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Novel mutations in *gyrA* and *parC* among *Shigella sonnei* strains from Jiangsu Province of China, 2002-2011

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ABSTRACT

Background: To investigate fluoroquinolone resistance and associated mechanisms of *Shigella sonnei* isolates in Jiangsu Province of China between 2002 and 2011.
Methods: All 337 unduplicated *S. sonnei* isolates were collected from hospitals in Jiangsu Province from January 2002 to December 2011. Fluoroquinolone susceptibility was characterized by Kirby-Bauer disk diffusion method, and direct nucleotide sequencing of genes of the quinolone resistance determining regions were conducted. Also, the transferable quinolone resistance determinants, including *qnrA*,*qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac-(6′)-Ib-cr* and *qepA* were amplified by PCR.
Results: Among 950 *Shigella* isolates, 337 (35.5%) were identified as *S. sonnei*, of which 76.6% displayed nalidixic acid resistance and norfloxacin-resistant isolates appeared in 2005-2009, with an average resistance rate of 21.8%. Commonly reported point mutations of Ser83Leu and Asp87Asn/Gly in *gyrA* and Ser80Ile in *parC* were detected, with mutation rates of 78.0%, 9.5% and 30.3%, respectively, while no alteration in *gyrB* or *parE* were detected. Besides, His211Tyr mutation in *gyrA* was first reported in a *S. sonnei* strain in 2009 and two novel mutations in *parC* were found, of which Met86Trp occurred in another strain in 2009 and Ser129Pro appeared every year except 2011 (28.8%). Plasmid-mediated quinolone resistance determinants were found in 23 isolates and 19 of these isolates were resistant to both nalidixic acid and norfloxacin. *qnrB*, *qnrS*, *aac-(6′)-Ib-cr* and *qepA* were detected in 1, 7, 14 and 2 *S. sonnei* strains, relatively, and the most abundant PMQR gene found in this work was *aac-(6′)-Ib-cr* (4.2%).
Interpretation & conclusions: S. sonnei became increasingly important as fluoroquinolone-resistant isolates emerged, and further detection on the resistant genes would be useful in the treatment and control of this infection.

Introduction

Shigellosis (bacillary dysentery) caused by *Shigella* spp. is an enteric infectious disease and is one of the leading causes of morbidity and mortality in developing nations, particularly for young children (Wang et al., 2006; Niyogi, 2005 Niyogi, 2005), and is the sixth most common cause of death from infectious disease in China. Moreover, travellers from developed to developing regions and soldiers serving under field conditions are also at an increased risk to develop shigellosis (Niyogi, 2005). *Shigella* infection can result in a variety of symptoms including diarrhoea and/or dysentery with frequent mucoid bloody stools, abdominal cramps and tenesmus (Niyogi, 2005).

Shigella is classified on the basis of biochemical and serological differences: *S. dysenteriae* (consisting of 13 serotypes); *S. flexneri* (consisting of 15 serotypes [including subtypes]); *S. boydii* (consisting of 18 serotypes) and *S. sonnei* (consisting of a single serotype). A classical quantification indicated that *S. sonnei* was the predominant serogroup (77%) in industrialized countries, followed by *S. flexneri* (16%), while *S. flexneri* was the main serogroup found in developing countries (60%). However, recently the frequency of *S. sonnei* has increased in some areas that have undergone rapid improvements in their socioeconomic status (Zhang et al., 2014a; Qiu et al., 2015).

For the treatment of *Shigella* infection, antimicrobial therapy evolved from initial sulfonamides and tetracycline, followed by ampicillin and co-trimoxazole, to nalidixic acid, which was

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effective originally and is no longer recommended in the international guidelines over the years due to the emergence of nalidixic acid resistant strains, later it is documented that newer fluoroquinolones are effective both for children and adults (Bhattacharya et al., 1997; Gendrel and Moulin, 2001 Gendrel and Moulin, 2001). Nevertheless, the unfortunate increase in the fluoroquinolone resistance frequently resulting in treatment failure in *Shigella* isolates across the world has been an area for concern, especially in Asia (Wang et al., 2006; Gu et al., 2012 Gu et al., 2012).

Resistance to fluoroquinolones is usually caused by one of two main mechanisms that may occur singly or in combination: alterations in the targets of quinolones, and the impermeable status of the membrane and/or an overexpression of efflux pump systems, leading to decreased drug accumulation inside the bacteria, meanwhile, mobile elements carrying the *qnr* gene also confer a low level of resistance to fluoroquinolones (Ruiz, 2003; Hooper and Jacoby, 2015).

Most published studies in diverse geographical areas have focused on the bacillary dysentery infections and antimicrobial resistance among *Shigella* in China, with *S. flexneri* (86.0%) and *S. sonnei* (12.0%) as the two major causative subgroups (Wang et al., 2006), thus, more and more studies have been performed to analyze the molecular basis of the acquired resistance to fluoroquinolone among *S. flexneri* while mere attention is paid to *S. sonnei* isolates. However, the prevalence of *S. sonnei* during 2001-2010 was approximately 10% higher than that reported 10 years ago (Chang et al., 2012). Despite the increasing reports of *S. sonnei* species, studies on fluoroquinolone resistance mechanism of *S. sonnei* in China are truly few, especially in Jiangsu Province. To fill the knowledge gaps mentioned above, we performed analyses on the alterations in the quinolone resistance determining regions (QRDRs) and prevalence of plasmid-mediated quinolone resistance determinants (PMQR) in *S. sonnei* to aid in the control of fluoroquinolone resistance in Jiangsu Province of China.

Material and Methods

Bacterial isolates

In Jiangsu Province of China, from 2002 to 2011, a total of 950 unduplicated *Shigella* isolates were identified from patients in hospitals in different cities. According to the geographical distribution, Jiangsu Province is divided into three areas: Southern Jiangsu (Nanjing, Wuxi, Suzhou, Changzhou and Zhenjiang), Central Jiangsu (Nantong, Yangzhou and Taizhou) and Northern Jiangsu (Xuzhou, Lianyungang, Yancheng, Suqian and Huai'an). All isolates were confirmed as *Shigella* spp. using API 20E test strips (bioMerieux Vitek, Marcyl'Etoile, France) following the manufacturer's instructions.

Serotyping

The isolates were serotyped by slide agglutination with commercial antisera (Tianrun Bio-Pharmaceutical Co., Ltd, China).

Quinolone susceptibility test

Nalidixic acid and norfloxacin susceptibility of isolated *S. sonnei* strains were determined using Kirby-Bauer disc diffusion system on Mueller-Hinton agar in compliance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

PCR analyses for quinolone resistance genes

The *gyrA*, *gyrB*, *parC* and *parE* and PMQR genes of *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aac-(6′)-Ib-cr* and *qepA* were amplified using primers and conditions as described previously (Pu et al., 2009; Chau et al., 2007; Dutta et al., 2005; Robicsek et al., 2006; Wang et al., 2009; Cavaco et al., 2009; Rahman et al., 1994). Sequencing of the amplification products were performed by Genewiz (Suzhou, China) Company and sequences of *gyrA*, *gyrB*, *parC*, *parE* and positive PMQR genes were compared with sequences obtained from GenBank.

Statistical analysis

SPSS software (SPSS, Chicago, IL, USA) was used for the statistical analysis of the differences in fluoroquinolone resistance frequencies and mutation, and $P < 0.05$ were considered as statistically significant.

Results

Bacterial isolates

950 *Shigella* isolates were collected from 13 cities in Jiangsu Province of China during 2002–2011. Among the 950 *Shigella* isolates, 337 (35.5%) were identified as *S. sonnei* (Figure 1), which witnessed a significant increase during the year 2005-2007, in comparison to the first three years ($P<0.01$), and *S. sonnei* remained the dominant serogroup instead of *S. flexneri* in the period 2007-2009 despite that the frequency came down to less than 40.0% in the recent two years.

Quinolone susceptibility of S. sonnei *isolates*

The strains displayed high resistance (76.6%) against nalidixic acid throughout the study, ranging from 50.0% to 100.0%. The norfloxacin resistant *S. sonnei* only appeared in 2005-2009 in the study, reaching a peak of 40.7% in 2009.

Additionally, we made a comparison between different regions of Jiangsu Province and significant differences of drug resistance profiles were found (Table 1). The resistance rate to nalidixic acid in Southern Jiangsu was the highest, reaching 80.9%, followed by Central Jiangsu of 76.2%, and the proportion of nalidixic acid-resistant *S. sonnei* strains from Northern Jiangsu was the lowest ($P<0.05$). On the contrary, the norfloxacin resistance profiles distinct from nalidixic acid show a decreasing trend in the order Central Jiangsu (32.1%), Northern Jiangsu (18.6%) and Southern Jiangsu (9.8%). Meanwhile, there was a significant overall decrease in resistance for nalidixic acid during the periods 2007–2011 in comparison with that between 2002 and 2006 ($P<0.05$).

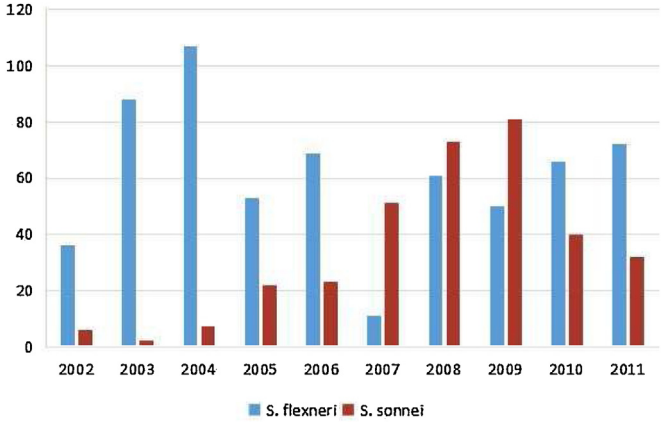

Figure 1. *Shigella* species during the study periods.