaminase; IND, indole production, VP, voges proskauer; GEL, gelatinase; GLU, glucose fermentation; MAN, mannitol fermentation; INO, inositol fermentation; SOR, sorbitol fermentation; RHA, rhamnose fermentation; SAC, saccharose fermentation; MEL, melibiose fermentation; AMY, amygdalin fermentation; ARA, arabinose fermentation.
Symbols: +, 90–100% of strains positive; d, 26–75% positive; -, 0–10% positive.