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Emergence of KPC-2-Producing *Pseudomonas aeruginosa* Sequence Type 463 Isolates in Hangzhou, China

Yan-yan Hu, Dan-xia Gu, Jia-chang Cai, Hong-wei Zhou, Rong Zhang

2nd Affiliated Hospital of Zhejiang University, Zhejiang University, Hangzhou, People's Republic of China

Thirty-nine *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Pseudomonas aeruginosa* isolates, all exhibiting high-level resistance to carbapenems and other β-lactam antibiotics, were isolated in Hangzhou, China. Molecular epidemiology analysis indicated the presence of two dominant clones, namely, clones A and B, both of which belong to sequence type 463 (ST463). A genetic environment analysis demonstrated that both clones harbor an IS*Kpn8* transposase, bla_{KPC-2}, and an IS*Kpn6*-like transposase. These findings depict the features of clonal expansion and transmission of KPC-2-producing *P. aeruginosa* strains in Hangzhou, China.

Pseudomonas aeruginosa is one of the most common and clinically important pathogens worldwide, causing both high morbidity and mortality among infected patients (1). According to an antimicrobial resistance surveillance of bacterial pathogens in China in 2012 (CHINET), the isolation rate of *P. aeruginosa* ranked second among the nonfermentative bacteria (2). Carbapenems are considered to be the most efficient antibiotics for the treatment of serious infections caused by multidrug-resistant Gram-negative bacilli. However, with the widespread use of such agents, the resistance rates of *P. aeruginosa* to carbapenems have increased rapidly. The findings of CHINET also showed that the rates of resistance of *P. aeruginosa* to imipenem and meropenem in 2013 reached 29.0% and 27.0%, respectively (2). The common resistance mechanisms of *P. aeruginosa* to carbapenems are the loss of the outer membrane protein OprD and the overexpression of efflux pumps and/or the intrinsic chromosomally encoded AmpC β-lactamase (3). The production of carbapenemases, which is recognized as another mechanism for carbapenem resistance, varies between countries. A survey conducted in the United States on 452 carbapenem-resistant *P. aeruginosa* isolates revealed that 90.0% of the isolates displayed the loss of OprD, 55.0% exhibited the overexpression of efflux pumps, and 25.0% produced AmpC β-lactamase; yet only four isolates were observed to produce carbapenemases (3). Conversely, longitudinal surveillance of carbapenem-resistant *P. aeruginosa* isolates in Belarus, Kazakhstan, and Russia demonstrated that the incidence of carbapenemase production increased yearly, from 4.5% in 2002 to 2004 to 28.7% in 2008 to 2010 (4).

In our study, 398 carbapenem-resistant *P. aeruginosa* isolates were collected from 10 hospitals in Zhejiang Province, China, in 2013. The geographical distribution of *P. aeruginosa* isolates was as follows: 55 isolates from the Second Affiliated Hospital of Zhejiang University (Hangzhou), 30 isolates from the First Affiliated Hospital of Zhejiang University (Hangzhou), 21 isolates from the Sir Run Run Shaw Hospital of Zhejiang University (Hangzhou), 20 isolates from the Red Cross Hospital (Hangzhou), 108 isolates from Zhejiang Provincial People's Hospital (Hangzhou), 20 isolates from the Taizhou Hospital of Zhejiang Province (Taizhou), 45 isolates from the Cixi People's Hospital (Ningbo), 48 isolates from the Third People's Hospital of Wenzhou City (Wenzhou), and 42 isolates from the Second People's Hospital of Jiaxing City (Jiaxing). Species identification was performed using the Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France).

All 389 imipenem-resistant isolates detected by the Kirby-Bauer disk diffusion method, as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines (5), were screened for the most common carbapenemase genes, including bla_{KPC} (6), bla_{NDM-1} (7), bla_{VIM}, and bla_{IMP} (8). For the bla_{KPC}-positive isolates, analyses of the other β-lactamase genes, including bla_{OXA-50}, bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{PER}, and bla_{VEB}, were performed. DNA sequence analysis indicated that 38 *P. aeruginosa* isolates harbored the bla_{KPC-2} gene only, one harbored both the bla_{KPC-2} and bla_{PER-1} genes, and the bla_{VIM-2} gene and bla_{IMP-4} gene were each detected in one strain. None of the other β-lactamases genes were detected. All of the 39 KPC-producing *P. aeruginosa* isolates were collected from Hangzhou (10 isolates from the 2nd Affiliated Hospital of Zhejiang University, 6 isolates from Sir Run Run Shaw Hospital of Zhejiang University, and 23 isolates from Zhejiang Provincial People's Hospital). The features of the geographical spread of the KPC-2 carbapenemase-producing *P. aeruginosa* isolates were similar to those of *Enterobacteriaceae* in Zhejiang Province, as reported previously (9, 10), which showed that such resistant isolates were first found in a big city and subsequently spread rapidly to the surrounding cities. The resistance mechanisms of the remaining 350 non-KPC-producing carbapenem-resistant *P. aeruginosa* isolates remain unknown but are most likely due to a loss of the OprD porin and/or the overexpression of efflux pump genes.

An analysis of the genetic environment of the bla_{KPC-2} gene in *P. aeruginosa* isolates was analyzed using primer-walking sequenc-

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Address correspondence to Rong Zhang, brigitte_zr@163.com.

Y.-Y.H. and D.-X.G. contributed equally to the work.

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TABLE 1 Summary of antimicrobial susceptibility, β-lactamase production characteristics, and results of molecular typing of selected *P. aeruginosa* isolates

Isolate(s)[a]	Molecular typing results			β-lactamase(s) present	Clone	MIC (mg/liter) for[b]:								Genetic environment of bla_{KPC-2} gene
	PFGE	No. of isolates	ST			IPM	MEM	CAZ	FEP	LEV	CI	AK	GM	
SRM9–SRM13, SRM15–SRM22	A1	13	463	KPC-2	A1	64 to 512	128 to >512	128 to 512	>512	1 to 16	0.125 to 6	3 to 8	2 to 6	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE2, ZE3	A2	2	463	KPC-2	A2	128 to 512	32 to >512	128	256 to >512	1 to 8	0.38 to 4	0.75 to 4	0.19 to 4	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM4–SRM6	A3	3	463	KPC-2	A3	128 to 256	512	128 to 256	>512	8 to 16	4	4	2 to 3	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE4	A4	1	463	KPC-2	A4	256	>512	64	>512	8	2	2	3	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM23	A5	1	463	KPC-2	A5	512	>512	512	>512	16	3	6	6	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SYF2, SYF3, SYF5	B1	3	463	KPC-2	B1	128 to 256	512 to >512	64 to 128	512	64	>256	3 to 4	1.5 to 3	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE7	B2	1	463	KPC-2	B2	128	512	64	256	16	4	4	4	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SYF6	B3	1	463	KPC-2	B3	256	512	64	512	16	6	6	3	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SYF1, SRM2	C	2	1076	KPC-2	C	128 to 256	512	32 to 64	256 to 512	8	3	4 to 6	3	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE1	D	1	209	KPC-2	D	256	>512	256	>512	0.5	0.25	8	4	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM1	E	1	1755	KPC-2	E	32	64	32	256	32	>256	2	1.0	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE5	F	1	836	KPC-2	F	128	>512	128	>512	0.5	0.125	4	1.5	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE10	G	1	463	KPC-2	G	256	>512	256	>512	8	3	4	3	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE6	H	1	463	KPC-2	H	64	32	64	128	8	2	2	>256	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE8	I	1	463	KPC-2	I	128	>512	512	>512	8	4	4	3	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SYF4	J	1	463	KPC-2, PER-1	J	64	64	>512	>512	8	3	0.50	>256	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
ZE9	K	1	463	KPC-2	K	16	4	256	256	1	0.19	3	2	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM14	L	1	244	KPC-2	L	128	>512	512	>512	16	2	4	3	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM7	M	1	463	KPC-2	M	128	512	128	>512	16	4	6	4	IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM8	N	1	357	KPC-2	N	16	32	16	64	8	1.0	6	4	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*
SRM3	O	1	850	KPC-2	O	8	16	16	64	4	0.75	12	4	Tn*3*-IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*

[a] ZE1 to ZE10, 10 strains of KPC-2-producing *P. aeruginosa* isolated from 2nd Affiliated Hospital of Zhejiang University; SYF1 to SYF6, 6 strains of KPC-2-producing *P. aeruginosa* isolated from Sir Run Run Shaw Hospital of Zhejiang University; SRM1 to SRM23, 23 strains of KPC-2-producing *P. aeruginosa* isolated from Zhejiang Provincial People's Hospital.

[b] IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; LEV, levofloxacin; CI, ciprofloxacin; AK, amikacin; GM, gentamicin. The MICs of colistin and polymyxin B for all *P. aeruginosa* isolates were 1 to 2 mg/liter. The MICs of aztreonam were >256 mg/liter, except for strain SRM3, with an MIC of 128 mg/liter. The MICs of cefoperazone-sulbactam and piperacillin-tazobactam were >256 mg/liter, except for SRM3 and SRM8 (MICs, 32 to 128 mg/liter).

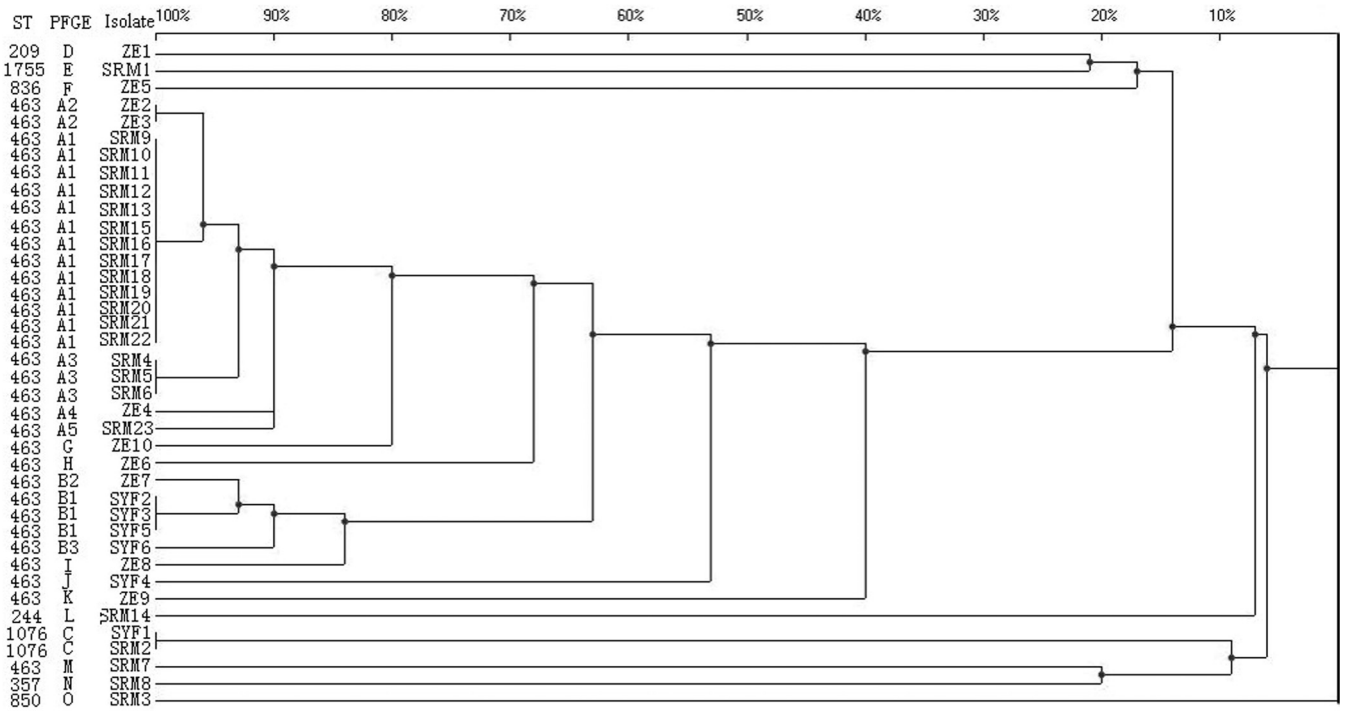

FIG 1 PFGE profile of SpeI-digested DNA from 39 KPC-producing *P. aeruginosa* isolates. An unweighted-pair group method using average linkages (UPGMA) dendrogram based on Dice similarity coefficients was generated using the UVIBand software (Bio-Rad). Eighty-five percent similarity was used as the cutoff point.

ing, as previously described (11). Twenty-six isolates were completely identical to the plasmid PE1, which was extracted from a KPC-2 carbapenemase-producing *Escherichia coli* isolate from our previous study (11). The same five major genes, encoding the Tn*3* transposase, Tn*3* resolvase, IS*Kpn*8 transposase, KPC-2, and an IS*Kpn*6-like transposase, were present in both the *P. aeruginosa* and *E. coli* isolates (Table 1). The identical bla_{KPC-2} nucleotide segments recovered in both *P. aeruginosa* and *E. coli* isolates indicates the probable transmission of bla_{KPC-2} from *Enterobacteriaceae* to *P. aeruginosa*.

For the 39 bla_{KPC}-positive isolates, the MICs were determined using Etest (bioMérieux, Marcy l'Etoile, France) for amikacin, ciprofloxacin, and gentamicin. The MICs for all other antibiotics were determined by the agar dilution method, as recommended by the CLSI (12). None of the isolates were susceptible to carbapenems, with MICs ranging from 8 mg/liter to 512 mg/liter for imipenem and 4 mg/liter to >512 mg/liter for meropenem (Table 1). All isolates were nonsusceptible to cephems and β-lactam–β-lactamase inhibitor combinations. All *P. aeruginosa* isolates were susceptible to polymyxin B, colistin, and amikacin, and 92.3% of the isolates were susceptible to gentamicin.

To investigate the molecular epidemiology of KPC-producing *P. aeruginosa* isolates, pulsed-field gel electrophoresis (PFGE) was performed as previously described but with a slight modification (13). Genomic DNA was digested using the restriction enzyme SpeI, and isolates with a Dice similarity index of ≥85% were defined as belonging to the same PFGE group (14). Multilocus sequence typing (MLST) was performed as recommended by the *P. aeruginosa* PubMLST website (http://pubmlst.org/paeruginosa/). The 39 carbapenem-resistant *P. aeruginosa* isolates were found to belong to different clones, designated clones A to O. The most prevalent clones were A (51.3% [20/39]) and B (12.8% [5/39]). The isolates of clones A and B were then divided into five subclonal groups (A1 to A5) and 3 subclonal groups (B1 to B3), respectively, on the basis of genetic similarity (Fig. 1). Isolates from clones A and B were found in several different hospitals, suggesting interhospital clonal spread. Thirty-one of the 39 carbapenem-resistant *P. aeruginosa* isolates were found to belong to sequence type 463 (ST463). The remaining eight isolates belonged to various single-sequence types, with ST1755 (11-5-5-11-4-4-7) among them being the most notable. To our best knowledge, this is the first report of the discovery of ST1755 (11-5-5-11-4-4-7). A large-scale emergence of clonally related KPC-2-producing *P. aeruginosa* ST463 isolates has never been reported elsewhere. Considering the experience given by previous reports (9, 10), the KPC-producing *P. aeruginosa* ST463 isolates we identified surely have a high chance of spreading from Hangzhou to the surrounding cities.

In summary, the present study provides the first report of the clonal spread of KPC-2-producing ST463 *P. aeruginosa* isolates. In view of the rapid emergence and transmission of the KPC-producing *P. aeruginosa* isolates in Zhejiang, China, carbapenem-resistant *P. aeruginosa* isolates should be carefully monitored, and increased care must be taken to prevent the spread of KPC-producing *P. aeruginosa* isolates in China.

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We declare no conflicts of interest.

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