Outbreak of Infections Caused by Shigella flexneri 2a with ESBL-Producing and Quinolone-Resistance in a Mental Healthcare Center in China

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Abstract

An outbreak of bacillary dysentery occurred in the Xiaoshan Mental Healthcare Center from November 8 to 16, 2012. Eight strains of Shigella flexneri 2a (S. flexneri 2a) were isolated from the same ward. The aim of the investigation was to clarify the origin of outbreak. Rectal swabs or fecal specimens from 7 doctors, 15 nurses, 2 nursing workers, 3 canteen workers, as well as 18 swabs from environmental sample in the ward were screened for Shigella. Phenotypic analysis included standard microbiological identification techniques, serotyping and antimicrobial susceptibility testing. PCR and sequencing were used to characterize drug resistance and virulence genes. Pulsed field gel electrophoresis (PFGE) was carried out for all Shigella isolates. S.flexneri 2a with multidrug resistance was isolated from eight patients and one canteen worker, which carried multiple antibiotics resistance genes and virulence determinants. The results of PFGE confirmed that nine strains of S. flexneri 2a belonged to the same clone. It was suggested that the outbreak strain spread directly from person-person or indirectly from person-food-person from the canteen worker. The outbreak was controlled after quarantine of ill residents, replacement of antibiotics and improvement of hygienic condition.

Background

Shigella species, which are important pathogens of the gastrointestinal tract, are responsible for causing severe diarrhea and acute gastroenteritis, especially in developing countries. It has been estimated that *Shigella* causes 165,000 deaths worldwide every year, of which 55,000 are children younger than 5 years of age [1,2]. In mainland China, the incidence of *Shigella* infections is 20.28 cases per 100,000 people per year [3]. *Shigella* is transmitted by the fecal-oral route and can cause large outbreaks, thus posing a great threat to human public health [4-9].

Currently, third-generation cephalosporins and fluoroquinolones are the main antimicrobial agents in the treatment of *Shigella* infection in China; nevertheless, increasing multidrug resistant *Shigella* has become a serious public health threat [10]. Multidrug resistance to fluoroquinolones, third-generation cephalosporins and azithromycin has been reported worldwide as a growing problem of multidrug resistance in *Shigella* [11-14].

Here, we report an outbreak of infection caused by *S. flexneri 2a* with ESBL-producing and quinolone-resistance in Xiaoshan District, Hangzhou, China. From November 8, 2012 to November 16, 2012, eight strains of *S. flexneri 2a* were isolated form 12 psychiatric patients form Xiaoshan Mental Healthcare Center of Zhejiang Province suffering from diarrhea to varying degrees. Patients were isolated, treated and comprehensive measures were taken to control further outbreak. An investigation started on 15 November 2012. Fortunately, the outbreak was effectively controlled in a short period of time and all patients recovered and there were no deaths.

Methods

Outbreak investigation

During the outbreak, we firstly isolated all infectious patients who received treatment with antibiotics. Next, we started examining the possible reasons of the outbreak. Since all patients were mentally ill and unable to communicate effectively, all relevant medical data (frequency of diarrhea, dietary process, the patient's clinical manifestations and external contact) were provided by the patients' physicians and nurses. Next, stool sample from patients and personal (doctors, nurses, nursing workers, canteen staff), as well as environmental sample in male wards were screened for *Shigella*. The ethics committee of the Mental Healthcare Center approved this study.

Laboratory investigation

Stool specimens from all patients with diarrhea were cultured for the detection of enteric pathogens. Suspected isolates were identified by VITEK2 system (BioMérieux, France) and the serotype of *Shigella* isolates were confirmed by slide agglutination using commercial antisera (Denka Seiken Co. Ltd, Tokyo, Japan), following the manufacturer's recommendations. Antimicrobial susceptibility testing of the *Shigella* isolates was performed by broth microdilution method and the susceptibility was interpreted according to Clinical and Laboratory Standards Institute guidelines [21]. Polymerase Chain Reaction(PCR) and sequencing were used to characterize topoisomerases (*gyrA*, *gyrB*, *parC*, *parE*) in the quinolone-resistance determining regions (QRDRs) and the ESBL genes (bla$_{TEM}$, *bla*$_{OXA}$, *bla*$_{CTX\text{-}M\text{-}1,\ 2,\ 8,\ 9}$ and *bla*$_{SHV}$) and virulence determinants (*ial*, *iapH*, *set1A*, *set1B*, *sen* and *virA*) [14,22]. Pulsed-field gel electrophoresis (PFGE) after the XbaI digestion of chromosomal DNA was carried out for all *Shigella* isolates [23].

Results

Epidemiologic investigation

Eight strains of *S. flexneri 2a* were isolated from fresh fecal specimens of 12 patients (average age 48.9±18.9 years old; youngest patient was seventeen and the oldest one was seventy-four years old) with diarrhea from the same ward of Xiaoshan Mental Healthcare Center in Hangzhou, China. The Mental Healthcare Centre is a fully enclosed medical and rehabilitation institution, which is divided into male ward, female ward and intensive care area. The outbreak occurred in the male ward with twenty-five patients; twelve patients had different degrees of diarrhea accompanied by high temperature (>38 °C) with blood-free mucus feces. Finally, a total of eight *Shigella* strains were isolated from twelve samples and the serotype of all isolates were *flexneri 2a*.

The investigation showed that no doctors, nurses or nursing workers were infected with *Shigella*. Interestingly, *S. flexneri 2a* was detected in stool sample of a canteen staff worker (his job was to distribute food to patients in the male wards). The staff member suffered from diarrhea and abdominal pain. Those symptoms accrued before the outbreak in patients, which suggested the worker was likely to

be a source of infection. Moreover, the serotype, drug sensitivity, resistance and virulence genes of the strain were the same as that in the *Shigella* presented in the outbreak.

Furthermore, in March 2013, we isolated the *S. flexneri 2a* from feces of two patients who have been infected with *Shigella*. In February 2014, one *S. flexneri 2a* was isolated from patient with initial infection. Then we confirmed that these isolates belong to the same clone as the outbreak strains. After that, we continued the monitoring for three years, except for four patients who had been discharged from the hospital. The investigation ended in December 2016. This phenomenon suggests that patients infected with *Shigella* do not have long-term immunity; it may turn into a latent infection under the intervention of antibiotics, and relapse when the body's immune system decreases.

Laboratory results

All 8 *S. flexneri 2a* isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, tetracycline, cefotaxime, ceftazidime, cefepime, ciprofloxacin, but were sensitive to piperacillin/tazobactam, imipenem, and the MIC of azithromycin was 2 μg/mL (no interpretation standard) (**Table 1**). Moreover, all strains produced extended-spectrum β-lactamase (ESBL). In addition, the PCR and sequencing results showed that all outbreak isolates carried $bla_{CTX\text{-}M\text{-}57}$, $bla_{OXA\text{-}30}$, $bla_{TEM\text{-}1}$ resistant genes and *ia1*, *ipaH*, *setlB*, *sen* and *virA* virulence genes. Meanwhile, the *gyrA* (Ser83Leu) and *parC* (Ser80Ile) amino acid mutations were discovered in quinolone resistance determining regions (QRDRs) (**Table 2**). Also, the PFGE results showed that all 9 *S. flexneri 2a* isolates (including the strain isolated from canteen staff) had the same band patterns, which indicated that they belong to the same clone (**Figure 1**).

Control measures

During the outbreak of the epidemic, the hospital took emergency preventive and control measures, including patient isolation, environmental disinfection, propaganda and education on hand hygiene, sanitary management of food and water, improvement of ventilation conditions, medical observation of close contacts.

All patients infected with *S. flexneri 2a* were treated with antibiotics. At the initial stage of infection, 3 patients were treated with intravenous cefotaxime (3.0gV/BID), 3 patients with oral norfloxacin (0.2g/TID), 1 patient with oral sulfamethoxazole (2g/BID) and 1 with intravenous piperacillin/tazobactam (3.375g/Q8H). However, five patients failed to receive effective treatment. Because the *S. flexneri 2a* isolates produce ESBL and quinolone-resistance, we recommended piperacillin/tazobactam or azithromycin as the main treatment approach for those patients. Consequently, the antibiotics were substituted with intravenous piperacillin/tazobactam 3.375g/Q8H and intravenous cefoperazone/sulbactam 3.0g/BID. All the infected patients were cured and five consecutive cultures of *Shigella* revealed to be negative.

Discussion

This study investigated a small-scale outbreak of *S. flexneri 2a* infection in a Xiaoshan Mental Healthcare Center in Hangzhou, China. From March 2012 (observed outbreak) to December 2016 (end of the investigation), 12 strains of *S. flexneri 2a* were isolated, including the infection of a canteen worker and the secondary infection of three patients. After a series of investigations, the canteen staff was identified as the source of *Shigella* infection (direct contact with patients or food intended for patients). All strains were the same clone type, showing ESBL-Producing and Quinolone-Resistance as well as the same PFGE bands. Because of the multi-drug resistance of the strain, most patients failed to respond to an initial antibiotic treatment and underwent a longer course of treatment. Fortunately, no patients died following episodes of shigellosis.

Laboratory results showed that these outbreak isolates carry multiple antibiotics resistance genes, including CTX-M gene. So far, at least 109 variants of CTX-M enzymes (CTX-M-1 to 124) have been described. Among these enzymes, some exhibit increased hydrolysis activity against ceftazidime, while others display a significantly higher rate of hydrolysis of cefotaxime than ceftazidime [15]. The presence of D240C mutations in $bla_{CTX\text{-}M\text{-}57}$ in this study resulted in the increased hydrolysis activity against ceftazidime [16]. Besides, the outbreak *S. flexneri 2a* isolates carried a $bla_{OXA\text{-}30}$, $bla_{TEM\text{-}1}$ genes and conferred resistance to cefotaxime, ceftazidime and cefepime. The main mechanism of quinolone resistance in the *Shigella* spp. was the mutation of *gyrA*, such as at codon 83 or 87 and of *parC* at codon 80 [17, 22]. So, the resistance of ciprofloxacin and levofloxacin may be caused by the mutation of *gyrA* (Ser83Leu) and *parC* (Ser80Ile) in the outbreak isolates.

The ability of *Shigella spp*. to cause shigellosis is attributed to the expression of arrays of virulence genes associated with colonization, invasion/penetration and toxin-mediated disease, such as the invasion-associated locus (*ial*), the invasion plasmid antigen H gene (*ipaH*), *Shigella* enterotoxin 1 (ShET-1) gene (*set1A* and *set1B*), *Shigella* enterotoxin 2 (ShET-2) gene (*sen*) and *virA* gene [18-20]. Estimating the existence of virulence determinants in *Shigella* would help us to further understand its pathogenicity. The *S. flexneri 2a* isolates harbored *virA*, *ial*, *ipaH*, *set1B*, *sen* virulence genes, which indicated that the strain possesses a strong virulence, which in turn can cause diarrhea and abdominal pain.

This study has several limitations. First, although it has been determined that the outbreak was transmitted by a canteen staff personnel who distributed food to patients in the male ward, the source of infection for the staff remains unclear due to limited information. Second, due to the lack of in-depth investigation, it is not clear whether workers introduced *Shigella* into the community before isolation thus causing infection among residents.

Conclusions

This is the first report on the outbreak of bacillary dysentery in a psychiatric ward in China. Our data suggested that the outbreak was caused by an ESBL-Producing and Quinolones-Resistance *S. flexneri 2a* strain which was introduced into the Mental Health Center by a canteen staff who distributed food to patients in the male wards. It was likely that the outbreak strain spread via direct person-person or indirect

person-food-person mode of transmission. Our findings suggested that personnel engaged in the catering industry should earnestly carry out pre-employment medical examinations, frequently attend food hygiene training and develop good hygiene practices; long-term monitoring should be carried out in patients previously infected with *Shigella* for prevention and timely treatment of reinfection, especially those who live in a closed or semi closed environment.

Abbreviations

S.flexneri: *Shigella flexneri*; PFGE: Pulsed-field gel electrophoresis; ESBL: extended-spectrum β-lactamase; QRDRs: quinolone resistance determining regions

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article

Authors' contributions

CLZ designed the study, performed the experiments, analyzed the clinical data and wrote the manuscript; TTL, CY, and YYG collected the clinical samples and investigated the case. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Our study was approved by the Ethics Review Committee of the Mental Healthcare Center of Xiaoshan District. The guardians of all these patients with mental illness provided consent on their behalf.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1

MICs for the outbreak *Shigella flexneri 2a* isolates.

Isolation no.	MICs(μg/mL)									
	AMP	CIP	SXT	FEP	CTX	CAZ	IPM	TZP	AZM	TET
jw001	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw002	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw003	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw004	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw005	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw006	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw007	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw008	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64
jw009	>256	>32	>4	128	>256	>256	0.5	0.5	2	>64

AMP, ampicillin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; IPM, imipenem; TZP, piperacillin/tazobactam; AZM, azithromycin; TET, tetracycline

Table 2

Characterization of resistance genes and virulence genes of the outbreak *Shigella flexneri 2a* isolates

Isolation No.	ESBL-genes			mutation in QRDR		Virulence genes
	CTX-M	OXA	TEM	gyrA	parC	
jw001	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw002	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw003	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw004	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw005	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw006	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw007	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw008	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*
jw009	CTX-M-57	OXA-30	TEM-1	Ser83Leu	Ser80Ile	*vira- ial- ipaH- set1*B- *sen*

Figures

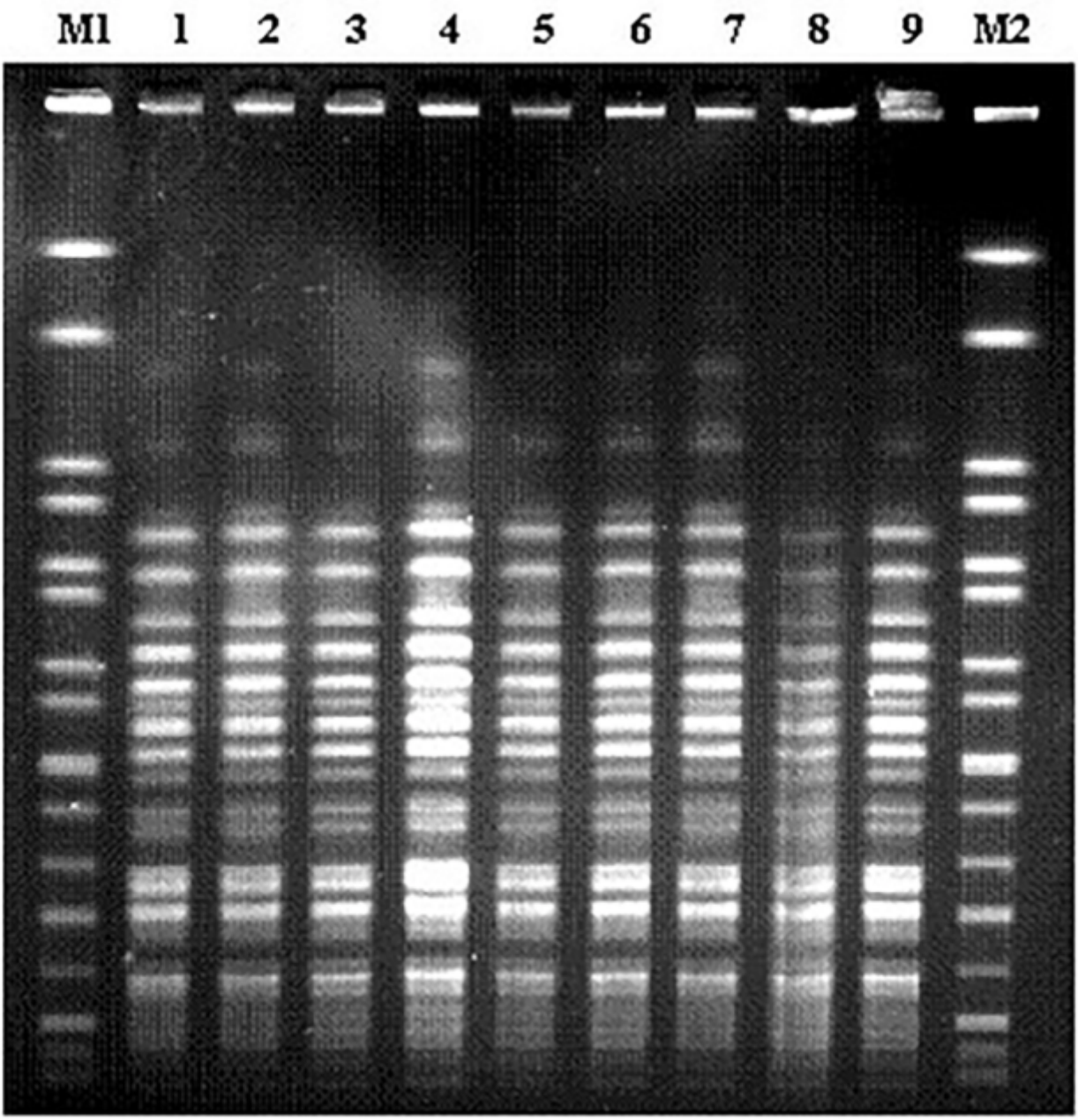

Figure 1

The pulsed-field gel electrophoresis patterns of 9 isolates. Lane M1, M2, molecular mass markers. Line 1-8, patient. Line 9, canteen staff