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SHORT REPORT
First report of the *qnrA* determinant in *Shigella sonnei* isolated from China

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SUMMARY

We investigated the first presence of *qnrA* among *Shigella sonnei* clinical isolates in Jiangsu Province, China. The *qnrA*-positive isolates coexisted with the mutation in *gyrA* at codon 83, these isolates were resistant to nalidixic acid and 22·2% (2 of 9) of them were resistant to norfloxacin.

Key words: *Shigella sonnei*, *qnrA*, China, fluoroquinolones.

Shigella spp. are a group of enteropathogenic bacteria consisting of four major species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. Despite the improvement in personal hygiene and heightened awareness of people about the importance of preventing infections, the global burden of shigellosis remains considerable. Based on data from the Chinese Center for Disease Control and Prevention, shigellosis is the third most commonly reported infectious disease in China, calling for urgent attention. Over years, a significant shift has occurred in the species of *Shigella* contributing to clinical disease, especially in developing countries, i.e. *S. sonnei* has become the prevalent species [1, 2]. For instance, Chang *et al*. reported a significantly higher rate of *S. sonnei* in eastern, northern, and northeast regions of China, probably due to unbalanced economic growth [3].

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Fluoroquinolones (FQs) are the most commonly used drugs for the treatment of shigellosis. However, the inexorable development of resistance by *Shigella* to the drugs has constrained the effective and adequate treatment of acute dysentery. *Shigella* resistance to FQ agents is produced by several mechanisms. Mutational alterations in DNA gyrase and/or topoisomerase IV genes, associated with high-level FQ resistance in clinical isolates, play a main role in FQ resistance. FQ resistance can also be acquired through quinolone resistance genes associated with plasmids including Qnr families, a variant of aminoglycoside acetyltransferase *aac(6')-Ib-cr* and efflux pump *qepA*. Qnr proteins interfere with quinolone binding to DNA gyrase and topoisomerase IV. Plasmid-mediated *qnrA* was initially identified in clinical isolates from *Klebsiella pneumoniae* from the USA, in 1988 [4], and since then, other plasmid-mediated quinolone resistance (PMQR) genes have been reported.

Recently, there have been numerous surveys on FQ resistance, mutations in quinolone resistance-determining regions (QRDRs) and the presence of PMQR in *Shigella* strains around the world. Studies describing the prevalence of PMQR determinants

and mutations in QRDRs among *S. sonnei* in Jiangsu Province of China are limited. The objective of the present study was to examine the extent of FQ resistance and investigate the prevalence of PMQR and mutations in QRDRs of *gyrA*, *gyrB*, *parC,* and *parE* among a collection of *S. sonnei* clinical isolates between 2012 and 2015.

Sponsored by Jiangsu Province Center for Disease Control and Prevention (Nanjing, JS, China), 108 unduplicated clinical *S. sonnei* isolates were obtained from hospitalized patients in Jiangsu Province between January 2012 and December 2015, comprising 19, 25, 30, and 34 *S. sonnei* isolates during the period between 2012 and 2015 respectively, which indicated a slight annual increase in the number of isolates detected. All strains were confirmed by API system (bioMerieux, France) and slide agglutination test using *Shigella* antisera (Ningbo Tianrong Bio-pharmaceutical Co., Ltd., China). According to the guidelines of the CLSI (Clinical and Laboratory Standards Institute) [5], susceptibility testing to nalidixic acid and norfloxacin of all the isolates was performed by the disc diffusion (Kirby–Bauer) method. Of the 108 isolates, 92 (85·2%) were categorized as nalidixic acid resistant (NAL^R) and 8 (7·4%) displayed norfloxacin resistance (NOR^R). The proportion of NAL^R strains was higher than that during 2008 and 2010 in the same area while the number of NOR^R *S. sonnei* isolates was decreased in this study (χ^2 test; $P < 0{\cdot}05$) [6]. In addition, the number of NOR^R isolates collected during 2012 and 2013 was significantly greater than that collected during the subsequent 2 years (χ^2 test; $P < 0{\cdot}05$). Periodic monitoring and reporting of FQ resistance circulating in the area are of immense importance.

The gyrase and topoisomerase IV genes (*gyrA*, *gyrB*, *parC,* and *parE*) of all strains were amplified through polymerase chain reaction (PCR) using previously described primers [7–9]. The amplification products were then purified and sequenced, and sequence alignment was done using BLAST program. Of the 108 *S. sonnei* isolates, 94 contained mutations in the *gyrA* gene, and only three contained mutations in the *parC* gene, while no mutations in the *gyrB* or *parE* genes was detected. Of *S. sonnei* containing mutations, codon Ser83 was the most frequently affected (91/108), and these strains were resistant to nalidixic acid, indicating that the mutation at position 83 of *gyrA* was crucial for resistance to nalidixic acid, which is in agreement with a previous study reporting that 94·9% of the quinolone-resistant *Shigella* isolates had at least mutations at position 83 of *gyrA* [10].

Table 1. *FQ resistance, amino acid substitutions in gyrA and parC genes in terms of amino acid positions and PMQR determinants in Shigella sonnei isolates*

Number of isolates tested	NAL	NOR	Substitutions in QRDRs		PMQR genes
			gyrA	*parC*	
14	S	S	–	–	–
72	R	S	S83L	–	–
7	R	S	S83L	–	*qnrA*
1	R	I	S83L	–	–
6	R	R	S83L	–	–
2	R	R	S83L	–	*qnrA*
2	S	S	D87Y	–	–
1	R	S	D87Y	–	–
2	R	S	S83L, D87N	S80I	–
1	R	I	S83L, D87G	S80I	–

NAL, nalidixic acid; NOR, norfloxacin; R, resistant; S, susceptible; I, intermediate resistant; –, negative.

Additionally, six isolates were observed with Asp87Asn/Gly/Tyr substitution, and replacement of Asp (GAC) to Tyr (TAC) was observed in three isolates without the occurrence of Ser83 mutation (Table 1). It is notable that two of the three Asp87Tyr strains showed susceptible to both nalidixic acid and norfloxacin. An earlier report pointed that mutation in codon 87 of *gyrA* was associated with nalidixic acid resistance [11]. We previously reported that mutations in QRDRs also occurred to FQ susceptible *Shigella flexneri* isolates, separately or together [12]. Curiously, three *S. sonnei* strains in this study that exhibited NAL^R and possessed double mutations in *gyrA* (Ser83 and Asp87) and a single mutation in *parC* (Ser80), were not resistant to norfloxacin. This was not consistent with the previous report that multiple QRDR mutations are largely responsible for FQ resistance among *Shigella* isolates [13]. However, some studies have already reported similar phenomenon. In southeast China, high mutation rate of mutations both in *gyrA*83 and *parC*80 was detected among ciprofloxacin-susceptible *S. flexneri* (138/142) [14]. Moreover, in our previous report, 28 of 410 norfloxacin-susceptible *S. flexneri* isolates had mutations of Ser83, Asp87 in *gyrA* and Ser80 in *parC* [12].

Then, the *S. sonnei* isolates were also screened for PMQR genes including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr,* and *qepA* by PCR [15–19]. The *qnrA* gene was detected in nine of the collected isolates, achieving a prevalence rate of 8·3% (Table 1), and no *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, and

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qepA genes were detected. All *qnrA*-positive isolates were resistant to nalidixic acid and only two of them were resistant to norfloxacin. It is worth noting that the *qnrA*-positive strains were observed with *gyrA* substitution at position Ser83 simultaneously. In China, *qnrA1* was first reported in *S. flexneri* isolates and even coexisted with other PMQR determinants in 2010 [20]. In China, the *qnrS* and *aac(6′)-Ib-cr* genes in *S. flexneri* were discovered in Zhejiang Province in 2009 [18]. Later in 2013, *aac(6′)-Ib-cr* appeared in *S. sonnei* and *qepA* gene was found [10]. Notably, all these reported PMQR-positive isolates were resistant to FQs except for the *qnrA*-positive isolates. Similarly, only two of the nine PMQR-positive isolates showed resistance to norfloxacin in this study. To the best of our knowledge, this is the first study reporting the existence of *qnrA* in *S. sonnei* isolates in China. Although PMQR genes provide a low level of quinolone resistance, they can promote mutations within the QRDR. In addition, considering the mobile characteristic of PMQR genes, it is essential to continue this type of surveillance and future research should focus on continual monitoring of the spread of PMQR determinants.

In conclusion, these new data demonstrate that there is a significant abundance of mutations in QRDR genes, which play a primary role in FQ resistance. In addition, we have reported the first incidence of *qnrA* gene in *S. sonnei* isolated from Jiangsu Province. Our findings emphasize on the need of continuing active surveillance of mechanisms of FQ resistance in *S. sonnei*, knowing that it is essential to safe and effective use of antimicrobial drugs for effective control of shigellosis.

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