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Insertion sequences in the CRISPR-Cas system regulate horizontal antimicrobial resistance gene transfer in *Shigella* strains

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ABSTRACT

Multidrug-resistant (MDR) *Shigella* strains are an enormous threat to public health. Antimicrobial resistance genes are frequently located on plasmids, phages and integrons, which enter bacterial cells by horizontal gene transfer (HGT). CRISPR-Cas systems are adaptive prokaryotic immune systems in bacteria that confer resistance to foreign genetic material such as phages and other mobile genetic elements. However, this may come at a cost of inhibiting the acquisition of other beneficial genes through HGT. This study investigated how *Shigella* strains regulate the activity of the CRISPR-Cas system spontaneously when they require an exogenous gene necessary for survival. Insertion sequence (IS) elements were identified in *cas* genes, such as IS*600* in *cse2*, IS*Sfl2* in *cas6e* and IS*629* in *cse1–cas3*. The number of spacers in CRISPR-Cas arrays in strains containing an IS was less than that for strains with no IS. Interestingly, fewer spacers were also found in MDR *Shigella* isolates. Furthermore, an antimicrobial-resistant strain was constructed by electrotransformation of a resistance plasmid in order to detect changes in the CRISPR-Cas system. It was found that the *cse2* gene had a new IS (IS*600*) in the antimicrobial-resistant strain. Bioinformatics analyses showed that the IS*600* insertion hotspot was TGC-GGC in the *cse2* gene, and the tertiary structure of the Cse2 protein was different with IS*600*. IS*600* caused a five-order of magnitude decrease in relative expression of the *cse2* gene. This study sheds mechanistic light on CRISPR-Cas-mediated HGT of antimicrobial resistance genes in *Shigella* spp. isolates.

1. Introduction

Shigella spp. are the leading cause of bacterial dysentery, which poses a serious threat to human health. Shigellosis remains a public-health concern because it is responsible for an estimated 200 million illnesses and 600 000 deaths per year worldwide and 500 000 illnesses and 200–500 deaths in China [1–3]. Owing to the wide use of antibiotics, the emergence of drug-resistant strains of *Shigella* has become a great challenge [4]. Mechanisms of antimicrobial resistance are associated with genetic point mutations, cellular impermeability, active efflux systems such as the AcrAB–TolC efflux pump, target site mutation(s), and horizontal transfer of resistance genes, which have been identified as the most important factors [5–7].

Clustered regularly interspaced short palindromic repeats (CRISPRs) and the CRISPR-associated (Cas) proteins constitute the CRISPR-Cas system in a number of bacteria and archaea hosts and confer resistance against invading foreign genetic material [8]. CRISPR arrays are composed of short repeats that are separated by unique sequences with a similar size called spacers. The mechanism of action of the CRISPR-Cas system can be divided into three stages, namely adaptation, expression and interference. Short nucleic acid sequences from foreign nucleic acids, such as phages or plasmids, are incorporated into the CRISPR array at the promoter (leader) end during adaptation, and the repeat array is transcribed into a long precursor CRISPR RNA (pre-crRNA), which is processed into short crRNAs in the expression phase. Finally, the crRNAs form a ribonucleoprotein complex with Cas protein(s) resulting in target degradation of the foreign nucleic acid (DNA or RNA) in the interference stage to maintain genomic stability [9,10]. It has been shown that CRISPR interference can prevent plasmid conjugation by sequence identity between a spacer and a plasmid target sequence, whereas it cannot for the mutant plasmid [11]. Significant associations have been found between the absence of CRISPR-Cas

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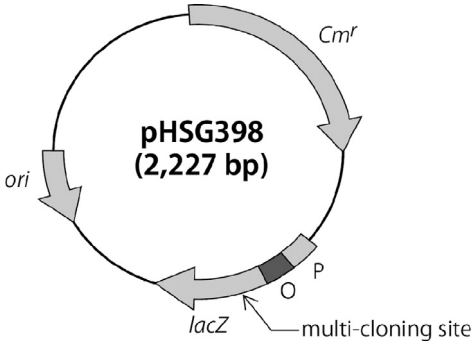

Fig. 1. Map of the chloramphenicol resistance plasmid pHSG398.

and the presence of antimicrobial resistance in *Enterococcus faecalis* strains [12,13]. Our previous studies also found that CRISPR-Cas was widely present in *Shigella*, the CRISPR1 spacer related to the resistance gene, and the existence of insertion sequence (IS) elements in the middle of the *cas* gene [14,15].

ISs, arguably the smallest and most numerous autonomous transposable elements, are a special part of the chromosome playing a critical role in shaping their host genomes; they can be transferred from one location to another or between different chromosomes [16]. Insertion of an IS element may lead to gene mutation and altered expression in transcription and translation. We speculate that existence of an IS element in the middle of the *cas* gene will affect expression of the Cas protein, thereby regulating the activity of the CRISPR-Cas system.

Thus, the aim of this study was to explore the relationship between antimicrobial resistance and the CRISPR-Cas system and how ISs impact on the activity of the CRISPR-Cas system in *Shigella* strains.

2. Materials and methods

2.1. Shigella strains

A total of 59 *Shigella* strains were isolated from stool samples of diarrhoeal patients in four Chinese hospitals (Tonggu, Jiangxi Province; Suixian, Henan Province; Zhengzhou, Henan Province; and General Hospital of the People's Liberation Army, Beijing) from 1995–2013, including 48 *Shigella flexneri*, 9 *Shigella sonnei* and 2 *Shigella dysenteriae* (see Supplementary Table S1 for the origin of the strains). All of the isolates were identified in the microbiology laboratory according to standard microbiological and biochemical methods and were typed using slide agglutination tests with *Shigella* polyvalent grouping (Mast Group Ltd., Bootle, UK) and monovalent antisera (Denka Seiken Co. Ltd., Tokyo, Japan). The chloramphenicol resistance plasmid pHSG398 (Fig. 1) was purchased from Takara Bio Inc. (Beijing, China).

2.2. CRISPR amplification and analysis

The primers used for amplification of the CRISPR-Cas regions are listed in Table 1. CRISPR1 and CRISPR3 were designed using sequences found in the GenBank nucleotide sequence database under the accession nos. **CP000038.1, AE014073.1** and **NC_016822.1**. The CRISPR2 array was obtained according to Díez-Villaseñor et al. [17]. Total bacterial DNA was extracted using a Bacterial DNA Extraction Kit (Lifefeng Biotechnology Co., Ltd., Shanghai, China). The PCR reaction mixture (50 µL) included PCR buffer, 10 pmol of primer, 200 mM dNTP, 1 U of *Taq* DNA Polymerase (Takara Bio Inc.) and 30 ng of template DNA. PCR was performed as follows: initial denaturation at 94°C for 5 min; followed by 32 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min; and a final extension step at 72°C for 10 min. Amplified PCR products were resolved in 2% agarose gels. PCR products were sequenced by Sangon Biotech (Shanghai, China). The length of the *cas* gene PCR products varied as some strains contained IS elements in these regions. According to the gene sequencing results, it was determined whether the *cas* gene contained an IS.

The numbers and sequences of repeats and spacers in every CRISPR locus were identified using an online CRISPR finder (http://crispr.i2bc.paris-saclay.fr), CRISPR Recognition Tool (CRT) and multiple sequence alignment [14,18,19].

2.3. Determination of Shigella resistance

The 59 *Shigella* strains were tested for antimicrobial susceptibility to β-lactams, tetracyclines, sulfonamides, fluoroquinolones and chloramphenicol by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. The following antimicrobial agents were tested: ampicillin (10 µg); cefalotin (30 µg); tetracycline (30 µg); trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 µg); chloramphenicol (30 µg); and norfloxacin (5 µg). The reference strain *Escherichia coli* ATCC 25922 (American Type Culture Collection, Manassas, VA) was used for quality control.

The *Shigella* strains were divided into two groups based on the presence or absence of an IS in the *cas* gene. Differences in CRISPR-Cas system spacer number and the relationship between IS and *Shigella* resistance in two groups were then assessed.

2.4. IS600 insertion hotspot analysis and protein tertiary structure prediction of Cse2

ClustalX software (http://www.clustal.org) and BLAST (https://blast.ncbi.nlm.nih.gov) were used to compare the sequencing results of the *cse2* gene. SWISS-MODEL (http://www.swissmodel.expasy.org) and PDBsum Generate (http://www.ebi.ac.uk/pdbsum) were used to predict the Cse2 protein tertiary structure.

2.5. Quantitative detection of cse2 mRNA

Reverse transcription quantitative PCR (RT-qPCR) was designed to detect the *cse2* mRNA level. 16S rRNA was used as the reference gene. Briefly, total RNA of 31 *Shigella* strains was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA) and was then reverse transcribed to cDNA with random primers using a PrimeScript™ RT Reagent Kit (Takara Bio Inc.).

RT-qPCR was performed on an ABI FAST 7500 Real-Time PCR System (Life Technologies) using a SYBR® Premix Ex Taq™ II Kit (Takara Bio Inc.) according to the manufacturer's instructions. The *cse2* relative gene expression values were obtained by normalisation to the 16S rRNA reference gene using the $-2^{\Delta\Delta Ct}$ method, where $-2^{\Delta\Delta Ct} = \Delta Ct$ sample$-\Delta Ct$ calibrator. The primers for *cse2* were designed according to *S. sonnei* Ss046 strain (**CP000038.1**).

2.6. Induction of resistant strain by electroporation

Antimicrobial-susceptible *Shigella* strains (mel-sf2005127/hn and mel-sf1998024/zz) were grown overnight on Luria–Bertani (LB) agar and a single colony was picked and inoculated in 2 mL of LB medium for 10 h at 37°C (220 rpm). Then, 500 µL of culture was diluted in 50 mL of fresh LB medium and continued to grow for ca. 3 h to reach an optical density at 600 nm (OD_{600}) of 0.4. Cells

Table 1
PCR primer sequences of the CRISPR-Cas system.

Gene	Primer	Sequence (5′→3′)	Approx. PCR product size (kb)	Annealing temperature (°C)
CRISPR1	CR1 F	AGCGACTAACTGGAATCTTG	0.7	56
	CR1 R	CAATCTGGCTACTGGAAGTG		
CRISPR2	CR2 F	CGATCCAGAGCTGGTCGAATG	1	56
	CR2 R	AGTGCTCTTTAACATAATGGATG		
CRISPR3	CR3 F	TTGTYAGGTAGGTTGGTGAAG	0.7	56
	CR3 R	GCGAAGAGAAAGAACGAGTA		
cas2–cas1	*cas2*F	CCCATCCAAATCCACCGGAA	1	55
	cas1 R	CGCCTGCATTATGCTCGAAC		
cas6–cas5	*cas6*F	TTGCTGTTGTCGGTAGGCAT	2.6/1.3	55
	cas5 R	ATGAACTTCCTTCGCGCTCA		
cas7	*cas7*F	CACGTCCTTCATGCTTCCCT	1	55
	cas7 R	AACTGTGGTGCTGGATGGAG		
cse2	*cse2*F	GCCCAGCGGATACGGATAAA	1.6/0.4	55
	cse2 R	ACGGATGGATTTCGCCTGTT		
cse1–cas3	*cse1* F	TTTCTCCCTGGCGGCTTTAG	2.6	55
	cas3 R	GTCATTCCTGCTTCCAGCCT		

were then cooled on ice for 20 min before being collected by centrifugation (5000 × *g* for 10 min) at 4°C. Cells were re-suspended in 1/10 vol of glycerol after three washes using double-distilled water and then 100 µL aliquots were prepared for storage at –80°C.

The chloramphenicol resistance plasmid pHSG398 was electrotransformed into *Shigella* strains mel-sf2005127/hn and mel-sf1998024/zz as follows. A volume of 100 µL of competent cells was placed in a pre-chilled electroporation cuvette (Bio-Rad, Richmond, CA) and then 1 µL of plasmid (50 ng/µL) was added to the cuvette. The transformation system was treated with a Bio-Rad Gene Pulser with the following parameters: 200 Ω; 25 µF; and 2.0 kV. Following electroporation, cells were immediately resuspended in SOB (Super Optimal Broth) medium and were recovered at 37°C for 2 h with gentle shaking (150 rpm). The culture was then placed on LB agar containing chloramphenicol (30 µg/mL). Following incubation for 20 h at 37°C, a single resistant colony was selected and was verified by PCR and DNA sequencing.

The chloramphenicol-resistant strains were named mel-sf2005127/pHSG398 and mel-sf1998024/pHSG398. The *cse2* gene sequence was obtained by PCR with specific primers and was compared with the susceptible strain to identify DNA differences.

2.7. Statistical methods

Statistical significance was two-tailed and a *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed by Student's *t*-test, χ^2 test and Fisher's exact test using IBM SPSS Statistics v.21.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Insertion sequence distribution in CRISPR-Cas system

PCR amplification of the CRISPR region gave positive products for all 59 *Shigella* strains: 47 strains possessed CRISPR1, which had an intact *cas* gene cluster, whereas only a repeat sequence and a pseudo *cas* gene were present in the remaining 12 *Shigella* strains (Table 2). An IS was identified in CRISPR-Cas of 32 *Shigella* strains, including IS*Sf13* in *cas6* (between *cas6e* and *cas5*), IS*600* in *cse2*, IS*629* in *cse1–cas3*, and IS*1* in CRISPR2. The difference in the number of spacers and the presence of an IS was statistically significant (Table 3).

Table 2
Distribution of CRISPR-Cas system in 59 *Shigella* strains.

CRISPR type [a]	No. (%) of strains	*cas* gene		IS	
		+	–	+	–
A	38 (64.41)	38	0	31	7
B	9 (15.25)	9	0	1	8
C	3 (5.08)	0	3	0	3
D	9 (15.25)	0	9	0	9

IS, insertion sequence.
[a] A, CRISPR1+CRISPR2+CRISPR3; B, CRISPR1+CRISPR2; C, CRISPR2+CRISPR3; and D, CRISPR3.

Table 3
Relationship between number of spacer sequences and presence of an insertion sequence (IS).

IS	No. of CRISPR1 spacer sequences				No. of CRISPR2 spacer sequences				
	2	3	9	*P*-value	1	2	5	6	*P*-value
+	28	2	2	0.000 *	29	0	1	2	0.000 *
–	0	15	0		0	15	0	0	

* Fisher's exact test.

3.2. Relationship between insertion sequences in the CRISPR-Cas system and Shigella resistance

Combining the results of the antimicrobial susceptibility tests and their CRISPR-Cas system information for the 59 *Shigella* strains, a statistically significant difference was found in the distribution of ISs and multidrug resistance. In the 24 CRISPR-Cas-positive multidrug-resistant (MDR) strains, 19 strains harboured an IS in the CRISPR-Cas system. The difference between IS presence and single drug resistance, such as resistance to ampicillin, cefalotin, tetracycline, SXT, chloramphenicol or norfloxacin, showed no statistically significant difference ($P > 0.05$) (Table 4).

3.3. Electroporation-induced resistant bacteria

The pHSG398 plasmid contains the chloramphenicol resistance gene (cm^r), which was transferred into *Shigella* mel-sf1998024/zz and mel-sf2005127/hn. Transformants with resistance to chloramphenicol (mel-sf1998024/pHSG398 and mel-sf2005127/pHSG398) were obtained on a screening plate. PCR amplification of the cm^r gene verified the results of electrophoresis (Fig. 2).

Table 4
Relationship between CRISPRs and presence of insertion sequence (IS) and *Shigella* resistance phenotype.

Phenotype		CRISPR1				CRISPR2				IS [a]				
		–	+	χ^2	*P*-value	–	+	χ^2	*P*-value	–	±	+	χ^2	*P*-value
MDR	No	2	23	4.077	0.055 *	1	24	2.874	0.090	2	10	13	6.913	0.032
	Yes	10	24			8	26			10	5	19		
AMP	S	1	16	–	0.134 *	1	16	–	0.219 *	1	6	10	–	0.320 *
	I	1	6			0	7			1	1	5		
	R	10	25			8	27			10	8	17		
CEF	S	7	21	–	0.059 *	5	23	–	0.146 *	7	9	12	–	0.080 *
	I	0	15			0	15			0	3	12		
	R	5	11			4	12			5	3	8		
TET	S	2	14	–	0.482 *	1	15	–	0.421 *	2	7	7	–	0.172 *
	R	10	33			8	35			10	8	25		
SXT	S	2	17	–	0.303 *	1	18	–	0.247 *	2	7	10	–	0.253 *
	R	10	30			8	32			10	8	22		
CHL	S	3	25	–	0.188 *	2	26	–	0.277 *	3	9	16	–	0.288 *
	I	0	1			0	1			0	0	1		
	R	9	21			7	23			9	6	15		
NOR	S	9	39	–	0.535 *	7	41	–	0.700 *	9	12	27	–	0.563 *
	I	0	1			0	1			0	1	0		
	R	3	7			2	8			3	2	5		

MDR, multidrug-resistant (resistant to at least three drugs); S, susceptible; I, intermediate; R, resistant; AMP, ampicillin; CEF, cefalotin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol; NOR, norfloxacin.

* Fisher's exact text.

[a] – indicates no CRISPR-Cas system, ± indicates a CRISPR-Cas system but no IS, and + means CRISPR-Cas system with IS.

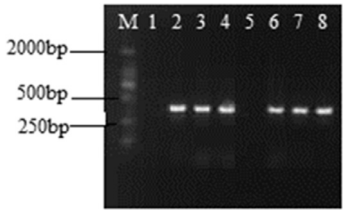

Fig. 2. Results of PCR amplification of the chloramphenicol resistance gene (*cm*^{r}). Lanes 1 and 5, mel-sf2005127/hn and mel-sf1998024/zz, respectively; lanes 2–4, transformants mel-sf2005127/pHSG398; and lanes 6–8, transformants mel-sf1998024/PHSG398.

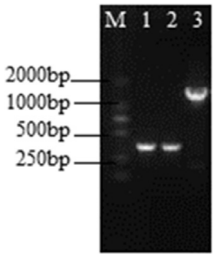

Fig. 3. Electrophoresis of amplification products of the *cse2* gene in mel-sf2005127/hn and mel-sf2005127/pHSG398. M, 2000-bp DNA marker; lanes 1 and 2, mel-sf2005127/hn; lane 3, mel-sf2005127/pHSG398.

3.4. Detection of the cse2 gene in resistant bacteria

Electrophoresis results of the amplification product of the *cse2* gene in the transformants of *Shigella* mel-sf2005127/hn showed that the PCR amplification products varied from 536 bp to 1692 bp (Fig. 3). IS*600* gene amplification was carried out using *Shigella* mel-sf2005127/hn, plasmid pHSG398 and the *cse2* gene of *Shigella* mel-sf2005127/pHSG398 as templates. IS*600* was found in *Shigella* mel-sf2005127/hn and the *cse2* gene of *Shigella* mel-sf2005127/pHSG398 (Fig. 4).

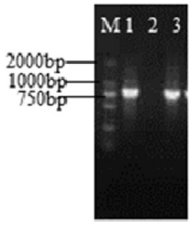

Fig. 4. PCR amplification products of insertion sequence IS*600* in *Shigella* strains and the pHSG398 plasmid. M, 2000-bp DNA marker; lane 1, mel-sf2005127/hn; lane 2, pHSG398, not amplified; lane 3, PCR *cse2* gene of mel-sf2005127/pHSG398.

3.5. Bioinformatics analyses of the cse2 gene

The sequencing results of 20 *cse2* gene (*IS*600+) strains were compared using ClustalX software and it was found that IS*600* inserted into the *cse2* gene from the 263 bases, which was generally located in the middle part of the *cse2* gene. The IS*600* insertion hotspot in the *cse2* gene was the TGC-GGC gene motif (Fig. 5). Further analysis revealed IS*600* inserted at this site (Fig. 6).

From the predicted tertiary structure model, it was deduced that the tertiary structure of the Cse2 protein in IS*600*(+) and IS*600*(–) strains had obvious differences. Changes in the Cas protein tertiary structure would influence the function of the Cas protein and the CRISPR-Cas system (Fig. 7).

3.6. Effect of IS600 on expression and tertiary structure of the Cse2 protein

The *cse2* gene was detected in 31 *Shigella* strains by PCR. IS*600* was found in 19 strains, but not the other 12 strains. RT-qPCR results showed that the relative expression level of *cse2* mRNA was 1.76×10 in IS*600*-positive *Shigella* strains and 1.07×10^6 in IS*600*-negative strains (Table 5). Insertion of IS*600* led to a decrease in the relative expression of CRISPR-related protein *cse2* mRNA ($t = -3.551$, $P = 0.004$).

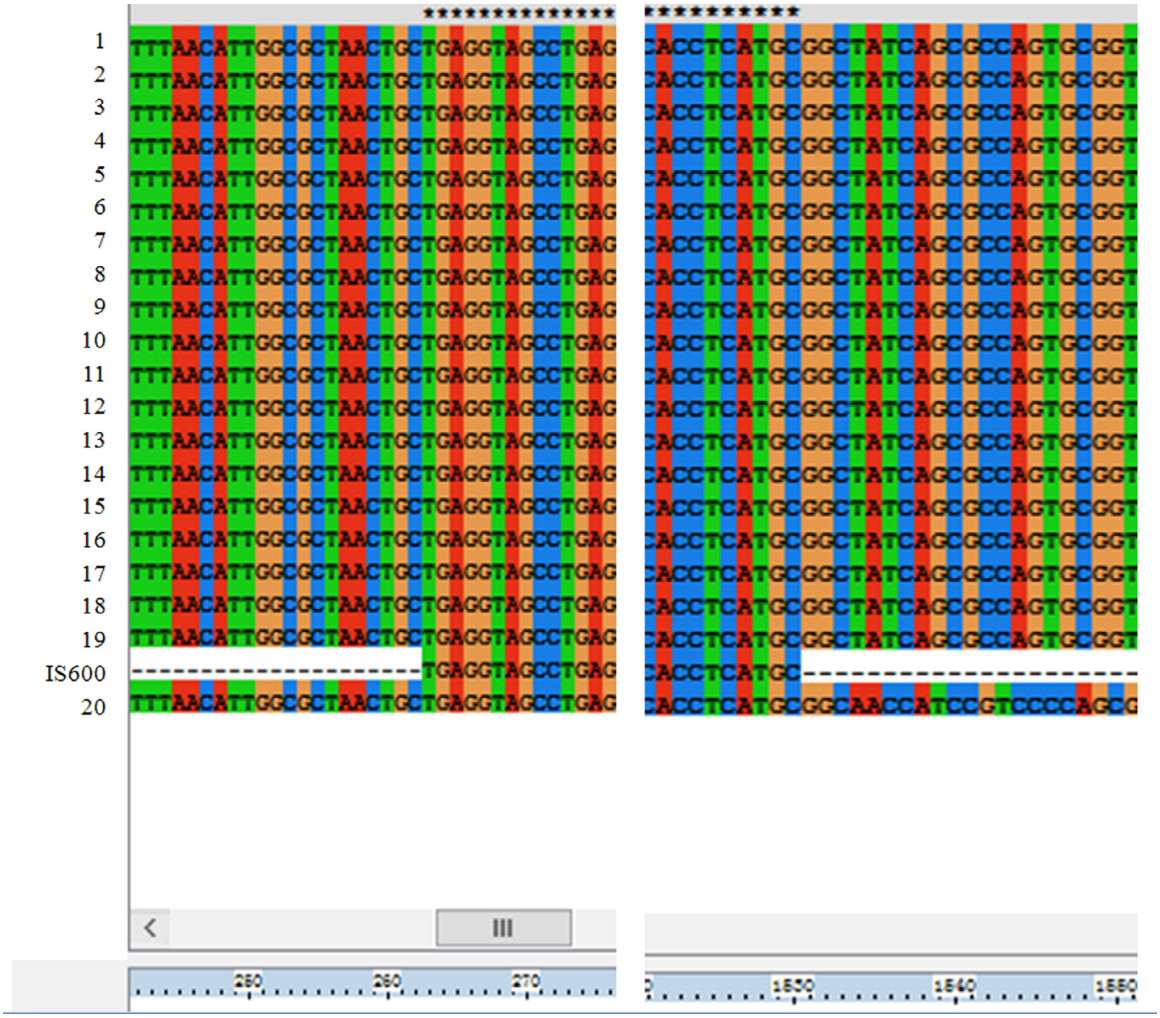

Fig. 5. Comparison of *cse2* gene sequencing results. Rows 1–19, *cse2* gene sequencing results from insertion sequence IS*600*-positive *Shigella* strains; row 20, *cse2* gene (IS*600*+) from *Shigella* Ss046 (**NC_007384.1**).

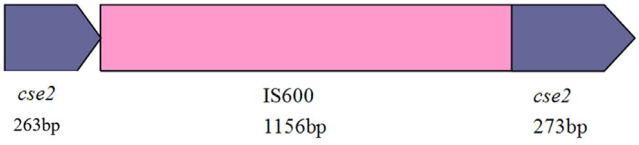

Fig. 6. Schematic diagram of the *cse2* gene sites with insertion sequence IS*600* insertion. The pentagons represent the inserted *cse2* gene; the two lengths are 263 bp and 273 bp, respectively. The rectangle represents the gene for transposable protein IS*600*, with a length of ca. 1156 bp.

Table 5
Relative expression of *cse2* mRNA with or without insertion sequence IS*600*.

IS*600*	mRNA relative expression (mean ± S.D.)	*t*-value	*P*-value
IS*600*-negative ($n = 12$)	$1.07 \times 10^6 \pm 1.41 \times 10^6$	−3.551	0.004
IS*600*-positive ($n = 19$)	$1.76 \times 10 \pm 3.75 \times 10$		

S.D., standard deviation.

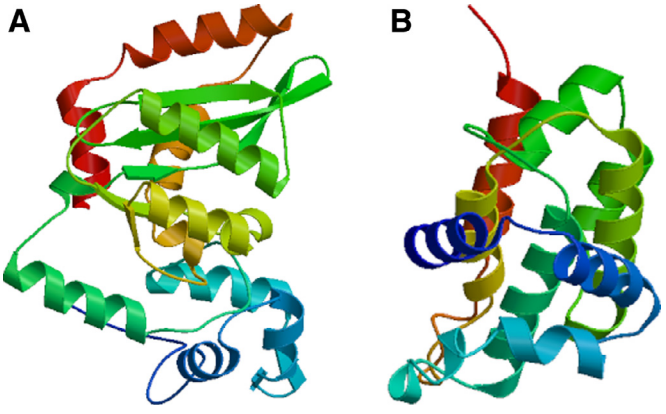

Fig. 7. Cse2 protein tertiary structure: (A) with insertion sequence IS*600*; and (B) without IS*600*.

4. Discussion

Mobile genetic elements such as plasmids, integrons, transposons, ISs and other elements can be transferred in the same or different types of bacterial strains through horizontal gene transfer (HGT), which causes recipient bacteria to acquire resistance to a variety of drugs/agents. In *Shigella*, only 5% of antimicrobial resistance genes are located on the bacterial chromosome and the remaining 95% are generally presented in plasmids, integrons, transposons and other Mobile genetic elements [6].

The CRISPR-Cas system is a bacterial immune system that degrades specific nucleic acids of invasive elements through expression of the Cas/crRNA complex [21]. It is a specific defence

mechanism for phage infection, plasmid conjugation and transformation by lateral gene transfer and can maintain the stability of the bacterial genome. Although the benefits of resisting phage infection are evident, this can come at a cost of inhibiting the acquisition of other beneficial genes through HGT [22,23].

As the simplest highly mobile transposable element, ISs are a special component of chromosomes, generally with a length between 0.7–2.5 kb, that insert into one or more target loci of the genome. ISs in the genome may lead to bacterial gene mutations and the normal expression of interference target sequence both at transcriptional and translational levels [24]. Insertion in *cse2, cas6e* and *cse1–cas3* genes may affect transcription and expression of the corresponding protein. Cas2 and Cas6 proteins are core parts of the Cascade complex and alteration of these essential components is bound to affect the function of the CRISPR-Cas system.

Analysis of the CRISPR-Cas system of 59 clinical *Shigella* isolates revealed that 32 strains contained an IS in their *cas* gene, and strains containing an IS had fewer interval sequences. There was a statistically significant difference among the distribution of ISs and MDR bacteria. Bacteria with ISs were mostly MDR. A study has shown that the fewer interval sequences in the CRISPR-Cas system, the lower the activity [25]. *Shigella* containing low activity of the CRISPR-Cas system are more likely to acquire antimicrobial resistance genes by HGT and to transform into MDR bacteria.

Clinical bacterial isolates face enormous antibiotic selection pressure and can acquire resistance through HGT of resistance genes to survive [26]. CRISPR-Cas can resist foreign genes to some extent. Therefore, bacteria deactivate the CRISPR-Cas system via a variety of mechanisms, such as loss of integrity of the CRISPR-Cas system, containing a transposable element in the *cas* gene, single nucleotide substitution or deletion to make the *cas* gene lose its function, and the conjugation of plasmid matching spacer sequences [27]. Thus, *Shigella* can regulate the activity of the CRISPR-Cas system spontaneously when it needs to acquire an exogenous gene necessary for its survival.

In the current study, susceptible *Shigella* strains were induced by electroporation with a plasmid containing the chloramphenicol resistance gene (cm^r). Comparing the CRISPR-Cas system of two strains, it was found that the *cse2* gene in resistant strains was inserted with the IS*600* gene sequence and that the IS*600* sequence was found in the bacterial genome. Expression results showed that insertion of IS*600* resulted in a five-order of magnitude (10^5) decrease in relative expression of the *cse2* gene. Following insertion of IS*600* into the *cse2* gene in *Shigella*, the physical and chemical properties, conserved domains and tertiary structure of the Cas protein are altered. This will definitely affect the structure of Cascade complexes and the function of the entire CRISPR-Cas system.

In the *E. coli* type I-E CRISPR-Cas system, Cse1, Cse2, Cse7, Cse5 and Cse6 assemble with a 61-nucleotide crRNA to form the Cascade (CRISPR-associated complex composed of the Cascade for antiviral defence) complex, which is combined with the target gene. Cascade is a 405-kDa complex comprising five functionally essential CRISPR-associated (Cas) proteins. Its overall shape is similar to the 'hippocampus', composed of Cse1, Cse2, Cas7, Cas5 and Cas6e according to the ratio 1:2:6:1:1. The Cse2 protein is a specific subtype of Cas protein belonging to type I-E CRISPR-Cas system and is an important part of Cascade protein complexes [28,29].

Cas1 and Cas2 proteins are related to the acquisition of new spacers and Cas3 is the tool enzyme to degrade the target gene. Cse2 is in the middle of the compound and its function is to stabilise the target DNA, to increase the affinity between Cascade and target DNA that plays a role in the degradation of exogenous DNA [30].

The length of the *cse2* gene containing IS*600* is 1692 bp, comprising the *cse2* gene (536 bp) and IS*600* (1156 bp). The IS*600* insertion site is in the *cse2* gene and *cse2* will be divided into two parts. IS*600* in *cse2* may influence the activity of the CRISPR-Cas system, which allows *Shigella* to access exogenous resistance genes in order to survive under external antibiotic pressure.

In conclusion, in this study we demonstrated in *Shigella* strains that IS*600* was inserted into the *cse2* gene under the pressure of external antibiotics, which decreased expression of *cse2* mRNA, modified the Cse2 protein and influenced the construction of the Cascade complex, which cannot combine with its target gene. Thus, the CRISPR-Cas system appeared to lose the corresponding CRISPR interference function. Bacteria regulate the activity of the CRISPR-Cas system via bacterial ISs and could acquire more resistance and virulence genes, which is more conducive to its survival in an environment containing antibiotics.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.09.020.

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