AAC Journals.ASM.org

Plasmid-Mediated Quinolone Resistance in Extended-Spectrum-β-Lactamase- and AmpC β-Lactamase-Producing *Serratia marcescens* in China

Hai-Fei Yang,[a] Jun Cheng,[a] Li-Fen Hu,[a] Ying Ye,[a,b,c] and Jia-Bin Li[a,b,c]

Department of Infectious Disease, the First Affiliated Hospital of Anhui Medical University, Hefei, China[a]; Institute of Bacterium Resistance, Anhui Medical University, Hefei, China[b]; and Anhui Center for Surveillance of Bacterial Resistance, Hefei, China[c]

We investigated the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants and examined the association of these determinants with extended-spectrum β-lactamases (ESBLs) and/or plasmid-mediated AmpC β-lactamases (pAmpCs) in *Serratia marcescens* isolates in China. In this study, the presence of PMQR determinants was significantly related to the co-production of ESBLs and/or pAmpCs (CTX-M-14, SHV-5, DHA-1, and ACT-1), among which CTX-M-14 was the most common gene type.

Serratia marcescens is a prominent opportunistic pathogen responsible for serious infections in immunocompromised individuals. For many Gram-negative bacteria, including *S. marcescens*, production of chromosomally encoded AmpC-type β-lactamase is the intrinsic mechanism of resistance to β-lactam antibiotics. *S. marcescens* generally has inducible expression of this enzyme and has been thought to be a natural chromosomal AmpC producer (9). In China, quinolones and β-lactam antibiotics are widely used in the treatment of many bacterial infections, including infection caused by *S. marcescens*. Three kinds of plasmid-mediated quinolone resistance (PMQR) determinants [*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac*(6′)*-Ib-cr*, and *qepA*] conferring low-level resistance to quinolones by different mechanisms [quinolone resistance (Qnr) proteins, AAC(6′)-Ib-cr, and QepA efflux] have been detected(8). The existence of these enzymes is of great concern because they not only are capable of conferring resistance against most clinically important quinolones but also are often associated with extended-spectrum β-lactamases (ESBLs) and/or plasmid-mediated AmpC β-lactamases (pAmpCs) (4). Although ESBLs have been identified in *S. marcescens* isolates repeatedly, there are few reports of pAmpCs being found in *S. marcescens*. To our knowledge, there was only one case report of an *S. marcescens* isolate harboring a pAmpC in a study carried out in Spain in 2010 (5). The aims of this study were to investigate the prevalence of PMQR determinants and to examine the association of these determinants with ESBLs and/or pAmpCs in *S. marcescens*.

A total of 146 nonduplicate *S. marcescens* isolates were collected from 2005 to 2011 at 34 hospitals in Anhui, China. Species identification was performed with the Vitek 2 system (bioMérieux, Marcy l'Étoile, France) and confirmed with API 20E (bioMérieux). The isolates were from respiratory specimens (69.6%), wounds (8.7%), blood (5.8%), urine (2.9%), body fluids (3.9%), and other specimens (9.1%). The MICs of piperacillin (PIP), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), cefoxitin (FOX), aztreonam (ATM), imipenem (IMP), meropenem (MEM), ciprofloxacin (CIP), levofloxacin (LVX), gatifloxacin (GAT), gentamicin (GM), and amikacin (AMK) (Oxoid) were determined by agar dilution method in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (3). ESBL detection was based on a double-disk synergy test (10). The modified Hodge test (15) was used to screen AmpC β-lactamase-producing strains.

For the ESBL screen-positive isolates, a search for the bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{OXA} genes was performed by PCR amplification as previously described (1). For the isolates with cefoxitin MICs of ≥16 mg/liter, plasmid DNA was extracted using a Qiagen plasmid purification kit (Qiagen, Hilden, Germany), and *ampC* amplification was carried out using multiplex PCR, which can detect various types (MOX, CMY, LAT, DHA, ACC, MIR-1, ACT-1, and FOX) of pAmpCs (7). Conjugation experiments were carried out for all ESBL gene- and/or pAmpC gene-positive isolates with sodium azide-resistant *Escherichia coli* J53 as the recipient, as previously described (1). Transconjugants were selected on Luria-Bertani (LB) agar plates supplemented with sodium azide (100 mg/liter) (Sigma Chemical Co., St. Louis, MO) and FOX (16 mg/liter) or CAZ (2 mg/liter). Plasmid DNA was extracted from donors and transconjugants by using a Qiagen plasmid purification kit. The transconjugants were examined for the presence of β-lactamase genes by PCR using plasmid DNA as the template and tested for susceptibility as described above for the wild strains. For all 146 *S. marcescens* isolates, a search for the presence of *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac*(6′)*-Ib-cr*, and *qepA* was performed by PCR using the methods described previously (2, 6, 11, 13, 14). All the purified PCR products were sequenced on an ABI Prism 3730 sequence analyzer (Applied Biosystems, Foster City, CA). Sequence alignment was compared with the GenBank nucleotide database using the nucleotide BLAST program.

Of the 146 *S. marcescens* isolates, 25 (17.1%) were CTX resistant (MICs ≥ 64 mg/liter) and 32 (21.9%) were CAZ resistant (MICs ≥ 32 mg/liter) according to the CLSI guidelines. In addition, 11 (7.5%) isolates showed some potentiation of the inhibi-

Received 3 March 2012 Returned for modification 8 April 2012
Accepted 27 May 2012
Published ahead of print 4 June 2012
Address correspondence to Jia-Bin Li, lijiabin948@vip.sohu.com.
Copyright © 2012, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.00493-12

TABLE 1 Profiles of 8 ESBL- and/or pAmpC-gene positive *S. marcescens* isolates and their transconjugants

Isolate	Specimen	β-Lactamase(s)	PMQR determinant	MIC (mg/liter)												
				PIP	CTX	CAZ	FEP	FOX	ATM	IMP	MEM	CIP	LVX	GAT	GM	AMK
GN0199	Sputum	$bla_{\text{CTX-M-14}}$, $bla_{\text{SHV-5}}$, $bla_{\text{OXA-1}}$,[a] $bla_{\text{ACT-1}}$	*aac(6′)-Ib-cr*	512	32	64	8	>256	128	0.5	<0.25	>32	32	8	>128	16
T0199[d]		$bla_{\text{CTX-M-14}}$, $bla_{\text{SHV-5}}$, $bla_{\text{ACT-1}}$	*aac(6′)-Ib-cr*	256	16	32	2	256	32	<0.25	<0.25	1	0.5	0.5	8	2
GN0885	Wound	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$,[a] $bla_{\text{OXA-1}}$	*aac(6′)-Ib-cr*	512	256	>256	2	256	>256	1	<0.25	>32	>64	16	>128	>512
T0885		$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$	*aac(6′)-Ib-cr*	128	32	32	0.5	4	32	<0.25	<0.25	1	0.5	0.5	<0.5	<0.5
GN0480	Sputum	$bla_{\text{SHV-5}}$, $bla_{\text{OXA-1}}$, $bla_{\text{DHA-1}}$	—[b]	512	32	256	4	64	>256	0.5	<0.25	>32	32	16	>128	>512
T0480		$bla_{\text{SHV-5}}$, $bla_{\text{DHA-1}}$	—	128	8	16	0.25	32	32	<0.25	<0.25	<0.06	<0.125	<0.125	1	1
GN0818	Wound	$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$	*qnrB6*	512	256	32	0.5	256	64	0.5	<0.25	>32	>64	>64	>128	16
T0818		$bla_{\text{CTX-M-14}}$, $bla_{\text{TEM-1}}$	*qnrB6*	128	32	8	0.25	4	16	<0.25	<0.25	2	1	1	0.5	0.25
GN1237	Sputum	$bla_{\text{CTX-M-14}}$, $bla_{\text{DHA-1}}$	*aac(6′)-Ib-cr*	256	32	32	32	128	256	1	<0.25	8	8	16	>128	>512
T1237		$bla_{\text{CTX-M-14}}$, $bla_{\text{DHA-1}}$	*aac(6′)-Ib-cr*	64	8	8	1	128	16	<0.25	<0.25	1	1	0.5	0.25	0.25
GN1249	Sputum	$bla_{\text{CTX-M-14}}$, $bla_{\text{DHA-1}}$	*qnrS2*, *aac(6′)-Ib-cr*	512	128	32	4	256	>256	1	0.25	>32	>64	32	>128	>512
T1249		$bla_{\text{CTX-M-14}}$, $bla_{\text{DHA-1}}$	*qnrS2*, *aac(6′)-Ib-cr*	64	8	8	<0.25	128	32	<0.25	<0.25	2	1	1	0.5	0.25
GN1382	Sputum	$bla_{\text{DHA-1}}$	*aac(6′)-Ib-cr*	256	64	128	16	256	128	1	<0.25	4	8	8	>128	>512
T1382		$bla_{\text{DHA-1}}$	*aac(6′)-Ib-cr*	64	8	8	1	128	16	<0.25	<0.25	0.5	0.25	0.25	<0.5	<0.5
J53[c]		—	—	1	<0.5	<0.5	<0.25	<0.5	<0.5	<0.25	<0.25	<0.06	<0.125	<0.125	<0.5	<0.5
GN0889	Sputum	$bla_{\text{SHV-5}}$, $bla_{\text{OXA-1}}$	—	256	256	32	16	512	>256	2	0.25	0.5	0.125	0.125	>128	4
GN0413	Wound	$bla_{\text{TEM-1}}$	—	256	32	16	2	32	8	0.5	<0.25	2	1	0.5	>128	32
GN0447	Urine	$bla_{\text{TEM-1}}$	—	512	128	128	0.5	64	32	0.5	<0.25	>32	>64	32	>128	4
GN0132	Urine	$bla_{\text{TEM-1}}$	*aac(6′)-Ib-cr*	256	32	>256	8	64	>256	0.5	<0.25	>32	8	8	>128	>512
GN0526	Blood	$bla_{\text{TEM-1}}$	—	512	512	128	32	256	>256	0.5	<0.25	>32	16	4	>128	4

[a] $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$ are not ESBL genes.
[b] —, no gene was detected.
[c] Sodium azide-resistant *Escherichia coli* J53.
[d] Strains whose designation begin with "T" are transconjugants.

tory zones of the β-lactams by clavulanic acid, suggesting the presence of ESBL activity. In these 11 putatively ESBL-positive isolates, the structural genes for CTX-M-14 ($n = 5$) and SHV-5 ($n = 3$) were found either alone or in combination in 7 strains (Table 1). Also, bla_{TEM-1} and bla_{OXA-1} were detected either alone or in combination with ESBL or pAmpC genes in 6 and 4 strains, respectively. Of the 7 ESBL gene-positive strains, 6 (85.7%) were resistant to ciprofloxacin and levofloxacin. The rate of ciprofloxacin resistance was higher in the ESBL gene-positive strains (85.7%) than in the ESBL nonproducers (27/135, 20.0%) ($P < 0.05$). All of the ESBL gene-positive strains were resistant to gentamicin, and 4 (57.1%) of them were resistant to amikacin. However, all the ESBL gene-positive strains were susceptible to carbapenems.

Of the 146 *S. marcescens* isolates, 30 were not susceptible to cefoxitin (MICs ≥ 16 mg/liter). Among these 30 isolates, 6 had a positive reaction according to the modified Hodge test. PCR and sequencing analysis revealed that 5 of them harbored pAmpC genes (4 contained bla_{DHA-1}, 1 contained bla_{ACT-1}) (Table 1), but in one isolate no pAmpC gene was detected. pAmpC gene-harboring isolates also harbored $bla_{CTX-M-14}$, bla_{SHV-5}, and bla_{OXA-1} either alone or in combination.

Of the total of 146 isolates, 15 (10.3%) showed high-level resistance (MIC > 32 mg/liter) to both ciprofloxacin and levofloxacin. Five of the ESBL gene- and/or pAmpC gene-positive strains harbored the *aac(6′)-Ib-cr* variant, and one strain coexpressed *qnrS2*. One strain harbored *qnrB6* alone. None harbored a *qnrA*, *qnrC*, *qnrD*, or *qepA* gene. In this study, the rate of PMQR determinants was significantly higher in the ESBL gene-positive strains (5/7, 71.4%) than in ESBL nonproducers (12/135, 8.9%) ($P < 0.05$). PMQR determinants were detected in 4 pAmpC gene-positive strains (4/5, 80.0%). The presence of PMQR determinants was significantly related to the coproduction of ESBLs and/or pAmpCs, among which CTX-M-14 was the most common (Table 1). This finding corroborates the previous reports that the PMQR determinants are frequently associated with ESBLs and/or pAmpCs and they are cotransferred by conjugation (4). Wang et al. (12) reported that several *qnrA*-positive strains produced SHV-7 and CTX-M-9. In our study, CTX-M-14 and DHA-1 were common among PMQR determinant-positive strains.

Among the 8 ESBL gene- and/or pAmpC gene-positive strains, 7 had genes that were successfully transferred on transferable plasmids to the recipients. An increase in the MICs of quinolones and β-lactam was detected in the transconjugants compared to the recipients (Table 1). It is suggested that the dissemination of the PMQR determinants and pAmpC is mostly due to the transmission of plasmids by horizontal exchange.

In conclusion, we verified that PMQR determinants are widespread among quinolone-resistant *S. marcescens* isolates in Anhui, China. More importantly, we also determined that PMQR determinants are closely related to various ESBLs and pAmpCs. Due to the multidrug resistance in *S. marcescens* isolates, prudent antibiotic use and accurate detection of resistance are urgently needed to prevent the spread of these organisms.

ACKNOWLEDGMENTS

We thank all the contributing hospitals that provided isolates for this study.

This work was supported by a research grant from the National Natural Science Foundation of China (no. 30972631,81101288) and by the Natural Science Foundation of Anhui Province, China (no. 11040606Q23).

REFERENCES

1. **Barguigua A, et al.** 2011. Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J. Med. Microbiol. **60**:1344–1352.
2. **Cavaco LM, Hasman H, Xia S, Aarestrup FM.** 2009. *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. Antimicrob. Agents Chemother. **53**:603–608.
3. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement, M100–S22. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Jeong HS, et al.** 2011. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in *Enterobacteriaceae*. Korean J. Lab. Med. **31**:257–264.
5. **Mata C, et al.** 2010. In vivo transmission of a plasmid coharbouring *bla* and *qnrB* genes between *Escherichia coli* and *Serratia marcescens*. FEMS Microbiol. Lett. **308**:24–28.
6. **Park YJ, Yu JK, Lee S, Oh EJ, Woo GJ.** 2007. Prevalence and diversity of qnr alleles in AmpC-producing *Enterobacter cloacae*, Enterobacter aerogenes, *Citrobacter freundii* and *Serratia marcescens*: a multicentre study from Korea. J. Antimicrob. Chemother. **60**:868–871.
7. **Perez-Perez FJ, Hanson ND.** 2002. Detection of plasmid-mediated AmpC b-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40**:2153–2162.
8. **Robicsek A, Jacoby GA, Hooper DC.** 2006. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect. Dis. **6**:629–640.
9. **Stock I, Grueger T, Wiedemann B.** 2003. Natural antibiotic susceptibility of strains of *Serratia marcescens* and the *S. liquefaciens* complex: *S. liquefaciens sensu stricto*, *S. proteamaculans* and *S. grimesii*. Int. J. Antimicrob. Agents **22**:35–47.
10. **Tzelepi E, et al.** 2000. Detection of extended spectrum β-lactamases in clinical isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*. J. Clin. Microbiol. **38**:542–546.
11. **Wang M, et al.** 2009. New plasmid-mediated quinolone resistance gene, *qnrC*, found in a clinical isolate of *Proteus mirabilis*. Antimicrob. Agents Chemother. **53**:1892–1897.
12. **Wang M, et al.** 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. **47**:2242–2248.
13. **Xiong Z, et al.** 2008. Investigation of *qnr* and *aac(6′)-Ib-cr* in *Enterobacter cloacae* isolates from Anhui Province, China. Diagn. Microbiol. Infect. Dis. **62**:457–459.
14. **Yamane K, et al.** 2007. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. Antimicrob. Agents Chemother. **51**:3354–3360.
15. **Yong D, et al.** 2002. Further modification of the Hodge test to screen AmpC β-lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. J. Microbiol. Methods **51**:407–410.