Identification of a *Serratia marcescens* Clinical Isolate with Multiple Quinolone Resistance Mechanisms from China

Serratia marcescens, a Gram-negative bacillus that belongs to the family *Enterobacteriaceae*, is an opportunistic pathogen that can cause many types of nosocomial infections (1). In the meantime, widespread use of quinolones has resulted in an increasing incidence of quinolone-resistant *S. marcescens*. However, only preliminary investigations concerning quinolone resistance mechanisms in *S. marcescens* have been reported (14). Recently, four kinds of plasmid-mediated quinolone resistance (PMQR) determinants conferring low-level resistance to quinolones have been detected: quinolone resistance proteins (Qnr), *aac(6′)-Ib-cr*, QepA efflux, and OqxAB (6, 10).

A strain of *S. marcescens*, labeled GN0188, was isolated from the wound drainage fluid of a 46-year-old male inpatient who suffered from chest trauma and was admitted to the intensive care unit in September 2010. During the period of hospitalization, symptoms of lower respiratory tract infection emerged and this patient received treatment with levofloxacin and cefotaxime for 7 days. But the effect was unsatisfactory. The patient continued to be febrile until appropriate therapy with imipenem-cilastatin (500 mg intravenously [i.v.] every 8 h [q8h]) was initiated. Imipenem-cilastatin was continued with a 7-day course, and the symptoms gradually disappeared during the patient's hospital stay. Species identification was performed with the Vitek2 system (bioMérieux, Marcy l'Étoile, France) and confirmed with API 20E (bioMérieux). The clinical strain showed high resistance to ampicillin and piperacillin (MICs ≥ 256 μ g/ml), cefotaxime, ceftazidime, aztreonam, and cefoxitin (MICs ≥ 32 μ g/ml). The isolate was susceptible to cefepime and imipenem.

The MICs for ciprofloxacin, levofloxacin, and gatifloxacin (Oxoid) were further determined by agar dilution in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (3). Amplification of *qnrA*, *qnrB*, and *qnrS* was carried out by multiplex PCR using the primers described by Robicsek et al. (11). *aac(6′)-Ib-cr* was amplified by PCR as Park et al. described (9). The *qnrC*, *qnrD*, and *qepA* genes were screened by using the primers described previously (2, 12, 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 previously described (5). All the purified PCR products were sequenced on both strands. Conjugation experiments were carried

out in Luria-Bertani (LB) broth with sodium azide-resistant *Escherichia coli* J53 as the recipient, as previously described (13). Transconjugants were selected on LB agar plates supplemented with sodium azide (100 μ g/ml) (Sigma Chemical Co., St. Louis, MO) and ciprofloxacin (0.25 μ g/ml).

S. marcescens GN0188 had high-level quinolone resistance, with a ciprofloxacin MIC of 64 μ g/ml and levofloxacin and gatifloxacin MICs of 32 μ g/ml. The genes *qnrB6*, *aac(6′)-Ib-cr*, *gyrA*, and *parC* were detected in GN0188. GN0188 showed one mutation in *gyrA* and nine mutations in *parC* compared with *gyrA* (GenBank accession no. U56906) and *parC* (GenBank accession no. AF227958) of *S. marcescens*, respectively (Table 1). The plasmid of GN0188 was transferred into the recipient *E. coli* J53 strain by conjugation. The genes *qnrB6* and *aac(6′)-Ib-cr* were detected in the transconjugant of GN0188.

Earlier studies have described Ser83 alterations in the *S. marcescens* GyrA protein that might lead to high-level quinolone resistance, but those studies did not examine the topoisomerase IV genes in *S. marcescens* (7, 14). Mutation at the conserved Ser84 in *parC* was documented in many bacterial species to cause quinolone resistance (4, 8). Moon et al. reported that a mutation at codon 84 in *parC*, combined with mutations in *gyrA* at codons 83 and 87, led to high levels of resistance to fluoroquinolones in 10 *E. coli* isolates (8). To our knowledge, we reported the substitution at codon 84 (Ser [AGC]→Ile [ATT]) in *parC* for the first time in *S. marcescens*. Substitutions of P13A, T16E, P33R, and Y37F, which lie outside the quinolone resistance-determining regions (QRDRs) of *parC*, were observed in *S. marcescens* GN0188, but their roles in resistance to fluoroquinolones have not been determined in our study. The changes at Ile50, Thr59, Asn60, and Ala133, observed in GN0188, have not been documented before. These four substitutions lie in the QRDRs of *parC*, and their roles in resistance to fluoroquinolones need to be further studied. Mutations in *gyrA* are a prerequisite for

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TABLE 1 MICs of quinolones, PMQR genes, and amino acid changes in the GyrA and ParC subunits of GN0188

Strain ^a	MIC (μ g/ml) ^b			Nucleotide change (amino acid change) at ^c										
	CIP	LVX	GAT	PMQR genes	ParC position									
					GyrA position Ser83 (TCG)	Pro13 (CCG)	Thr16 (ACA)	Pro33 (CCG)	Tyr37 (TAC)	Ile50 (ATC)	Thr59 (ACC)	Asn60 (AAC)	Ser84 (AGC)	Ala133 (GCC)
<i>E. coli</i> J53 Az ^r	≤ 0.0625	≤ 0.125	≤ 0.125											
<i>S. marcescens</i> GN0188	64	32	32	<i>aac(6′)-Ib-cr</i> , <i>qnrB6</i>	Leu (TTG)	Ala (GCG)	Glu (GAA)	Arg (CGT)	Phe (TTT)	Val (GTG)	Asn (AAT)	Ala (GCC)	Ile (ATT)	Ser (TCC)
Transconjugant of GN0188	2	1	1	<i>aac(6′)-Ib-cr</i> , <i>qnrB6</i>										

^a Az^r, sodium azide resistant.

^b CIP, ciprofloxacin; LVX, levofloxacin; GAT, gatifloxacin.

^c The nucleotide and amino acid changes in GN0188 are shown in comparison with the nucleotides and amino acids of *gyrA* (GenBank accession number U56906) and *parC* (GenBank accession number AF227958) of *S. marcescens*.

resistance to fluoroquinolones through target alteration. The accumulation of mutations in *parC* confers high levels of resistance to fluoroquinolones.

In conclusion, these mechanisms were likely to have contributed individually to the high-level quinolone resistance in *S. marcescens* GN0188. Additionally, the dissemination of the PMQR determinants is mostly due to the transmission of plasmids by horizontal exchange.

Nucleotide sequence accession numbers. The sequences of the *qnrB6*, *aac(6')-Ib-cr*, *gyrA*, and *parC* genes reported in this article have been deposited in the GenBank database under the accession numbers [JQ034317](#), [JQ034318](#), [JQ034320](#), and [JQ034319](#), respectively.

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