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NOTE

Detection of the plasmid-mediated quinolone resistance determinants in clinical isolates of *Serratia marcescens* in China

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Serratia marcescens is a prominent opportunistic pathogen responsible for serious infections in immunocompromised individuals, due primarily to its high intrinsic antibiotic resistance. Quinolones are commonly prescribed antimicrobial agents in China, and quinolone-resistant *S. marcescens* isolates have emerged. Yang *et al.*[1] reported that the resistance rate of ciprofloxacin and levofloxacin in *S. marcescens* collected from 15 teaching hospitals located in different areas in China in 2005 was 15.0% and 7.5%, respectively. Three kinds of plasmid-mediated quinolone resistance (PMQR) determinants have been detected, conferring low-level resistance to quinolones by different mechanisms: quinolone-resistance proteins (Qnr), AAC(6′)-Ib-cr, and QepA efflux.[2] Recently, oqxAB has been identified as another mechanism of PMQR.[3] As the discovery of Qnr, the presence of different *qnr* (A, B, S, C and D) in plasmids has been found worldwide in different bacterial pathogens.[4,5] Resistance to quinolones can also be caused by mutations in the chromosomal genes that code for DNA gyrase and/or DNA topoisomerase IV.[6] Fujimaki K *et al.*[7] reported that DNA gyrase alterations are the basis of quinolone resistance in clinical isolates of *S. marcescens.* Watanabe M *et al.*[8] showed that the mutation in DNA gyrase and mutations that appear to decrease drug permeation occurred frequently in quinolone-resistant isolates of *S. marcescens*. It has been found that the amino-acid residues most frequently mutated in GyrA from *S. marcescens* occur at Gly-81, Ser-83 and Asp-87. Park *et al.*[9] examined 166 isolates of *S. marcescens* and reported one positive for *qnrA* and three for *qnrB*. We conducted this study to estimate the prevalence of PMQR determinants and the role of mutations in DNA gyrase and/or DNA topoisomerase IV in *S. marcescens* in Anhui, China.

A total of 146 nonduplicate *S. marcescens* isolates were collected in 34 hospitals from 2005 to 2011 in Anhui, China. Species identification was performed with the Vitek 2 system (bioMérieux, Marcy l' Étoile, France) and confirmed with API 20E (bioMérieux). The minimum inhibitory concentrations (MICs) for ciprofloxacin, levofloxacin and gatifloxacin (Oxoid) were further determined by the agar dilution method in accordance with the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2012).[10] All isolates were screened for the presence of *qnrA*, *qnrB*, *qnrS* by multiplex PCR using the primers as Robicsek *et al.*[11] as described. Amplification products were provisionally identified from their sizes in ethidium bromide-stained agarose gels. Positive results were confirmed by amplification with primers 5′-ATGACGCCATTACTGTATAA-3′ and 5′-GATCGCAATGTGTGAAGTTT-3′ for *qnrB*.[12] *aac(6′)-Ib* was amplified by PCR as Park *et al.*[12] described. All positives were further analyzed by direct sequencing of the PCR products with primer 5′-CGTCACTCCATACATTGCAA-3′ to identify *aac(6′)-Ib-cr*. *qnrC*, *qnrD*, *qepA*, and *oqxA* genes were screened by using the primers as described previously.[3,13–15] The *gyrA* and *gyrB* genes of DNA gyrase and the *parC* and *parE* genes of topoisomerase IV were amplified by PCR using the primers, as previously described.[16] All the purified PCR products were sequenced on an ABI PRISM3730 sequencer analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analyzed with software available from the National Center for Biotechnology Information. Conjugation experiments were carried out in Luria–Bertani (LB) broth with sodium azide-resistant *Escherichia coli* J53 as the recipient, as previously described.[17] Transconjugants were selected on LB agar plates supplemented with sodium azide (100 μg ml^{-1}) (Sigma Chemical Co., St Louis, MO, USA) and ciprofloxacin (0.25 μg ml^{-1}).

Among the 146 *S. marcescens* isolates, the total resistance rates to ciprofloxacin, levofloxacin and gatifloxacin were 27.4% (40/146), 17.1% (25/146), and 15.8% (23/146), respectively. The quinolone resistance rates for all isolates per year were shown in Table 1. The quinolone resistance rates in our study were higher than the reports of Yang *et al.*,[1] which shown that the resistance rate of ciprofloxacin and levofloxacin in *S. marcescens* collected from 15 teaching hospitals in China in 2005 was 15.0% and 7.5%, respectively. The differences may

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Table 1 The quinolone resistance rates in *S. marcescens* during recent 7 years in China

	Years						
	2005 (N = *14*)[a]	*2006* (N = *30*)	*2007* (N = *16*)	*2008* (N = *22*)	*2009* (N = *15*)	*2010* (N = *16*)	*2011* (N = *33*)
Quinolones	*R%* (n)[b]	*R%* (n)	*R%* (n)	*R%* (n)	*R%* (n)	*R%* (n)	*R%* (n)
Ciprofloxacin	42.9 (6)	33.3 (10)	12.5 (2)	18.2 (4)	26.7 (4)	31.3 (5)	27.3 (9)
Levofloxacin	28.6 (4)	16.7 (5)	6.25 (1)	9.1 (2)	20.0 (3)	31.3 (5)	15.2 (5)
Gatifloxacin	14.3 (2)	16.7 (5)	6.25 (1)	4.5 (1)	26.7 (4)	12.5 (2)	24.2 (8)

[a]*N* = the total number of isolates in that year.
[b]R% = the resistance rates; *n* = the number of resistant isolates.

Table 2 PMQR determinants and alterations in gyrA and parC in *S. marcescens* isolates

		MICs ($\mu g\,ml^{-1}$)				*Nucleotide and amino-acid change*		
						gyrA		*parC*
Strain	*Year*	*CIP*[a]	*LVX*[a]	*GAT*[a]	*PMQR determinants*	*Ser83 (TCG)*	*Asp87 (GAC)*	*Ser80 (AGC)*
9	2005	16	8	4	*aac(6′)-Ib-cr*	Leu (TTG)	—	Ile (ATC)
12	2005	>32	32	16	*aac(6′)-Ib-cr, qnrS2*	Leu (TTG)	Asn (AAC)	Ile (ATC)
T12[b]		2	2	2	*aac(6′)-Ib-cr, qnrS2*	—	—	—
22	2006	32	16	16	*qnrB6*	Leu (TTG)	Asn (AAC)	—
T22[b]		1	0.25	0.25	*qnrB6*	—	—	—
60	2007	16	16	8	*aac(6′)-Ib-cr*	—	—	Ile (ATC)
T60[b]		0.5	0.25	0.25	*aac(6′)-Ib-cr*	—	—	—
64	2008	32	32	8	*qnrS2*	Leu (TTG)	Asn (AAC)	—
T64[b]		1	0.25	0.25	*qnrS2*	—	—	—
86	2009	>32	32	32	*aac(6′)-Ib-cr*	Leu (TTG)	Asn (AAC)	Ile (ATT)
T86[b]		0.5	0.25	0.25	*aac(6′)-Ib-cr*	—	—	—
91	2009	>32	32	32	*aac(6′)-Ib-cr, qnrB6*	Leu (TTG)	—	Ile (ATC)
T91[b]		2	1	1	*aac(6′)-Ib-cr, qnrB6*	—	—	—
J53AZ^{R} [c]		<0.0625	<0.125	<0.125	—[d]	—	—	—

Abbreviations: CIP, Ciprofloxacin; GAT, Gatifloxacin; LVX, Levofloxacin; MICs, minimum inhibitory concentrations; PMQR, plasmid-mediated quinolone resistance.
[a]The resistance breakpoints for CIP, LVX and GAT are ⩾4, ⩾8, and ⩾8, respectively.
[b]T = transconjugant of.
[c]Sodium azide-resistant *Escherichia coli* J53.
[d]No alterations and genes.

be due to the extensive use of quinolone in clinic condition in recent years in China. PMQR determinants were present in seven (4.8%) isolates with *qnr* and *aac(6′)-Ib-cr* detected alone or in combination. Mutations in *gyrA* and/or *parC* were identified in those seven PMQR-positive isolates simultaneously (Table 2). Those seven PMQR-positive isolates were all resistant to fluoroquinolones according to CLSI 2012. The most frequently found gene was *aac(6′)-Ib-cr*, which was identified in 5 (3.4%) isolates. Two *aac(6′)-Ib-cr*-positive isolates carried *qnrS2* and *qnrB6*, respectively. This agrees with the previous reports that *qnr* alleles were coexpressed with *aac(6′)-Ib-cr* on the same plasmid.[18] *qnrS* and *qnrB* were identified in two isolates, respectively. However, other PMQR genes (*qnrA*, *qnrC*, *qnrD*, *qepA* and *oqxA*) were not found in these isolates. Kim SY *et al.*[19] observed significant association of *aac(6′)-Ib-cr* with *qnrA* and *qnrS* in a Korean study. And most isolates with both *aac(6′)-Ib-cr* and *qnr* genes showed higher levels of quinolone resistance than those with *aac(6′)-Ib-cr* alone.

Mutations in the quinolone resistance-determining regions of *gyrA* and/or *parC* were identified amongst the seven PMQR-positive isolates, whereas no *gyrB* or *parE* mutations were found. The most common mutation found in PMQR-positive isolates was at codon 83 of *gyrA* (TCG→TTG transition), resulting in the replacement of serine by leucine. Four isolates were found with a combination of a mutation in *parC* at codon 80 and a mutation in the *gyrA* at codon 83 or codon 87. The mutations of *parC*, plus other *gyrA* mutations, conferred a higher level of resistance to fluoroquinolones than the mutation at codon 80 of *parC* in the study. Earlier studies have described alterations in DNA gyrase in quinolone-resistant *S. marcescens* isolates but did not examine the topoisomerase IV genes.[20,21] Weigel LM *et al.*[21] reported that the fluoroquinolone-resistant clinical isolates of *S. marcescens* displayed the greatest diversity in mutations, including Gly-81 to Cys, Ser-83 to Ile or Arg and Asp-87 to Asn. Interestingly, in contrast with other enterobacterial species, mutation of Ser-83 was not required for high-level fluoroquinolone resistance in *S. marcescens.* Kim JH *et al.*[20] reported that the Ser-83-to-Arg substitution in GyrA protein might lead to high-level quinolone resistance in *S. marcescens* by introducing a bulky amino-acid residue into the protein and also by decreasing the hydrogen-bonding capacity between amino-acid residues. In our study, the mutation at codon 83 (C→T transition) in *gyrA* has also

been detected, resulting in the replacement of serine (TCG) by leucine (TTG).

Among the seven PMQR-positive isolates, plasmids from six of the isolates were successfully transferred to the recipients, suggesting that the dissemination of the PMQR determinants is mostly due to the transmission of plasmids by horizontal exchange. An increase in the MICs of quinolones was detected in the transconjugants compared with the recipients (Table 2).

Our study described clinical isolates of *S. marcescens* in China carrying plasmid-mediated quinolone-resistant genes (*qnrB6*, *qnrS2* and/or *aac(6′)-Ib-cr*) together with mutations in *gyrA* and *parC* genes. These mechanisms were likely to have contributed individually to the high level of ciprofloxacin, levofloxacin and gatifloxacin resistance in *S. marcescens*.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The sequences of the *qnrB6*, *qnrS2* and *aac(6′)-Ib-cr* reported in this article have been deposited in the GenBank database and assigned accession numbers **JQ034317, JQ041635** and **JQ034318**, respectively. *gyrA* and *parC* genes have also been deposited in GenBank under accession number **JQ034320** and **JQ235843** for *gyrA*, **JQ235844** for *parC*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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