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Original Article

The relationship between integrons, antibiotic resistance genes and SXT resistance in *Shigella flexneri* strains

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Abstract: Objective: To investigate the correlation between *sulfamethoxazole-trimethoprim* (SXT) resistance in *Shigella flexneri* (*S.flexneri*) and the presence of integrons and relevant antibiotic resistance genes. Methods: We collected 115 strains of *Shigella flexneri* isolated from feces of children with diarrhea in Jinan from 2012 to 2020 and determined the minimum inhibitory concentration (MIC) of SXT by Etest method. The presence of class 1, class 2, and class 3 integron genes, variable region antibiotic resistance gene cassettes, and *sul1*, *sul2*, *sul3*, and SXT elements were detected using polymerase chain reaction (PCR). Positive results were further analyzed by DNA sequencing and BLAST comparison. Results: In total, the resistance rate to SXT was 60.9% among the 115 *S.flexneri* strains. The prevalence of class 1 and class 2 integrons were 88.7% and 87.0%, respectively, with no class 3 integrons detected. Among the strains, 13.0% carried typical class 1 integrons with variable region antibiotic resistance gene cassettes *dfrA17-aadA5* and *dfrV*, while 85.2% carried atypical class 1 integrons with variable region antibiotic resistance gene cassette *blaoxa-30-aadA1*. The variable region antibiotic resistance gene cassettes of class 2 integrons were all *dfrA1+sat1+aadA1*. There was a statistical difference between the presence of class 1 integrons and class 2 integrons between the SXT-sensitive and resistant *S.flexneri* strains (χ^2=22.800, χ^2=16.365, P<0.01, P<0.01). Integrons carrying *dfrV* and *dfrA1* by integrons also showed a statistical difference in SXT resistance (χ^2=9.422, χ^2=16.365, P<0.01, P<0.01). PCR revealed the presence of *sul1* and *sul2* in 13.0% and 47.0% of strains, respectively, with neither *sul3* nor SXT elements detected. There was a significant difference between the presence of *sul1*, *sul2* between the SXT-sensitive and resistant *S.flexneri* strains (χ^2=9.588, χ^2=65.445, P<0.01, P<0.01). Conclusion: In summary, integrons are involved in SXT resistance of *S.flexneri*, and *dfrV*, *dfrA1*, *sul1*, *sul2* are closely related to SXT resistance of *S.flexneri*.

Keywords: *S.flexneri*, sulfanilamide, integron, drug resistance genes

Introduction

Shigella spp. are a Gram-negative, rod-shaped, immotile, and non-spore - forming bacteria and a causative agent of acute diarrhea that may progress to bloody mucoid diarrhea, also known as bacillary dysentery (or shigellosis). Shigella is a predominant cause of diarrheal disease and a major pathogen responsible for increased morbidity and mortality caused by dysentery each year around globally, particularly affecting children aged <5 years in developing countries. *Shigella* is reported as one of the top three major pathogens responsible for childhood diarrhea mortality [1] and has been recognized by the World Health Organization (WHO) as a bacteria with increasing antibiotic resistance, posing a significant threat to human health [2]. Recent studies indicate a steady increase in the resistance of *Shigella* to antimicrobials [3-5]. The Clinical and Laboratory Standards Institute (CLSI) 2019 edition [6] recommended ampicillin, fluoroquinolones, and sulfonamides for the treatment of bacterial dysentery. Historically, sulfonamides were effective in treating bacterial dysentery caused by *Shigella* in the 1930s. However, in recent years, SXT resistance in *Shigella* has become increasingly con-

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cerning. Shigella spp. are emerging as significant public health threats due to the increase and global spread of multidrug-resistant (MDR) strains [4]. The study of integron-carried antibiotic resistance gene cassettes [7-9] and related gene-mediated resistance [10, 11] has become a research hotspot.

In this study, we investigated the relationship between SXT resistance, integrons, and relevant genes in *Shigella* strains isolated from fecal samples of children with diarrhea. We assessed the resistance profiles of 115 *Shigella* strains and performed PCR amplification to explore the correlation between SXT resistance and variable region antibiotic resistance gene cassettes, *sul1*, *sul2*, *sul3*, and SXT elements.

Materials and methods

Strain source

A total of 115 strains of S.*flexneri* were isolated from fecal samples of children aged 6 months to 14 years who visited the Children's Hospital of Jinan, Fourth People's Hospital of Jinan and Infectious disease Hospital of Jinan from 2012 to 2020. The *Shigella* strains were isolated using conventional cultivation and automated biochemical identification methods and serotyped using *Shigella* diagnostic antisera. The preserved strains were retrieved from a -86°C ultra-low temperature freezer, thawed at room temperature, and re-identified. Escherichia coli ATCC 25922, a quality control strain for antimicrobial susceptibility testing, was kept at the Clinical Microbiology Laboratory of the Fourth People's Hospital of Jinan.

Main reagents and instruments

Key materials and equipment included SXT Etest strips (AB Biodisc, Sweden), Mueller-Hinton agar (OXOID, UK), *Shigella* diagnostic antisera (Lanzhou Institute of Biological Products, China), agarose (Invitrogen, USA), automated bacterial identification system (VITEK-2, BioMérieux, France), DNA amplification instrument (Biometra, Germany), electrophoresis instrument (Beijing Liuyi Instrument Factory, Model 10C), biological safety cabinet (Shanghai Lishen Scientific Instrument Factory, 1200IIA2). PCR primer synthesis and DNA sequencing of amplification products were performed by Bao Biological Engineering (Dalian) Co., Ltd.

Antimicrobial susceptibility testing

The Etest method was employed to assess the antimicrobial susceptibility of Shigella flexneri. Three to four colonies of *S.flexneri* grown at 35°C for 16-18 hours were picked and adjusted to a turbidity of 0.5 McF using sterile saline on a turbidimeter. Within 15 minutes, a sterile cotton swab was dipped into the bacterial suspension, excess liquid was squeezed from the swab against the inner wall of the tube, and the swab was streaked onto Mueller-Hinton agar. The plate was rotated 60 degrees three times and streaked along the edge. After a few minutes, an SXT Etest strip was placed on the agar surface. The plate was incubated at 35°C for 16-18 hours, and the MIC results of SXT were determined according to the instructions provided with the Etest strip. The experimental procedures and interpretation of results were strictly in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines of 2019 [6]. MIC values ≤2/38 mg/L were considered sensitive, and MIC values ≥4/76 mg/L were considered resistant. Escherichia coli ATCC 25922 was used for quality control.

PCR amplification experiment

Single colonies were picked and inoculated on M-H agar plates, incubated at 35°C for 16-18 hours. A sterile cotton swab was used to pick an appropriate amount of bacterial, which was then resuspended in 50 μl of double-distilled water. The mixture was then subjected to a 95°C water bath for 5 minutes, followed by a 30-second centrifugation at 12,000 rpm. The supernatant was stored at -20°C for further use. Primers for intI and variable regions were synthesized according to references [12-15], and the primer sequences are shown in **Table 1**. The PCR reaction for intI and the 3' end was performed as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at the temperature specified in **Table 1** for 30 seconds, and extension at 72°C for 60 seconds. A final extension step was performed at

Table 1. Primer sequences for PCR

Primer	Sequence (5'→3')	Fragment Length (bp)	Annealing Temperature (°C)	Reference
intI1F	ACATGTGATGGCGACGCACGA	569	50	[12]
intI1R	ATTTCTGTCCTGGCTGGCGA			
in1F	GGCATCCAAGCAGCAAGC	-	52	[13]
in1R	AAGCAGACTTGACCTGAT			
qacEΔ1	ATCGCAATAGTTGGCGAAGT	798	52	[13]
sul1	GCAAGGCGGAAACCCGCGCC			
intI1ca	CGTAGAAGAACAGCAAGG	-	52	[14]
IS1ca	AGTGAGAGCAGAGATAGC			
intI2F	GTAGCAAACGAGTGACGAAATG	789	51	[12]
intI2R	CACGGATATGCGACAAAAAGGT			
intI2ca-F	CGGGATCCCGGACGGCATGCACGATTTGTA	-	55	[15]
intI2ca-R	GATGCCATCGCAAGTACGAG			
intI3F	GCCTCCGGCAGCGACTTTCAG	980	55	[12]
intI3R	ACGGATCTGCCAAACCTGACT			
*sul1*F	CTTCGATGAGAGCCGGCGGC	338	55	[16]
*sul1*R	GCAAGGCGGAAACCCGCGCC			
*sul2*F	GCGCTCAAGGCAGATGGCATT	286	55	[16]
*sul2*R	GCGTTTGATACCGGCACCCGT			
*sul3*F	GAGCAAGATTTTTGGAATCG	799	55	[16]
*sul3*R	CATCTGCAGCTAACCTAGGGCTTTGGA			
SXT-F	ATGGCGTTATCAGTTAGCTGGC	1035	56	[17]
SXT-R	GCGAAGATCATGCATAGACC			

72°C for 5 minutes. The PCR reaction for the variable regions was performed as follows: initial denaturation at 94°C for 3 minutes, followed by 10 touchdown PCR cycles with a decreasing annealing temperature from 68°C to 54°C in 1.4°C increments, followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 75°C for 180 seconds, and a final extension step at 72°C for 8 minutes. After the reaction, 10 μl of the PCR product was subjected to 1% agarose gel electrophoresis at 120 V for 20 minutes. The gel was stained with ethidium bromide and visualized using a UV analyzer. Positive strains were subjected to sequencing of the intI variable region, and if necessary, the obtained sequences were analyzed for restriction enzyme cutting sites. Restriction fragment length polymorphism analysis was performed using HinfI as the restriction enzyme. The reaction mixture consisted of 1 μl of HinfI, 2 μl of 10× buffer, up to 1 μg of DNA, and double-distilled water to a total volume of 20 μl. The mixture was incubated at 37°C for 1 hour and then terminated by adding 10× loading buffer. A 10 μl aliquot was subjected to 2.5% agarose gel electrophoresis at 120 V for 1 hour. Primers for *sul1*, *sul2*, *sul3*, and SXT elements were synthesized according to references [16, 17], with sequences displayed in **Table 1**. The PCR reaction was performed as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at the temperature specified in **Table 1** for 30 seconds, and extension at 75°C for 40 seconds. A final extension step was performed at 75°C for 5 minutes. After the reaction, 10 μl of the PCR product was subjected to 1% agarose gel electrophoresis at 120 V for 20 minutes. The gel was stained with ethidium bromide and visualized using a UV analyzer. The PCR products were sent to Bao Biological Engineering (Dalian) Co., Ltd. for gene sequencing, and the sequences were analyzed and compared using NCBI/BLAST to determine the genotype. The drug resistance gene sequencing map of positive strains was summarized, and critical fragments were archived for future reference and result validation.

Table 2. Antimicrobial resistance and distribution of integron resistance gene cassettes in 115 strains of *S.flexneri*

SXT resistance phenotype in SXT (Number of strains)	Class 1			Class 2	
	Class 1 integrase genes	Canonical resistance gene	Atypical drug resistance gene	Class 2 integrase gene	Drug resistance gene cassette
Sensitive (45)	- *(n=11)*	-	-	- *(n=11)*	-
	intI1 (n=2)	-	*blaoxa-30-aadA1*	-	-
	-	-	-	*intI2 (n=2)*	*dfrA1-sat1-aadA1*
	intI1 (n=30)	-	*blaoxa-30-aadA1*	*intI2 (n=30)*	*dfrA1-sat1-aadA1*
Drug Resistance (70)	*intI1 (n=2)*	-	*blaoxa-30-aadA1*	-	-
	intI1 (n=4)	*dfr V*	-	*intI2 (n=4)*	*dfrA1-sat1-aadA1*
	intI1 (n=9)	*dfr V*	*blaoxa-30-aadA1*	*intI2 (n=9)*	*dfrA1-sat1-aadA1*
	intI1 (n=2)	*dfrA17-aadA5*	*blaoxa-30-aadA1*	*intI2 (n=2)*	*dfrA1-sat1-aadA1*
	intI1 (n=53)	-	*blaoxa-30-aadA1*	*intI2 (n=53)*	*frA1-sat1-aadA1*
Total (115)	102	15	98	100	100

Table 3. Serotypes and resistance genotypes of the 115 strains of *S.flexneri*

Serotype	Strains	Constituent ratio (%)	Class 1 In	Class 2 In	*dfrA17*	*dfrV*	*dfrA1*	*sul1*	*sul2*
1a	12	10.4	12 (10.4)	10 (8.7)	0 (0.0)	0 (0.0)	10 (8.7)	0 (0.0)	10 (8.7)
2a	68	59.1	56 (48.7)	56 (48.7)	0 (0.0)	12 (10.4)	56 (48.7)	12 (10.4)	14 (12.2)
2b	7	6.1	6 (5.2)	6 (5.2)	0 (0.0)	1 (0.9)	6 (5.2)	1 (0.9)	2 (1.7)
4a	26	22.6	26 (22.6)	26 (22.6)	2 (1.7)	0 (0.0)	26 (22.6)	2 (1.7)	26 (22.6)
X varietas	2	1.7	2 (1.7)	2 (1.7)	0 (0.0)	0 (0.0)	2 (1.7)	0 (0.0)	2 (1.7)
Total	115	99.9	102 (88.6)	100 (86.9)	2 (1.7)	13 (11.3)	100 (86.9)	15 (13.0)	54 (46.9)

Statistical analysis

SPSS 22.0 software was used for statistical analysis. Chi-squared test was used for comparing qualitative data between groups to analyze drug resistance, with $P<0.05$ indicating a statistically significant difference.

Results

Antimicrobial susceptibility results

Among the 115 strains of *S.flexneri*, 45 strains demonstrated SXT MIC values ≤2/38 mg/L, while 70 strains showed MIC values ≥4/76 mg/L, demonstrating a resistance rate to SXT of 60.9% (70/115). *S.flexneri* subtypes 1a, 2a, 2b, 4a, and X accounted for 10.4% (12/115), 59.1% (68/115), 6.1% (7/115), 22.6% (26/115), and 1.7% (2/115), respectively (**Tables 2** and **3**).

PCR detection results

Of the 115 *S.flexneri* strains, 104 strains carried the integrase gene, accounting for 90.4% (104/115). The class 1 integrase genes (intI1) were detected in 102 strains (88.7%, 102/115), the class 2 integrase genes (intI2) were detected in 100 strains (87.0%, 100/115), and both intI1 and intI2 were simultaneously detected in 98 strains (85.2%, 98/115), while no strains showed amplification of class 3 integrase genes. intI1 and intI2 were detected in all serotypes, with the highest detection rates in *S.flexneri* 2a and 4a, accounting for 48.7% (56/115) and 22.6% (26/115), respectively. Among the intI1 positive strains, 15 strains (13.0%, 15/115) carried typical class 1 integrons, with a positive 3' conserved segment including intI1, In, and *qacEΔ1-sul1*, while 98 strains (85.2%, 98/115) carried atypical class 1 integrons with a positive 3' conserved segment including intI1 and intI1-IS1. 11 strains carried both typical and atypical class 1 integrons (9.6%, 11/115). The variable region of class 1 integrons contained resistance gene cassettes *dfrA17-aadA5*, *dfrV*, and *blaoxa-30-aadA1*, accounting for 1.7% (2/115), 11.3% (13/115), and 85.2% (98/115), respectively. The variable region of class 2 integrons con-

tained gene cassette *dfrA1+sat1+aadA*, which was detected in 87.0% (100/115) of the strains and showed the same restriction enzyme profile after HinfI digestion. Among them, two strains of *S.flexneri* 1a were resistant to SXT and positive for intI1 and intI1-IS1 but negative for intI2. The resistance gene cassette identified was *blaoxa-30-aadA1*, accounting for 1.7% (2/115). Two strains of *S.flexneri* 2a were susceptible to SXT and positive for intI1 and intI1-IS1 but negative for intI2, with the resistance gene cassette being *blaoxa-30-aadA1*, also accounting for 1.7% (2/115). Two strains of *S.flexneri* 2a were susceptible to SXT and negative for intI1 and intI1-IS1 but positive for intI2, with the resistance gene cassette being *dfrA1+sat1+aadA.* Eleven strains of *S.flexneri* 2a were susceptible to SXT and negative for both intI1 and intI2, accounting for 9.6% (11/115) (**Tables 2**, **3**; **Figures 1-4**).

Among the 115 strains of *S.flexneri*, primers targeting *sul1*, *sul2*, *sul3*, and the SXT element were used for amplification. Positive fragments were obtained in 67 strains, accounting for 58.3% (67/115). Specifically, 15 strains showed positive amplification with the *sul1* primer, representing 13.0% (15/115), while 54 strains showed positive amplification with the *sul2* primer, accounting for 47.0% (54/115). No positive fragments were obtained for *sul3* or the SXT element. DNA sequencing and BLAST comparison confirmed that these positive products corresponded to dihydropteroate synthase genes *sul1* and *sul2*. Among the strains, 11.3% (13/115) carried only *sul1*, 45.2% (52/115) carried only *sul2*, and 1.7% (2/115) of the strains (*S.flexneri* 4a) carried both *sul1* and *sul2*. *Sul1* was detected in *S.flexneri* subtypes 2a, 2b, and 4a, with the highest detection rate in subtype 2a, accounting for 10.4% (12/115). *Sul2* was detected in all subtypes, with the highest detection rates in subtypes 4a, 2a, and 1a, representing 22.6% (26/115), 12.2% (14/115), and 8.7% (10/115), respectively. One strain of *S.flexneri* 2a and two strains of *S.flexneri* 1a (carrying only atypical class 1 integrons with the resistance gene cassette *blaoxa-30-aadA1*) were resistant to SXT but did not amplify *sul1* or *sul2*. Among the 45 SXT susceptible strains, no *sul1* or *sul2* was detected, while among the 70 SXT resistant strains, 67 strains (95.7%, 67/70) carried both *sul1* and *sul2* (**Table 3**; **Figures 5**, **6**).

Analysis of the presence of integrons and resistance genes in S.flexneri strains

The chi-square test revealed significant differences in the presence of class 1 integrons (χ^2=22.800, P<0.01), class 2 integrons (χ^2= 16.365, P<0.01), *dfrV* (χ^2=9.422, P<0.01), *dfrA1* (χ^2=16.365, P<0.01), *sul1* (χ^2=9.588, P<0.01), and *sul2* (χ^2=65.445, P<0.01) between SXT-sensitive and resistant strains. However, there was no significant difference in the presence of *dfrA17* between SXT-sensitive and resistant strains (χ^2=1.308, P>0.05) (**Table 4**). Both class 1 and class 2 integrons were detected in the same strains of *S.flexneri* subtypes 2a, 2b, 4a, and X. Among the *S.flexneri* 1a strains, 16.7% (10/12) carried only class 1 integrons, while 83.3% (10/12) carried both class 1 and class 2 integrons. Only 7.7% (2/26) of the *S.flexneri* 4a strains were positive for *dfrA17*. *DfrV* was detected in 17.6% (12/68) of the *S.flexneri* 2a strains and 14.3% (1/7) of the *S.flexneri* 2b strains, while *dfrA1* was present in all serotypes. *Sul1* was detected in 17.6% (12/68) of the *S.flexneri* 2a strains, 14.3% (1/7) of the *S.flexneri* 2b strains, and 7.7% (2/26) of the *S.flexneri* 4a strains. Interestingly, *sul1* and *dfrV* coexisted in 12 strains of *S.flexneri* 2a and 1 strain of *S.flexneri* 2b. Additionally, *sul1*, *sul2*, and *dfrA17-aadA5* were found to coexist in 2 strains of *S.flexneri* 4a. *Sul2* was detected in all serotypes, predominantly in *S.flexneri* 4a, with a detection rate of 100% (**Table 3**).

Discussion

In recent years, the drug resistance of *Shigella* has been increasing, leading to the global spread of multidrug-resistant *Shigella*, which is a pathogen that requires reevaluation due to its significant impact on public health, as recognized by the WHO [2]. The incidence of *Shigella* infections is related to the economic conditions, public health, lifestyle habits, and predominant serotypes in each region. Bacterial dysentery caused by *Shigella* is a serious public health problem in China.

In this study, 115 strains of *S.flexneri* were tested for their resistance to SXT, revealing a resistance rate of 60.9%, which is consistent with previously reported 61.3% in 10 cities in China [18]. However, it is lower than the reported resistance rates of 85% in Peru [19] and 91% in

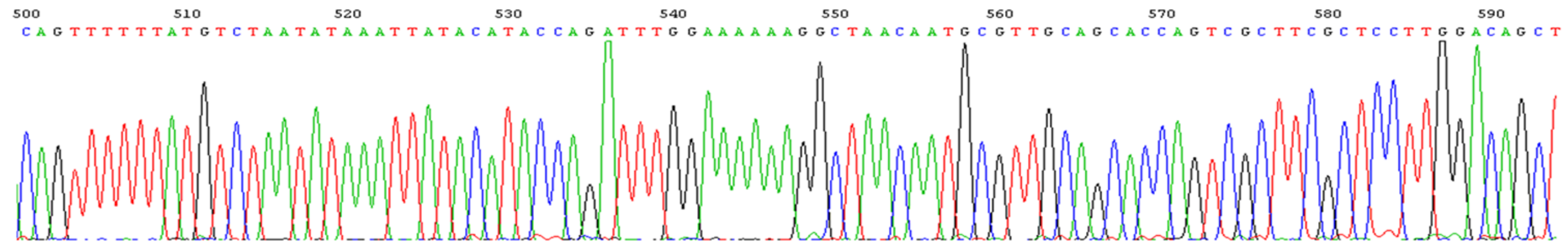

Figure 1. Partial sequence of the *dfrA17-aadA5* gene cassette.

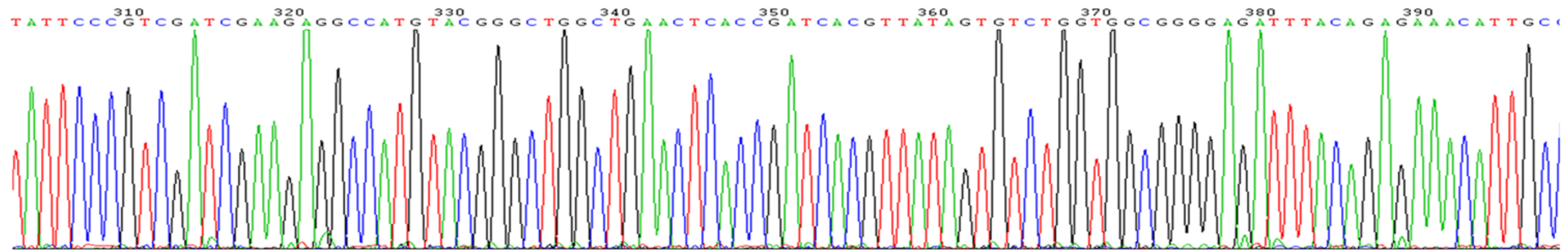

Figure 2. Partial sequence of the *dfrV* gene cassette.

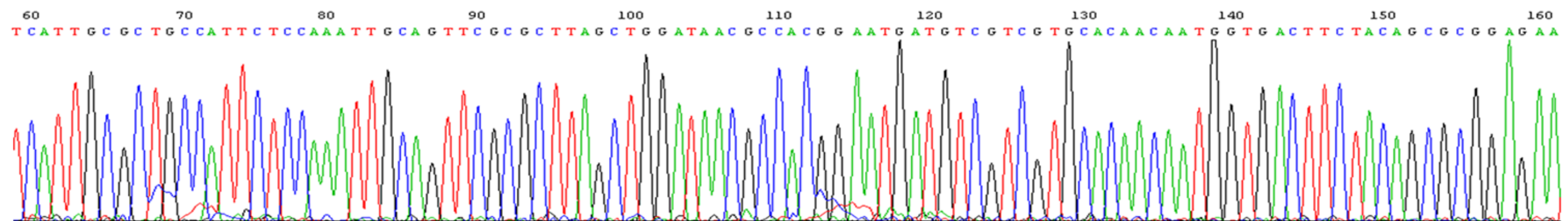

Figure 3. Partial sequence of the *blaoxa-30-aadA1* gene cassette.

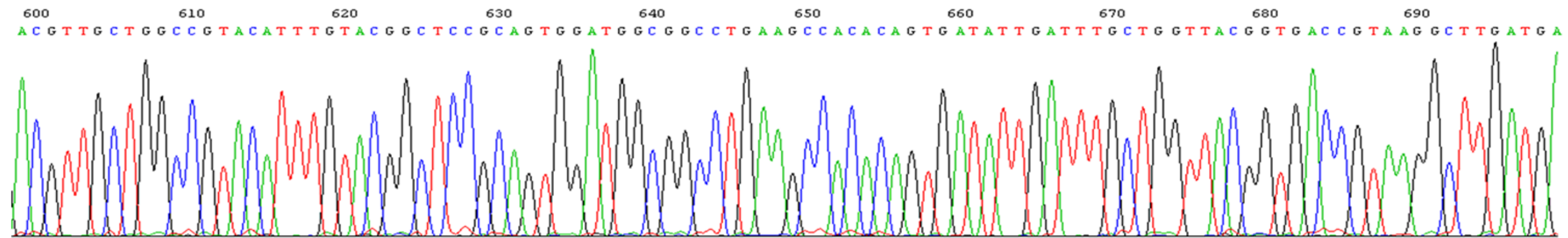

Figure 4. Partial sequence of the *dfrA1+sat1+aadA* gene cassette.

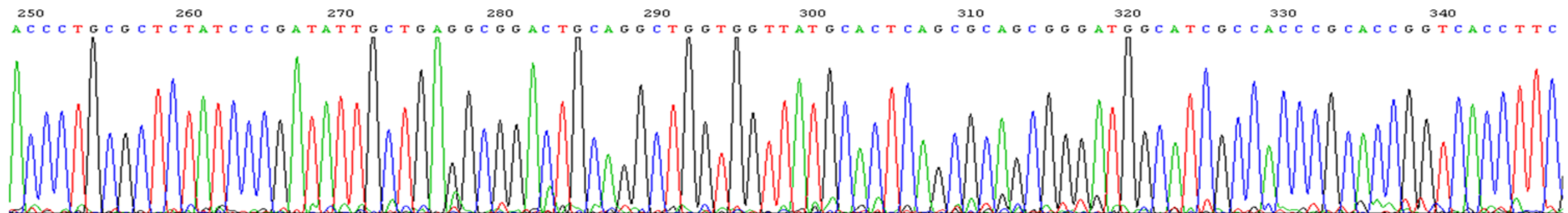

Figure 5. Partial sequence of the *sul1* gene.

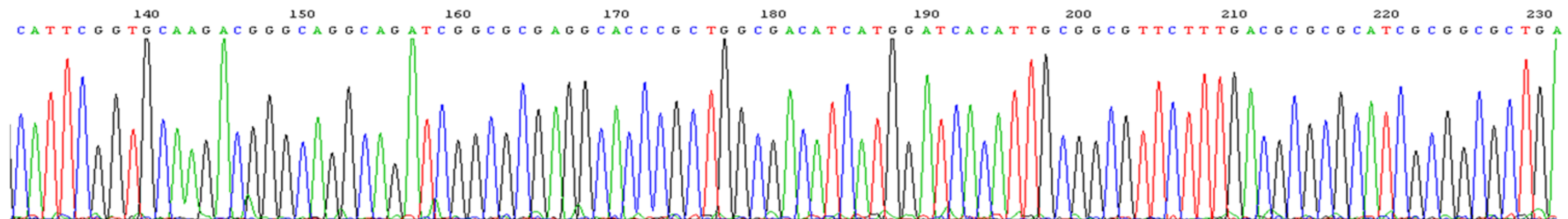

Figure 6. Partial sequence of the *sul2* gene.

Table 4. Analysis of integrons and resistance genes in the 115 strains of *S.flexneri*

Integrons and related genes	Sensitive (45)	Sensitive composition ratio (%)	Drug Resistance (70)	Ratio of drug resistance (%)	X^2	*P*
Class 1 In (intI1, in positive)	32	71.1	70	100.0	22.800	<0.01
Class 2 In (intI2, intI2ca positive)	32	71.1	68	97.1	16.365	<0.01
dfrA17	0	0.0	2	2.9	1.308	>0.05
dfrV	0	0.0	13	18.6	9.422	<0.01
dfrA1	32	71.1	68	97.1	16.365	<0.01
sul1	0	0.0	15	21.4	9.588	<0.01
sul2	0	0.0	54	77.1	65.445	<0.01

Iran [20], yet higher than the resistance rate of 44.4% in Xinjiang, China [21]. These variations may be attributed to the local use of antibiotics and prevalent serotypes.

Plasmids, integrons, and genomic islands are mobile genetic elements associated with bacterial multidrug resistance. Integrons, in particular, are significant due to their ability to capture, integrate, and disseminate resistance genes among bacteria, both within and across species, which is a major cause for concern. Cecilia S. Toro and colleagues [9] studied 349 strains of *Shigella* sonnei from 1995 to 2013, of which 55.6% carried class 1 integrons containing the *blaOXA-1-aadA1* gene and 18.3% carried class 2 integrons containing the *dfrA1* gene. Nabi Jomehzadeh et al. [8] detected 47 strains of *S.flexneri* in southwest Iran, and the carriage rates of class 1 and class 2 integrons were 87.2% and 93.6%, respectively. Lluque et al. [19] tested 55 strains of *S.flexneri* in Peru, and the carriage rates of class 1 and class 2 integrons were 51% and 52%, respectively. Kang Haiquan et al. [18] tested 31 strains of *S.flexneri* in China, and the carriage rates of class 1 and class 2 integrons were 80.6% and 83.9%, respectively. In our study, among the 115 strains of *S.flexneri*, 88.7% carried class 1 integrons, with 85.2% being non-typical integrons carrying the *blaoxa-30-aadA1* variable region, and 13.0% carrying typical integrons with the *dfrA17-aadA5* and *dfrV* variable regions. Furthermore, 87.0% carried class 2 integrons with the *dfrA1+sat1+aadA1* variable region. These results are similar to those reported in Iran [8] and by domestic scholars [18, 22], but different from the carriage rates and resistance gene profiles reported in Peru [19] and Chile [9]. The differences may be related to the tested regions, time periods, and *Shigella* strains. Whether regional clonal transmission exists requires further investigation.

The *OXA*, *aadA*, and *sat* genes encode resistance to β-lactams, aminoglycosides, and streptomycin, respectively, and are not related to SXT resistance. The *dfr* gene encodes resistance to trimethoprim, and the *sul* gene encodes resistance to sulfamethoxazole. Statistical analysis revealed that *dfrV*, *dfrA1*, *sul1*, and *sul2* are statistically related to SXT resistance, indicating the involvement of these four genes in *S.flexneri*'s resistance to SXT. In a study by Shuyu Wu et al. [23], *sul2* was detected in 65% of sulfonamide-resistant *Escherichia coli* in Denmark, along with 45% carrying *sul1* and 12% carrying *sul3*. These genes can be transferred through plasmids ranging in size from 33-160 kb, contributing to the spread of sulfonamide-resistant *E. coli* in the region. Lluque A et al. [19] detected *sul2* in 95% of clinical SXT-resistant *Shigella* strains and *dfrA1* in 47% of strains. In a study by Mohd SI et al. [24], *sul2* was detected in all 146 SXT-resistant *S.flexneri* 2a strains, mediated by a 4.3 MDa plasmid. In our study, 13.0% of the 115 *S.flexneri* strains tested positive for *sul1*, 46.9% tested positive for *sul2*, and 1.7% tested positive for both *sul1* and *sul2*. Among the 40 SXT-sensitive strains, no *sul* genes were detected, while among the 70 SXT-resistant strains, 67 strains (95.7%; 67/70) carried *sul* genes. These research findings suggest that various regions of integrons, *dfrV*, *dfrA1*, *sul1*, and *sul2*, contribute to SXT resistance in *S.flexneri*. However, one *S.flexneri* 2a strain and two *S.flexneri* 1a strains only carried *blaoxa-30-aadA1* genes and exhibited resistance to SXT, suggesting the presence of other resistance mechanisms.

The SXT element, initially discovered in Vibrio cholerae *O139*, encodes resistance to SXT

[25]. Over recent years, SXT elements have been identified in various other bacteria, carrying different resistance genes [26, 27]. In our study, the SXT element was not amplified in *S.flexneri*, yet its potential for horizontal gene transfer warrants close monitoring. Among the 115 *S.flexneri* strains, three showed resistance to SXT without amplifying the corresponding resistance genes, and 30 carried *dfrA1* but remained sensitive to SXT. Further exploration is needed to determine if *dfrA1* is a non-functional structural gene.

Given the limited number of strains examined, it is possible that some integrons and drug resistance genes might not be detected, and the high prevalence of certain resistance genes may suggest the presence of gene clones specific to this region. Our analysis focused solely on the presence of resistance genes and their statistical correlation with SXT resistance, revealing significant associations. However, without gene cloning of the positive markers, it remains uncertain whether these genes are functional within the strains.

In summary, integrons are involved in SXT resistance of S.flexneri, and dfrV, dfrA1, sul1, sul2 are closely related to SXT resistance of S.flexneri.

Disclosure of conflict of interest

None.

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