Two novel mutations in *parE* among *Shigella flexneri* isolated from Jiangsu Province of China, 2016

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Background: The study was conducted to assess the resistance capacity of quinolone against *Shigella flexneri*, and to investigate the involved quinolone resistance mechanism. The data were collected from Jiangsu Province, China in 2016.

Methods: The number of 81 *S. flexneri* was obtained from 12 cities in Jiangsu Province of China during 2016. Slide agglutination was taken for serotyping, and susceptibility test was identified by the disc diffusion method. PCR aimed to amplify the quinolone resistance-determining region (*QRDR*) genes and screen for plasmid-mediated quinolone resistance (PMQR) determinants. Chromosomal mutation was confirmed by sequencing and Blast comparison.

Results: 2a was the commonest serotype, accounting for 40.7% (33/81) of the 81 *S. flexneri*. 70.4% (57/81) isolates expressed resistance against nalidixic acid, and the resistance against ciprofloxacin even reached up to a high proportion of 58.0% (47/81). A total of 8 point mutations were identified, including 2 novel mutations discovered in *parE* (Ser458Leu and Gly408Asp). The common mutation Ser83Leu in gyrA was still the most prevalent here with a percentage of 70.4% (57/81), followed by the approximate mutation of 69.1% (56/81) in parC (Ser80Ile) and His211Tyr in *gyrA*. Meanwhile, 35.8% (29/81) isolates were confirmed with mutation of Gln517Arg in *gyrB*. In addition, *qnrS* positive isolates occupied a proportion of 7.4% (6/81), but only 1 strain was observed with *aac(6')-Ib-cr*. All PMQR positive isolates were resistant to nalidixic acid. However, 5 of them didn't stay susceptible to ciprofloxacin any more.

Conclusions: This is the first time that a study researches the occurrence of mutations in *parE* among *S. flexneri*, Ser458Leu and Gly408Asp included. The study indicates that the high resistance to fluoroquinolone remains a serious problem in Jiangsu, China. Thus, the prevention and control of current infection urge for a comprehensive and systematic surveillance based on persistent surveys.

Keywords: *S. flexneri*; Jiangsu; resistance; *parE*; plasmid-mediated quinolone resistance (PMQR)

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Introduction

With the frequent mobility of international population, bacillary dysentery (or called shigellosis), a severe intestinal infection disease caused by *Shigella* spp, has become a great threat on human's health (1,2). The endemic epidemic and widespread of pathogens have exposed the children and the elderly to be the primary targets of the diarrheal disease, especially for those in the areas with relatively poor economy and unqualified sanitation. People in these areas are more likely to develop bad living habits. As a result, they could easily get in touch with the infectious bacteria involved in the contaminative water and food (3-5). In China, *Shigella* has already been listed into the Chinese Center for Disease Control and Prevention as one of the most important pathogenic bacteria of gastrointestinal infection since 2006, and it calls for more attention and further development (6).

Furthermore, by the classification based on O antigen, *Shigella* consisted of 4 different subgroups, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Among some developed and (or) industrialized countries that were reported before, *S. sonnei* has not been limited to the main emergence yet, but it gradually increases the proportion in some regions with notable shift of socio-economic types (5,7,8). Even so, it was reported that the diverse *S. flexneri*, with 15 serotypes or subtypes, remains the most prevalent pathogenic factor leading to the gastrointestinal disease in many developing countries (9).

A lot of antibacterial agents, especially the ciprofloxacin, have been advised as the first-line antibiotics against the serious *Shigella* infections. These antibacterial agents have been widely applied into the clinical treatment for shigellosis in the past years (10,11). However, with the sharp development of fluoroquinolone resistance or the more severe multiple-drug resistance in Asia and European regions (4,12,13), it's actually a tremendous challenge to pick out the most appropriate antibiotic. In the previous studies, many target mutations of quinolone resistance-determining region (QRDR) have been reported. The mutations in *gyrA* and *parC* may be responsible for the fluoroquinolone-resistant *S. flexneri* as the primary mechanism (14,15). Besides the high prevalence of amino acid alterations in *gyrA*, *gyrB* and *parC*, no one can show that there are other mutations in *parE* gene within *S. flexneri* isolates. What's more, plasmid-mediated quinolone resistance (*PMQR*) genes were associated with mobile elements. It also plays an important role on the poor resistance capacity against fluoroquinolones, leading to the increase of the minimum inhibitory concentration (MIC) and the loss of original drug activity (15-17). Besides, as Liu and colleagues have reported in 2012, multiple-resistant clinical isolates of *S. flexneri* were correlated to the influence of some resistance determinants, such as integrons and β-lactamases (18).

We have successively carried out detailed characterization and studies on *S. flexneri* isolates in Jiangsu Province of China from 2001 to 2015, especially in the aspect of antimicrobial resistance to quinolones and relevant molecular mechanism. The results of these studies have displayed the essentiality of continuous monitoring in this area (19,20). Based on this, we collected the *S. flexneri* isolates in Jiangsu Province of China in 2016, trying to observe the changes of resistance to fluoroquinolones , the prevalence of PMQR determinants, and ulteriorly explore whether new point mutation associated with resistance mechanism exists in the *QRDR* genes or not.

Methods

Bacterial collection

With the great support of Jiangsu Provincial Center for Disease Control and Prevention, 81 strains of *S. flexneri* in Jiangsu Province of China during 2016 were collected. All these samples were from clinical patients of 12 cities, including Southern Jiangsu (n=27), Central Jiangsu (n=18) and Northern Jiangsu (n=36) in this area. By the way, Yangzhou was not included.

Strains identification and serotyping

After the recovery of strains, automatic VITEK2 COMPACT analysis system was applied into the bacterial identification under the guidance of the manufacturer (BioMerieux, Marcy l' Etoile, France). And serotyping of 81 isolates was confirmed by slide agglutination in combination with specific antisera of *Shigella* species (Tianrun Bio-Pharmaceutical Co., Ltd., China).

Susceptibility test in quinolones

Based on the disc diffusion method (Kirby-Bauer), M-H and N-A agar (Oxoid, Hampshire, UK) were employed to test the isolates' susceptibility to nalidixic acid and ciprofloxacin. *Escherichia coli* ATCC 25922 and ATCC 35218 were cultured for quality control, and the result of susceptibility test was judged by Clinical and Laboratory Standards Institute (CLSI) standards (21).

Table 1 Serotype distribution and separation rate in different cities [No.]

Cities	1a	1b	2a	2b	X	%
Wuxi	0.0 [0]	0.0 [0]	66.7 [2]	33.3 [1]	0.0 [0]	3.7 [3]
Changzhou	0.0 [0]	66.7 [4]	33.3 [2]	0.0 [0]	0.0 [0]	7.4 [6]
Nanjing	0.0 [0]	0.0 [0]	0.0 [0]	100.0 [2]	0.0 [0]	2.5 [2]
Zhenjiang	0.0 [0]	66.7 [4]	16.7 [1]	16.7 [1]	0.0 [0]	7.4 [6]
Suzhou	20.0 [2]	0.0 [0]	0.0 [0]	80.0 [8]	0.0 [0]	12.3 [10]
Nantong	11.1 [1]	0.0 [0]	33.3 [3]	55.6 [5]	0.0 [0]	11.1 [9]
Taizhou	66.7 [6]	0.0 [0]	0.0 [0]	0.0 [0]	33.3 [3]	11.1 [9]
Lianyungang	0.0 [0]	0.0 [0]	71.4 [5]	28.6 [2]	0.0 [0]	8.6 [7]
Suqian	0.0 [0]	0.0 [0]	0.0 [0]	100.0 [2]	0.0 [0]	2.5 [2]
Xuzhou	16.7 [2]	0.0 [0]	75.0 [9]	8.3 [1]	0.0 [0]	14.8 [12]
Huaian	12.5 [1]	0.0 [0]	87.5 [7]	0.0 [0]	0.0 [0]	9.8 [8]
Yancheng	42.9 [3]	0.0 [0]	57.1 [4]	0.0 [0]	0.0 [0]	8.6 [7]
Total	18.5 [15]	9.9 [8]	40.7 [33]	27.2 [22]	3.7 [3]	100 [81]

Genes amplification and products sequencing

Study on QRDR

PCR was applied to amplify the genes of QRDR, including *gyrA*, *gyrB*, *parC* and *parE*, using primers synthesised in Sangon Biotech (Shanghai, China). It also referred to the previous studies (22-24). PCR products were verified by agarose gel electrophoresis, and then were sent to Genewiz Company (Suzhou, China) for nucleotide sequencing after being purified. The results were analyzed by the Basic Local Alignment Search Tool (BLAST) comparison with sequences in the GenBank database.

Study on PMQR

Screening of PMQR determinants (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6')-Ib-cr* and *qepA*) was performed by PCR amplification, based on published primers (17,25-28). Agarose gel electrophoresis was used to observe the genes above. And then, the purified products with positive genes were sequenced in Genewiz Company (Suzhou, China) and compared with the sequences in the GenBank to ensure the existence of PMQR determinants.

Statistical analysis

The software SPSS 19.0 was used for data management and statistical analysis. Comparisons of serotypes distribution, mutations and resistance were judged by P value. Only when P≤0.05, the research was considered to be valuable.

Results

Shigella isolates and serotypes

Coming from 12 cities in Jiangsu Province, China during 2016, a total of 81 *S. flexneri* isolates were sponsored by Jiangsu Provincial Center for Disease Control and Prevention. All these strains were serotyped and 5 different serotypes were identified, among them 2a and 2b being the most common serotypes, accounting for 40.7% (33/81) and 27.2% (22/81) (P<0.05). Nevertheless, 1a, 1b and X only occupied a smaller proportion of 18.5% (15/81), 9.9% (8/81) and 3.7% (3/81). What's more, the serotypes distribution of *Shigella* in 3 regions had significant differences (P<0.001). When it comes to the type of serotypes, it's obvious that Northern Jiangsu obtained a higher proportion of 2a isolates with the percentage of 69.4%, while 2b isolates had a percentage of 44.4% in Southern Jiangsu. Central Jiangsu was different from both of the mentioned regions, for 38.9% proportion was occupied by 1a isolates there. Meanwhile, 3 X *S. flexneri* strains were all isolated from Taizhou in Central Jiangsu without exception (*Table 1*, *Figure 1*).

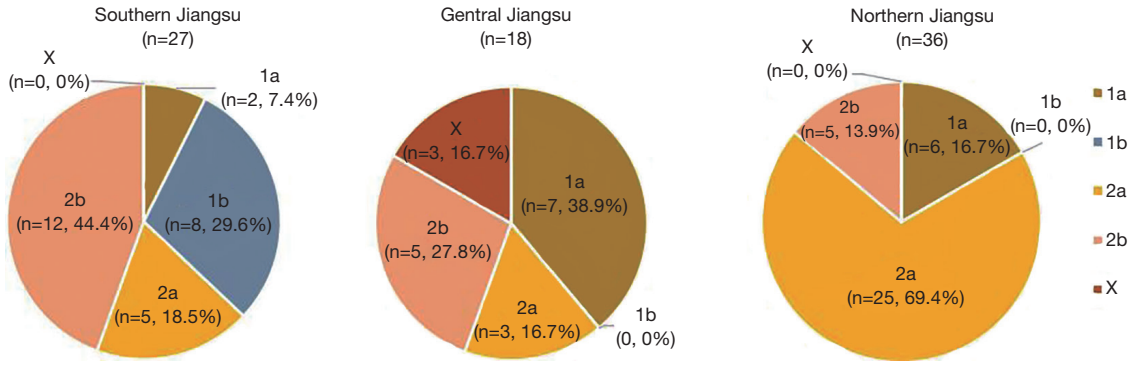

Figure 1 Comparison of serotypes distribution of *S. flexneri* in the 3 regions of Jiangsu Province.

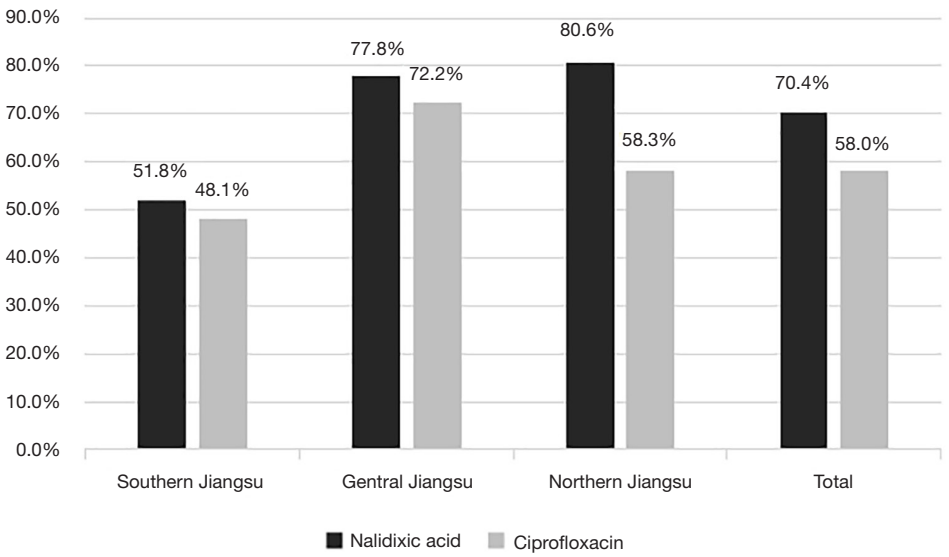

Figure 2 Comparison of the resistance to quinolones of *S. flexneri* in the 3 areas of Jiangsu.

Quinolone susceptibility test

Resistance and regions

Among these isolates, 70.4% were resistant to nalidixic acid and 58.0% (47/81) expressed resistance to ciprofloxacin. Overall, *S. flexneri* isolates in the 3 regions of Jiangsu Province conferred obviously different resistant level to nalidixic acid (P<0.05). In particular, the isolates in the region of Northern Jiangsu possessed the highest resistance rate of 80.6%, while the Southern Jiangsu (51.8%). Nevertheless, the ciprofloxacin-resistant strains mainly focused on the area of Central Jiangsu (72.2%), followed by the isolates in Southern Jiangsu (48.1%) and Northern Jiangsu (58.3%) (*Figure 2*).

Resistance and serotypes

Additionally, in some degree, the resistant level of *S. flexneri* isolates to quinolones also presented a slight difference between the diverse serotypes. Among the 5 serotypes detected, 1a isolates simultaneously possessed the highest resistance to nalidixic acid and ciprofloxacin, with the percentage of 80.0% and 66.7%, respectively. These two proportions were both a little higher than 2a (75.8% and 60.6%) and 2b (72.7% and 63.6%). The more interesting fact is that 1b *S. flexneri* expressed the same resistance rate to both 2 antibacterial drugs, accounting for 25.0%, but obviously lower than the other 4 serotypes (P<0.05) (*Figure 3*).

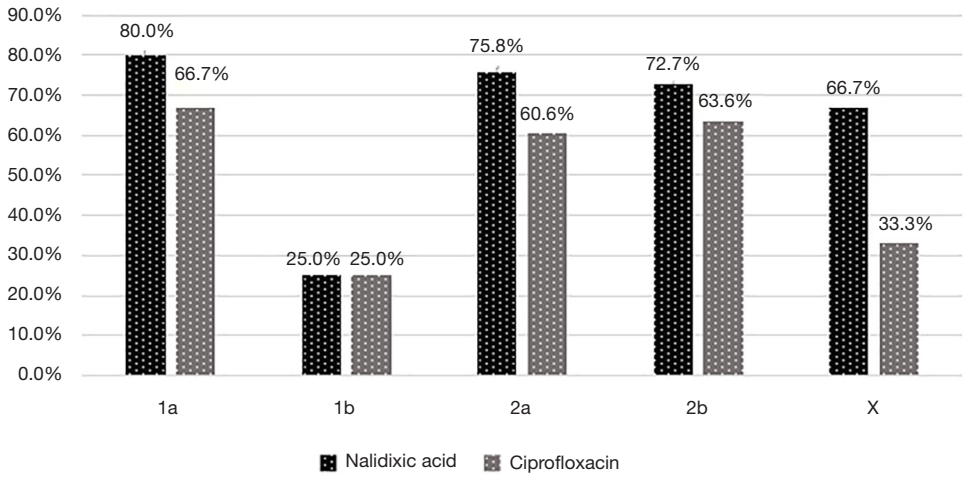

Figure 3 The drug resistance to 2 antibacterials of 5 serotypes S. *flexneri* isolates.

Target mutations in QRDR genes

Mutations in genes of topoisomerase IV

With the methods of sequences comparison, 2 novel mutations in *parE* were observed from 3 isolates, and 2 of them were detected with mutation at codon 458 (TCG→TTG), contributing to Serine replaced by Leucine. Meanwhile, another mutation presented with the substitution of Gly408Asp (GGC→GAC) within a single isolate. In our opinion, all these 2 mutations in parE had never been reported before, for the gene of *parC*, 69.1% isolates showed the common alteration of Ser80Ile. So it was the same with did the mutation His211Tyr in *gyrA*, higher than the other mutation at position of 129 in *parC* (Ser→Pro) with the percentage of 58.0% (*Table 2*).

Mutations in genes of DNA gyrase

Besides, a total of 3 point mutations were detected in *gyrA*, of which, the amino acid substitution at the position of Ser83 and His211 were the most 2 prevalent, accounting for 70.4% and 69.1%, respectively, followed by the mutation of Asp87Gly/Asn (56.8%). Besides, 35.8% of isolates showed the alteration of Gln517Arg in *gyrB*, which was much lower than the mutation rate in *gyrA* and *parC* (*Table 2*).

Mutations and resistance

Among 57 nalidixic acid-resistant isolates, mutation Ser83Leu and His211Tyr in *gyrA* played a very important part, accounting for 93.0% and 91.2%, followed by Ser80Ile within *parC*, which was obviously higher than other mutations (P<0.05). Additionally, with 3 point mutations, including Ser83Leu (*gyrA*), Ser80Ile (*parC*) and Asp87Gly/Asn (*gyrA*), isolates remained a high prevalence of 76.6% resistance rate to ciprofloxacin. Noticeably, almost half of ciprofloxacin-susceptible and nalidixic acid-susceptible *Shigella* isolates generated amino acid mutation at the position of Ser129 in *parC*, followed by the mutation in *gyrB* (Gln517Arg). For *parE*, carrying mutation Ser458Leu, 2 isolates didn't have susceptibility to ciprofloxacin and nalidixic acid any more, while the single isolate with mutation Gly408Asp was resistant to nalidixic acid but susceptible to ciprofloxacin. And we also observed that one strain of *Shigella* did not have any mutation, but resistant to both 2 drug detected (*Table 3*).

Mutations and serotypes

Besides, 86.7% 1a *S. flexneri* isolates generated the mutation of Ser83Leu, followed by Ser80Ile in *parC* (80.0%), remarkably higher than other mutations (P<0.05). While serotype 2a isolates presented a more prevalence of *gyrA* mutation, floating between 72.7% and 78.8%. It was different that serotype 2b isolates mainly carried with the mutation at codon 129 in *parC*, accounting for 77.3%. Moreover, 2 X serotype strains with mutations of Ser83Leu and His211Tyr in *gyrA* also generated the substitution in *parC* at the position of Ser80 synchronously. Contrary to other 4 serotypes, 1b tended to be the lowest mutation rate

Table 2 Mutation of *S. flexneri* isolates detected in 12 cities

Cities [N]	Amino acid changes caused by base mutation							
	gyrA			*gyrB*	*parC*		*parE*	
	TCG→TTG (83Ser→Leu)	GAC (87Asp)†	CAC→TAC (211His→Tyr)	CAG→CGA (517Gln→Arg)	AGC → ATC (80Ser → Ile)	TCC→CCC (129Ser→Pro)	GGC→GAC‡ (408Gly→Asp)	TCG→TTG‡ (458Ser→Leu)
Wuxi [3]	1	1	1	1	1	3	0	0
Changzhou [6]	2	2	2	4	5	4	0	0
Nanjing [2]	0	0	0	0	0	2	0	0
Zhenjiang [6]	2	2	2	4	2	0	0	0
Suzhou [10]	8	7	8	3	7	7	0	2
Nantong [9]	6	6	6	3	4	7	0	0
Taizhou [9]	9	7	9	0	9	5	0	0
Lianyungang [7]	5	5	5	4	4	6	0	0
Suqian [2]	2	1	2	0	2	2	0	0
Xuzhou [12]	12	9	11	8	11	4	1	0
Huaian [8]	6	4	6	1	6	3	0	0
Yancheng [7]	5	2	5	0	5	4	0	0
Total [81]	57	46	56	29	56	47	1	2

†, GAC → GGC/AAC; the corresponding amino acid changes: 87Asp→Gly/Asn; ‡, novel mutations in this study.

Table 3 Mutation in different quinolones sensitive phenotypic of *S. flexneri*

Mutation	NAL^S & CIP^S (n=20)	NAL^R (n=57)	NAL^R & CIP^S (n=10)	CIP^R (n=47)
None	20.0 [4]	1.8 [1]	0.0 [0]	2.1 [1]
Ser83Leu	0.0 [0]	93.0 [53]	90.0 [9]	93.6 [44]
Asp87Gly/Asn	0.0 [0]	75.4 [43]	40.0 [4]	85.1 [40]
His211Tyr	0.0 [0]	91.2 [52]	80.0 [8]	93.6 [44]
Gln517Arg	45.0 [9]	33.3 [19]	10.0 [1]	38.3 [18]
Ser80Ile	15.0 [3]	87.7 [50]	90.0 [9]	87.2 [41]
Ser129Pro	50.0 [10]	59.6 [34]	40.0 [4]	63.8 [30]
Ser458Leu	0.0 [0]	3.5 [2]	0.0 [0]	4.3 [2]
Gly408Asp	0.0 [0]	1.8 [1]	10.0 [1]	0.0 [0]
Ser83Leu & Ser80Ile	0.0 [0]	84.2 [48]	80.0 [8]	85.1 [40]
Ser83Leu & Ser80Ile & Asp87	0.0 [0]	68.4 [39]	40.0 [4]	76.6 [36]

NAL^S, nalidixic acid-susceptible; NAL^R, nalidixic acid-resistant; CIP^S, ciprofloxacin-susceptible; CIP^R, ciprofloxacin-resistant.

Table 4 Mutation in different serotypes of *S. flexneri* isolates

Serotype [N]	Amino acid changes caused by base mutation							
	gyrA			gyrB	parC		parE	
	TCG→TTG (83Ser→Leu)	GAC (87Asp)†	CAC→TAC (211His→Tyr)	CAG→CGA (517Gln→Arg)	AGC→ATC (80Ser→Ile)	TCC→CCC (129Ser→Pro)	GGC→GAC‡ (408Gly→Asp)	TCG→TTG‡ (458Ser→Leu)
1a [15]	86.7 [13]	40.0 [6]	53.3 [8]	20.0 [3]	80.0 [12]	53.3 [8]	0.0 [0]	0.0 [0]
1b [8]	12.5 [1]	12.5 [1]	12.5 [1]	62.5 [5]	50.0 [4]	25.0 [2]	0.0 [0]	0.0 [0]
2a [33]	78.8 [26]	72.7 [24]	75.8 [25]	36.4 [12]	69.7 [23]	31.9 [19]	3.0 [1]	0.0 [0]
2b [22]	68.2 [15]	63.6 [14]	68.2 [15]	40.9 [2]	59.1 [13]	77.3 [17]	0.0 [0]	9.0 [2]
X [3]	66.7 [2]	33.3 [1]	66.7 [2]	0.0 [0]	100.0 [3]	33.3 [1]	0.0 [0]	0.0 [0]
Total [81]	70.4 [57]	56.8 [46]	63.0 [51]	27.2 [22]	67.9 [55]	58.0 [47]	1.2 [1]	2.5 [2]

†, GAC→GGC/AAC; the corresponding amino acid changes: 87Asp→Gly/Asn; ‡, novel mutations in this study.

Table 5 Mutations in *QRDR* genes of *S. flexneri* isolates and *PMQR* positive genes

Strains' number	NAL	CIP	*PMQR* genes		*gyrA*			*gyrB*	*parC*		*parE*
17	R	R	*qnrS*	–	Ser83	–	His211	–	Ser80	–	–
21	R	R	*qnrS*	*aac(6')-Ib-cr*	Ser83	–	His211	Gln517	Ser80	–	–
68	R	S	*qnrS*	–	Ser83	Asp87	His211	Gln517		Ser129	–
103	R	R	*qnrS*	–	Ser83	Asp87	His211	–	Ser80	Ser129	–
114	R	R	*qnrS*	–	Ser83	Asp87	His211	–	Ser80	Ser129	–
120	R	R	*qnrS*	–	Ser83	Asp87	His211	–	Ser80	Ser129	–

NAL, nalidixic acid; CIP, ciprofloxacin.

in all the mutations within the gene of *gyrA* (P<0.05) (*Table 4*).

Analysis of PMQR genes

Among 81 *S. flexneri* isolates, 6 strains were detected with PMQR positive genes, including 1 *aac(6')-Ib-cr* and 6 *qnrS* positive isolates, without the participation of *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qepA*. Every positive isolate involved at least 3 mutations, especially all the 6 *qnrS* positive isolates mutated at Ser83Leu in *gyrA* in combination with the alteration of Ser80Ile in *gyrC* except the number 68. Even 4 of them generated the mutation simultaneously at codon 87 in *gyrA*. Furthermore, mutations Gln517Arg in *gyrB* and Ser129Pro in *parC* were also detected among 2 and 4 positive isolates. Besides, the single *aac(6')-Ib-cr* positive strain was observed with the emergency of *qnrS* gene. Unfortunately, all these PMQR positive isolates showed resistance to nalidixic acid and even 5 of them didn't keep susceptible to ciprofloxacin any more (*Table 5*).

Discussion

Looking back to our research, 5 novel mutations have been detected in *S. flexneri* isolated from Jiangsu Province of China during 2001–2011, including 2 mutations in *gyrA* (Asn57Lys and His80Pro) and 3 mutations in *parC* (Ala85Thr, Asp111His and Ser129Pro) (20). In this thesis, 2 novel mutations in *parE* of the topoisomerase IV at Ser458Leu and Gly408Asp were identified among 3 isolates. From our perspective, all the 2 mutations had never been reported before.

Meanwhile, susceptibility test result demonstrated that 2 isolates with the mutation

Ser458Leu all expressed resistance to nalidixic acid and ciprofloxacin, while the single isolate harboring the alteration of Gly408Asp was resistant to nalidixic acid but susceptible to ciprofloxacin. The change of amino acid

could indirectly affect the combination of enzymes and fluoroquinolones, and then resulted in the emergency of resistance (29). All the 2 novel mutations had amino acid changes, especially the serine at codon 458 in *parE* was replaced by leucine, so it's possible that the mutation Ser458Leu could also lead to the same level of fluoroquinolones resistance as Ser83Leu (*gyrA*) did. What's more, a previous study proved that the mutation Ser458Ala in *parE* found from *Escherichia coli* was in correlation with the increase in MIC for ciprofloxacin (30). Therefore, both the 2 novel mutations in *parE* of *S. flexneri* have the potential to increase the MIC for ciprofloxacin and mediate fluoroquinolone resistance. However, it still needs to explore in a further way.

Indeed, this is an interesting result that strains of *S. flexneri* in the 3 regions of Jiangsu Province had evident differences in serotype distribution. Serotype 2b was mainly reflected in Southern Jiangsu, which was discrepant to Central Jiangsu (serotype 1a) and Northern Jiangsu (serotype 2a). Unbalanced economic status or other social factors may be an explanation for this phenomenon. Obviously, 1a isolates seemed to be the most threatening type (with the highest resistance to both nalidixic acid and ciprofloxacin), revealing that Central Jiangsu should be highly aware of the serious situation of anti-infection, and solve the problem in a more rational way of controlling antibiotics. Meanwhile, they are supposed to bear in mind that human immune specificity, unknown cross protection, and diverse serotypes with distribution difference mean stricter requirements for the development of vaccines in order to prevent the dissemination of pathogenic bacteria (31-33).

In recent years, several regions in the Eastern provinces of China, such as Henan (34), Zhejiang (35) and Anhui (36), have reported a certain level of ciprofloxacin resistance of *S. flexneri*, ranging from 21.0% to 25.4%, which was much lower than our data (58.0%). Hence, the frequent occurrence of ciprofloxacin resistance requires a further specific test on whether fluoroquinolones can be applied into the treatment or not. Under this pressure, more efforts are supposed to be made to the study on fluoroquinolone resistance mechanism.

Up to now, chromosomal mutation in target genes of QRDR remains the most significant mechanism for fluoroquinolone resistance, particularly the amino acid substitution in *gyrA* and *parC* (15,37), and a recent study based on structural level has reconfirmed this (38). In this research, unsurprisingly, Ser83Leu within *gyrA* was still described as the most dominant point mutation, consistent with previous study (19), with *gyrA* (His211) and *parC* (Ser80) being the predominant. Besides, among 57 nalidixic acid-resistant isolates, mutation Ser83Leu and His211Tyr in *gyrA* also played an important part. It could not be ignored that since the mutation His211Tyr (*gyrA*) was first detected in Bangladesh during 2009 (39), the quinolone-resistant *S. flexneri* isolates seemed to get more attention (19,40,41), and this made us keep thinking that whether this mutation could confer resistance to antibacterial agents. Then, our data also showed that multiple mutations will highly increase the risk of acquiring fluoroquinolone resistance (37), for 76.6% *Shigella* isolates expressed resistance to ciprofloxacin with 3 critical mutations at the same time, which were located at the position of Ser83 and Asp87 within *gyrA* in company with Ser80 in *parC*.

However, no evidence could prove that the mutations have to appear in the antimicrobial-resistant strains, so neither the susceptible ones. In this study, almost half of strains showing susceptibility to both nalidixic acid and ciprofloxacin were observed with mutation in *parC* (Ser129Pro) or *gyrB* (Gln517Arg), and similar research findings also appeared in Zhejiang, China (35). The chance was that we need to combine the additional mutations in these target genes or determinants associated with plasmid together thus mediating the high level of resistance far from the decreased susceptibility (15,42). Nevertheless, 1 single *Shigella* isolate was corroborated to be resistant to both 2 drugs tested, but none of mutations occurred, which may be attributed to decreased drug accumulation or other mechanisms (15).

Besides, obvious difference between mutations and serotypes was also observed among 81 *S. flexneri* isolates. For instance, Ser83Leu in *gyrA* and Ser80Ile in *parC* among 1a isolates were more prevalent than other mutations, which may be a reason for the high resistance rate of this serotype to antimicrobial agents mentioned above. However, contrary to other 4 serotypes, the emergency of mutations in *gyrA* among 1b isolates only takes up a small proportion, indicating that the selection of mutation was associated with serotypes. What's more, it also certified that serological research was also an effective means to better analyze it.

Since the end of the twentieth Century, *qnr* families, *aac(6')-Ib-cr* and *qepA*, involved with protection, inactivity and efflux, gradually went into people's sight and were described as PMQR determinants by participating in low level of fluoroquinolone resistance (16,17,43,44). Here, *qnrS* and *aac(6')-Ib-cr* were observed within six clinical isolates. It

was different from our previous result (19) that *qnrS* became the most prevalent positive gene. In addition, all *qnrS* positive strains that conferred resistance to ciprofloxacin were involved in at least 3 mutations in *gyrA* and *parC*, especially at the position of Ser83 and Ser80. It suggested that *qnr* family played an important part in protecting the target sites of fluoroquinolone, and in accelerating the emergency of chromosomal mutation. Subsequently, all of these made preparations for the high level of resistance (17). However, 5 of 6 PMQR positive strains lost original susceptibility to ciprofloxacin, which tended to be more serious than other regions in China, such as Anhui (36). Besides, the single *aac(6')-Ib-cr* positive isolate was observed in co-existence of *qnrS* gene, which was in agreement with what Luo *et al.* has reported before (45). However, it has to be noticed that horizontal transmission of resistance gene could increase the risk of acquiring resistance. Thus, it's reasonable enough to constantly monitor the dynamic changes of these PMQR genes, and to seasonably develop evasive strategies according to the latest normalized data.

Unavoidably, there were some shortcomings in our study. To begin with, the current samples could only reflect the basic profile of *S. flexneri* in the year 2016, but could not make systematic review of the trend analysis. In addition, it is still unclear whether the new mutations in *parE* correlate with resistance, and which will be our next plan. Last but not least, there may be some differences between drug susceptibility test and the result *in vivo* test, because some regions show us the high drug resistance. Therefore, it is necessary to verify the true resistance combined with the clinical efficacy of patients.

Conclusions

In this study, we have made a specific analysis on the *S. flexneri* isolates from Jiangsu Province of China in 2016, concentrating on the resistance to quinolone and quinolone resistance mechanism involved. In our thesis, a breakthrough is that 2 novel mutations were identified in *parE* gene of QRDR. Apart from this, the resistance of *S. flexneri* isolates to fluoroquinolone was documented to be extremely high. Besides, given that the emergency of PMQR determinants, it's high time to strengthen the communication between laboratory and clinical. We should also make out effective strategies accordingly in order to control the spread of pathogenic bacteria and reduce the burden of shigellosis.

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Footnote

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