Original Article

Existence of virulence genes in clinical *Shigella sonnei* isolates from Jiangsu Province of China: a multicenter study

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Background: The ability of *Shigella* to invade, colonizes, and eventually kill host cells is influenced by many virulence factors. The aims of this study were to assess the presence of 11 virulence genes of *S. sonnei* strains isolated in this country.

Methods: A total of 166 *S. sonnei* was collected from 13 cities of Jiangsu province through the provincial Centers for Disease Control (CDC) from 2010 to 2015 and then the distribution of virulence genes was detected by polymerase chain reaction (PCR) technology.

Results: Invasive virulence genes included *ipaH* and *ial*, in which the positive rate of *ipaH* was 100% while the positive rate of *ial* was 15.1% in *S. sonnei*. The classic pathway of regulating expression of *Shigella* virulence gene involved *virF* and *virB* gene, which positive rates were 33.7% and 24.1% respectively. The most common serine protease autotransporters of Enterobacteriaceae among *S. sonnei* were *sigA* (100%), followed by *sep*A (3.0%), *sat* (3.0%), *pic* (1.2%). Shigella enterotoxin genes include sen, *set*1A, *set*1B were found in 16.3%, 6.0% and 1.8% of the isolates, respectively.

Conclusions: This study provides baseline information on the distribution of virulence genes in clinical *S. sonnei* trains in Jiangsu province in China, which will be important for implementation of effective control strategies.

Keywords: *Shigella sonnei*; virulence genes; distribution; pathogenesis

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Introduction

Shigellosis is an acute invasive enteric infection caused by any of the four species of *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*). *S. flexneri* is the most commonly isolated species in many developing countries (1,2), but *S. sonnei* in developed countries (3,4). With the development in China, *S. sonnei* plays an increasingly important part in Shigellosis (5). What's more, the control of *S. sonnei* is inseparable from the research of the bacteria, including resistance, epidemiology, and virulence gene characteristics.

Although these were many studies involved the prevalence and antimicrobial resistance of *S. sonnei* from different parts of the world and China, little report investigated virulence genes of *S. sonnei* in the worldwide. Virulence factors, however, contribute to colonization and

invasion of epithelial cells and eventually death of host cells. Different distribution of virulence genes in *Shigella* might cause different clinical manifestations (6,7). Invasion plasmid antigen H (*ipaH*) and invasion associated locus (*ial*) are responsible for the invasion of *Shigella* spp (8). Virulence genes encoded *Shigella* enterotoxin including *Shigella* enterotoxin 1 (ShET-1) and *Shigella* enterotoxin 2 (ShET-2). virF and virB (InvE) are two plasmid-borne proteins that control the expression of invasion genes (9). Finally, serin protease autotransporters of enterobacteriaceae (SPATEs), which has two phylogenetical classes, are present in *Shigella spp*. Secreted autotransporter toxin (sat) and Shigella IgA-like protease homologue (sigA) as two members of Class 1 are toxic to epithelial cells. pic (mucinase involved in colonization) and sepA as two members of Class 2 are non-toxic (10). The present study objects to investigate the prevalence and distribution of 11 virulence genes on *S. sonnei* isolated from patients with diarrhea in Jiangsu for the purpose of an epidemiological study.

Methods

A CDC-based active surveillance program was conducted in 13 cities of Jiangsu province from 2010 to 2015. Dysentery or diarrhea patients suspected of *Shigella spp* infection attending in different hospitals were enrolled for this study. Isolated samples were examined for *Shigella spp*. at each hospital using routine biochemical techniques. All collected isolates were further confirmed by Rapid ID32E strips (bioMérieux Corp., Singapore) and an automatic biochemistry analyzer (Hitachi 917; Boehringer Mannheim, Japan). By used of slide agglutination with hyperimmune sera (Ningbo Tianrong Bio-pharmaceutical Company Limited), O and H antigens were identified.

DNA extraction was performed using Qiagen DNA mini kit according to the manufacturer's protocol. PCR was performed to target virulence genes by using previously reported primers (*Table 1*). Green Taq Mix (Vazyme, Nanjing, China) reaction was carried out according to the manufacturer's instructions. The species were amplified under the following cycling conditions: initial denaturation at 95 ℃ for 5 min, followed by 30 cycles including denaturation for 50 s at 95 ℃, annealing for 45 s (annealing temperature is shown in *Table 1*) and 72 ℃ for 1 min and a single final extension at 72 ℃ for 7 min. A representative amplicon was sequenced for each gene to validate that the primers amplified the target genes.

Statistical analyses were performed by using the database software program SPSS 16.0. Distribution of different virulence genes in serotypes, periods and regions were analyzed by Chi-square test. Statistical significance was set at $P<0.05$.

Results

In the 6 years of the collection, A total of 166 strains of *Shigella* were collected (*Figure 1*). The prevalence of virulence genes among *S. sonnei* was shown in *Table 2*. All isolates were positive for *ipaH* gene, while justly 25 (15.1%) of the isolates were positive for *ial* gene in the present study (*Table 2*). a total of 40 (24.1%) and 56 (33.7%) isolates were found to be positive for *virB* and *virF* genes, respectively, and 30 (18.1%) strains found both *virF* and *virB*. All *S. sonnei* isolates harbored at least one SPATE proteins. The most common SPATE among *S. sonnei* strains was *sigA* (100% of strains), but another Class I SPATE, sat, was just existence in 5 strains of *S. sonnei*. The two Class 2 SPATEs, *sep*A and *pic*, were existence in 5 and 2 strains of *S. sonnei* respectively. The *set1A* gene was present in 10 (6.0%) *S. sonnei* isolates, and *set1B* was present in 3 (1.8%) *S. sonnei* isolates. Both *set1A* and *set1B* were detected in 2 (1.2%) strains of *S. sonnei*. The *sen* was present in 27 (16.3%) *S. sonnei* isolates. Interestingly, just one stain was positive for all virulence genes. In addition, the existence of virulence genes in *S. sonnei* changed in years (*Table 2*).

Discussion

Shigella remains to be the hallmark etiology of inflammatory diarrhea and dysentery and presents a serious challenge to public health, especially in developing countries and regions with substandard hygiene and poor quality water supplies. During the 6 years of this study, there was outbreak of *S. sonnei* in Jiangsu every year, and the numbers of isolated *S. sonnei* increased year by year after 2012, which showed a challenge for controlling infection of *Shigella*.

Multiple copies on large plasmid and chromosome may explain the *ipaH* gene being tested positive in all strains. Studies detected *Shigella* by a PCR assay targeting the *ipaH* gene, which found that the positive rate is higher than traditional culture method (17,18), and the present research confirmed *ipaH* is an appealing target for a diagnostic tool for it remains detectable even in the absence of the plasmid. Unlike *ipaH* gene, the *ial* gene located only on *inv* plasmid which was easily lost. The positive rate of *ial* gene

Table 1 Primers used in this study

Target gene	Primer	Sequence (5'-3')	Annealing temperature	size (bp)
ipaH	*ipaH*-F	TGGAAAAACTCAGTGCCTCT	55 ℃ (11)	423
	ipaH-R	CCAGTCCGTAAATTCATTCT		
ial	*ial*-F	GCTATAGCAGTGACATGG	55 ℃ (12)	320
	ial-R	ACGAGTTCGAAGCACTC		
virB	*VirB*-F	CGATAGATGGCGAGAAATTATATCCCG	56 ℃ (13)	766
	VirB-R	CGATCAAGAATCCCTAACAGAAGAATCAC		
virF	*VirF*-F	AGCTCAGGCAATGAAACTTTGAC	60 ℃ (14)	618
	VirF-R	TGGGCTTGATATTCCGATAAGTC		
sigA	*sigA*-F	CCGACTTCTCACTTTCTCCCG	58 ℃ (15)	430
	sigA-R	CCATCCAGCTGCATAGTGTTTG		
sepA	*sepA*-F	GCAGTGGAAATATGATGCGGC	58 ℃ (16)	794
	sepA-R	TTGTTCAGATCGGAGAAGAACG		
pic	*pic*-F	ACTGGATCTTAAGGCTCAGGAT	58 ℃ (16)	572
	pic-R	GACTTAATGTCACTGTTCAGCG		
sat	*sat*-F	TCAGAAGCTCAGCGAATCATTG	59 ℃ (15)	930
	sat-R	CCATTATCACCAGTAAAACGCACC		
Set1A	*set1A*-F	TCACGCTACCATCAAAGA	57 ℃ (12)	309
	set1A-R	TATCCCCCTTTGGTGGTA		
Set1B	*set1B*-F	GTGAACCTGCTGCCGATATC	57 ℃ (12)	147
	set1B-R	ATTAGTGGATAAAAATGACG		
sen	*sen*-F	ATGTGCCTGCTATTATTTAT	55 ℃ (12)	799
	sen-R	CATAATAATAAGCGGTCAGC		

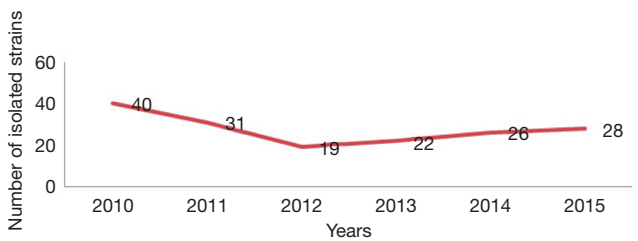

Figure 1 the numbers of isolated *S. sonnei* in 2010–2015.

in *S. sonnei* of Jiangsu was slightly lower than that in other regions (19,20). It should be noted that the existence of *ial* gene in *S. sonnei* was significantly lower than that in *S. flexneri* (2,21,22). The *ial* gene was involved in the invasion of intestinal cells (23), and the lower positive rate of this gene in *S. sonnei* might indicate lower aggressive.

When the growing condition is favorable for invasion, a transcriptional cascade is then initiated by activating *virF* gene to express the AraC-like protein virF, which in turn activates the transcription of the *virB* regulatory gene. The gene product virB protein consequently relieves the heat-stable nucleoid structural protein (H-NS) mediated transcriptional repression and activates the virulence genes on the plasmid-transcription of the virulence genes of *Shigella* is downregulates by H-NS in unfavorable growing condition (9,24). However, there were only 30 (18.1%) strains found both *virF* and *virB*. The low positive rate of those genes indicated that this classic pathway of regulating the expression of *Shigella* virulence gene does not play a major role in *S. sonnei*, and there might be other pathways

Table 2 Distribution of virulence genes in *S. sonnei* from 2011 to 2015

Genes	2010	2011	2012	2013	2014	2015	Total
ipaH	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
ial	2.5%	16.1%	10.5%	13.6%	38.5%	14.3%	15.1%
virF	32.5%	64.5%	21.1%	27.3%	42.3%	7.1%	33.7%
virB	5.0%	38.7%	15.8%	22.7%	46.2%	21.4%	24.1%
sigA	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
pic	0.0%	3.2%	5.3%	0.0%	0.0%	0.0%	1.2%
sepA	0.0%	6.5%	5.3%	4.5%	3.8%	0.0%	3.0%
sat	2.5%	3.2%	5.3%	9.1%	0.0%	0.0%	3.0%
set1A	2.5%	25.8%	5.3%	0.0%	0.0%	0.0%	6.0%
set1B	0.0%	3.2%	5.3%	4.5%	0.0%	0.0%	1.8%
sen	5.0%	16.1%	10.5%	18.2%	38.5%	14.3%	16.3%
Quantity	40	31	19	22	26	28	166

for regulating gene expression.

There is species specificity in the distribution of SPATE. The high presence of *sigA* gene indicated *sigA* toxin may play an important role in the pathogenesis of *S. sonnei* strains, which was agreed with the previous article (20,22). For another class I SPATE, *sat*, the positive rate of the gene in *S. sonnei* was significantly lower than that of the gene in *S. flexneri* (6,25). Probably *sat* toxin has a major contribution in the virulence of *S. flexneri* strains. Similar to *sat*, the class II SPATEs (*pic* and *sepA*) might haven't a significant effect on the pathogenicity of *S. sonnei*.

Shigella enterotoxin 1 (ShET-1) and ShET-2 could alter electrolyte and water transport in the small intestine, which could cause diarrhea and dehydration. ShET-1 is encoded in the set1 (A and B subunit) chromosomal gene that were almost exclusively found in *S. flexneri* isolates and rarely in other serotypes (26). Plasmid-encoded ShET-2 (encoded by *sen*) has been reported in different species of *Shigella* (27). the distribution of *Shigella* enterotoxin in *S. sonnei* was significantly lower than that in *S. flexneri* (21,26), which might mean that there is less danger of *S. sonnei* than *S. flexneri*.

In conclusion, this study provides baseline information on the distribution of virulence genes in clinical *S. sonnei* trains in Jiangsu province in China. Low distributions of genes encoding virulence factors in *S. sonnei* clinical isolates have been found compared with *S. flexneri*. The results obtained in this work contributed to a comprehensive understanding of the epidemiological status and characteristic of *S. sonnei* strains in Jiangsu Province.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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