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The Emergence of the 16S rRNA Methyltransferase RmtB in a Multidrug-Resistant *Serratia marcescens* Isolate in China

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The acquisition of multidrug resistance in nosocomial pathogens such as *Serratia marcescens* has been reported in the absence of antibiotic selection pressure. The broad antimicrobial spectrum of aminoglycosides and their synergistic antimicrobial effect in association with other drugs (such as β-lactams and fluoroquinolones) suggest the possible use of these antibiotics as a therapy for life-threatening infections. One of the most important mechanisms of aminoglycoside resistance is posttranscriptional methylation of 16S rRNA conferred by methyltransferases. To date, 10 kinds of 16S rRNA methyltransferase genes (*armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF, rmtG, rmtH,* and *npmA*), whose products confer high-level resistance to aminoglycosides, have been reported in various *Enterobacteriaceae* strains [1]. The *rmtB* gene was first identified in *S. marcescens* in Japan, but has not been reported in *S. marcescens* elsewhere [2]. In the present study, we described an isolate of *S. marcescens* harboring *rmtB*, together with quinolone resistance genes and various β-lactamase genes, for the first time in China.

A strain of *S. marcescens*, designated GN1384, was isolated from the urine of a 81-yr-old male patient who suffered from urinary tract infections in September 2009 and was admitted to the second hospital of Hefei, China. Species identification was performed with the Vitek2 system (bioMérieux, Marcy l'Étoile, France) and confirmed with API 20E (bioMérieux). The minimum inhibitory concentrations (MICs) of various antibiotics were determined by using the agar dilution method, and susceptibility data were interpreted by using the Clinical and Laboratory Standards Institute guidelines [3]. The isolate showed a multiple drug resistant pattern, including some β-lactams, all the aminoglycosides, and fluoroquinolones (Table 1).

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Table 1. Characteristics of clinical isolate *S. marcescens* GN1384 and its transconjugant

Strain	Resistance genes	MIC (mg/L)																		
		AMK	GM	KAN	TOB	NET	AMP	CSL	TZP	FEP	CTX	CRO	FOX	CAZ	CZ	IPM	MEM	ATM	GAT	CIP	LVX
J53*	-†	<1	<0.25	<1	<0.25	<0.5	2	<1	<1	<0.5	<0.5	<1	<0.5	<0.5	<0.5	<0.25	<0.25	<0.5	<0.12	<0.06	<0.12
86	*rmtB*, $bla_{CTX-M-14}$, bla_{TEM-1}, bla_{OXA-1}, *aac(6')-Ib-cr*, *gyrA*, *parC*	>512	>128	>512	>128	>256	>256	32	32	128	>512	512	8	256	128	<0.25	<0.25	>256	32	>32	32
T86	*rmtB*, $bla_{CTX-M-14}$, bla_{TEM-1}, bla_{OXA-1}, *aac(6')-Ib-cr*	256	128	>512	128	128	>256	16	16	128	>512	512	2	128	16	0.5	<0.25	256	1	2	1

*Sodium azide-resistant Escherichia coli J53; †Indicates no genes or no information needed.
Abbreviations: AMK, amikacin; GM, gentamicin; KAN, kanamycin; TOB, tobramycin; NET, netilmicin; AMP, ampicillin; CSL, cefoperazone-sulbactam; TZP, piperacillin-tazobactam; FEP, cefepime; CTX, cefotaxime; CRO, ceftriaxone; FOX, cefoxitin; CAZ, ceftazidime; CZ, cefizoxime; IMP, imipenem; MEM, meropenem; ATM, aztreonam; GAT, gatifloxacin; CIP, ciprofloxacin; LVX, levofloxacin.

Then, genomic and plasmid DNA of strain GN1384 was extracted. Using primers described previously, we identified the resistance genotype of this strain by detecting the following resistance determinants through PCR, based on the above resistance phenotypes: 16S rRNA methyltransferase genes (*armA, rmtA, rmtB, rmtC, rmtD, rmtE,* and *npmA*); plasmid-mediated quinolone resistance determinants (*qnrA, qnrB, qnrS, qnrC, qnrD, aac (6')-Ib-cr,* and *qepA*); chromosome mutations associated with fluoroquinolone resistance (*gyrA*, *parC,* and *parE*); and genes coding for β-lactamases (TEM, CTX-M, SHV, OXA-1) [4-7]. The positive amplicons were subsequently sequenced, and the results indicated the presence of *rmtB*, *aac(6')-Ib-cr*, $bla_{CTX-M-14}$, bla_{TEM-1}, and bla_{OXA-1}. In addition, two amino acid changes (Ser-83Leu and Asp87Asn) in GyrA and five amino acid changes (Thr57Ser, Ser80Ile, Ala129Ser, Val131Leu, and Gly134Ser) in ParC were observed.

Conjugation experiments were performed with *Escherichia coli* J53 as the recipient, as previously described [8]. The transconjugants were selected on agar plates containing sodium azide (100 mg/L) supplemented with amikacin (128 mg/L). Conjugation experiments showed that *aac(6')-Ib-cr*, bla_{TEM-1}, $bla_{CTX-M-14}$, and bla_{OXA-1} were cotransferred with *rmtB* to the recipient. The MIC results revealed that it simultaneously exhibited an elevated level of resistance to β-lactams, aminoglycosides, and fluoroquinolones (Table 1).

The extremely high MICs of all aminoglycosides tested against the multidrug-resistant *S. marcescens* were likely due to the presence of the *rmtB* gene. In contrast to the *rmtB* plasmid isolated by Doi *et al*. [2], the plasmid bearing *rmtB* in our study was self-transferable, as observed by Bogaerts *et al*. [9]. In accordance with a previous study, the close linkage between 16S rRNA methyltransferase and β-lactamase as well as plasmid-mediated quinolone resistance determinants revealed the significant relationship of these genes amongst *Enterobacteriaceae* [10]. It was also observed that the plasmid-mediated quinolone resistance gene together with the mutations in *gyrA* and *parC* contributed to the high-level quinolone resistance in *S. marcescens* GN1384. Further studies are required to assess the genetic environment of these genes and the role of these new substitutions in resistance to fluoroquinolones. The continuous and widespread use of aminoglycosides, fluoroquinolones, and β-lactams in human or animal infection is the major driving force leading to these sophisticated resistance gene linkages. The dissemination of these linked genes by horizontal transfer simultaneously is very worrisome, and continuous monitoring should be reinforced.

Nucleotide sequence accession numbers

The sequence of the *parC* gene reported in this work has been deposited in the GenBank database and assigned the accession number JQ034319.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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