




A Ceftazidime-Avibactam-Resistant and Carbapenem-Susceptible *Klebsiella pneumoniae* Strain Harboring *bla*_{KPC-14} Isolated in New York City

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ABSTRACT Ceftazidime-avibactam is a potent antibiotic combination against *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae*. Here, we describe a unique ceftazidime-avibactam-resistant and carbapenem-susceptible *K. pneumoniae* strain harboring a novel *bla*_{KPC-14} variant. This strain was isolated from a New York City patient in 2003, which predates the introduction of avibactam. Despite resistance to ceftazidime-avibactam, the strain was susceptible to imipenem-relebactam and meropenem-vaborbactam. Comprehensive genomic sequencing revealed that *bla*_{KPC-14} is harbored on an ST6 IncN plasmid associated with the early spread of *bla*_{KPC}.

IMPORTANCE KPC is currently the most common carbapenemase identified in the United States. More than 40 KPC variants have been described, of which KPC-2 and KPC-3 are the most frequent clinical variants. However, our understanding of the genetic structures and β -lactam resistance profiles of other novel KPC variants remains incomplete. Here, we report a novel *bla*_{KPC} variant (*bla*_{KPC-14}) and the complete genome sequence of *bla*_{KPC-14}-harboring *K. pneumoniae* strain BK13048, which is susceptible to carbapenems but resistant to ceftazidime-avibactam. To the best of our knowledge, this is one of the earliest KPC-producing *K. pneumoniae* strains exhibiting resistance to ceftazidime-avibactam.

KEYWORDS ceftazidime-avibactam, carbapenem, KPC, plasmid, antimicrobial resistance, selection

The rapid spread of carbapenemases among members of the *Enterobacteriaceae* family poses a major clinical concern, since it greatly limits therapeutic options. These β -lactamases are capable of hydrolyzing all generations of cephalosporins and carbapenems, the last-resort antibiotics for complicated infections with multidrug-

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TABLE 1 Susceptibilities of the strains studied to β -lactams

Strain	Description	Carbapenemase	MIC ^a (μ g/ml)								
			PIP	TZP	CRO	CAZ	ETP	MEM	IMP	ATM	CAZ-AVI
BK13048	Clinical isolate	<i>bla</i> _{KPC-14}	1,024	8	16	256	≤ 0.03	≤ 0.03	0.25	64	>16
KPC2-pET28	<i>bla</i> _{KPC-2} -harboring <i>E. coli</i> DH10B	<i>bla</i> _{KPC-2}	128	32	8	4	1	2	2	16	0.25
KPC3-pET28	<i>bla</i> _{KPC-3} -harboring <i>E. coli</i> DH10B	<i>bla</i> _{KPC-3}	128	32	8	16	1	1	2	32	0.25
KPC14-pET28	<i>bla</i> _{KPC-14} -harboring <i>E. coli</i> DH10B	<i>bla</i> _{KPC-14}	32	4	8	256	≤ 0.03	≤ 0.03	0.25	32	>16
<i>E. coli</i> DH10B			1	1		0.25	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.125	≤ 0.03

^aPIP, piperacillin; TZP, piperacillin-tazobactam; CRO, ceftriaxone; CAZ, ceftazidime; ETP, ertapenem; MEM, meropenem; IMP, imipenem; ATM, aztreonam; CAZ-AVI, ceftazidime-avibactam.

resistant Gram negative bacteria. Among the carbapenemases, *Klebsiella pneumoniae* carbapenemase (KPC), an Ambler class A serine β -lactamase, is particularly problematic, with major outbreaks in the northeastern United States, followed by its spread throughout the United States and worldwide (1). As a novel combination of a β -lactam and a β -lactamase inhibitor, ceftazidime-avibactam was highly active against KPC-producing bacteria. However, resistance to ceftazidime-avibactam has also been reported in patients who were treated with this combination, primarily due to amino acid substitutions in the KPC β -lactamase (2–4). In this study, we describe a novel KPC variant, KPC-14, isolated from *K. pneumoniae* strain BK13048, collected in 2003 from a New York City (NYC) patient. Surprisingly, this strain was both susceptible to carbapenems and resistant to ceftazidime-avibactam, a result indicating that this resistant KPC variant existed prior to 2015, when ceftazidime-avibactam was introduced.

Strain BK13048 was identified as a part of retrospective study screening of extended-spectrum cephalosporins and carbapenem-resistant *K. pneumoniae* from our archived strain collection. A molecular-beacon-based allelic discrimination real-time PCR assay (5) showed that strain BK13048 harbored a *bla*_{KPC-6-like} variant. PCR and Sanger sequencing of the full-length *bla*_{KPC} gene revealed a novel *bla*_{KPC} variant, *bla*_{KPC-14}. Nucleotide alignment of different *bla*_{KPC} variants showed that *bla*_{KPC-14} differs from *bla*_{KPC-2} by a 6-bp deletion (nucleotide positions 721 to 726), resulting in a 2-amino-acid deletion at Ambler positions 242Gly and 243Thr. KPC-28 has the same 242Gly and 243Thr deletion, but an additional His274Tyr substitution distinguishes this variant from KPC-14 (6).

Broth microdilution susceptibility testing showed that BK13048 is resistant to ceftriaxone (MIC, >16 μ g/ml), ceftazidime (MIC, >256 μ g/ml), piperacillin (MIC, >1,024 μ g/ml), aztreonam (MIC, >64 μ g/ml), and ceftazidime-avibactam (MICs, >16 and 4 μ g/ml) but susceptible to imipenem (MIC, ≤ 0.25 μ g/ml), ertapenem (MIC, ≤ 0.03 μ g/ml), and meropenem (MIC, ≤ 0.03 μ g/ml).

The MIC results from BK13048 showed an unusual profile: susceptibility to carbapenems but resistance to ceftazidime-avibactam. To investigate this finding, the full-length *bla*_{KPC-2}, *bla*_{KPC-3}, and *bla*_{KPC-14} genes and the same promoter sequences were cloned into pET28a vectors, followed by electroporation into *Escherichia coli* DH10B cells (Invitrogen). Susceptibility testing of the *bla*_{KPC-14}, *bla*_{KPC-2}, and *bla*_{KPC-3} *E. coli* DH10B constructs showed that the *bla*_{KPC-14} construct demonstrated a ceftazidime-avibactam MIC of >16 μ g/ml, which is at least 64-fold higher than that of the *bla*_{KPC-2} or *bla*_{KPC-3} construct (MICs, ≤ 0.25 μ g/ml) (Table 1). Similarly, the MIC of ceftazidime was much higher for the *bla*_{KPC-14} plasmid construct (256 μ g/ml) than for the cloned *bla*_{KPC-2} (4 μ g/ml) or *bla*_{KPC-3} (16 μ g/ml) gene.

In contrast, the MICs of the different carbapenems (ertapenem, meropenem, and imipenem) for the *bla*_{KPC-14} construct were 8- to 32-fold lower than the MICs for the *bla*_{KPC-2} or *bla*_{KPC-3} construct (Table 1). The susceptibility testing results presented above were consistent with a previous study by Oueslati et al. testing KPC-14 and KPC-28 using a different plasmid vector (pTOPO) (6). Those results demonstrated that the 242Gly and 243Thr amino acid deletions in KPC-14 decreased carbapenem activity but increased potency against ceftazidime and ceftazidime-avibactam (Table 1), and

TABLE 2 Steady-state kinetic parameters of purified KPC-2 and KPC-14 β -lactamases

β -Lactam	KPC-2			KPC-14 ^a		
	k_{cat} (s ⁻¹)	K_m (μ M)	k_{cat}/K_m (μ M ⁻¹ ·s ⁻¹)	k_{cat} (s ⁻¹)	K_m (μ M)	k_{cat}/K_m (μ M ⁻¹ ·s ⁻¹)
Meropenem	8.078	16.263	0.497	ND ^b	ND	ND
Imipenem	28.797	98.350	0.293	19.490	548.805	0.036
Ceftazidime	3.274	590.717	0.006	24.600	73.860	0.333
Aztreonam	12.601	2398.451	0.005	2.875	192.335	0.015
Cefepime	4.748	310.480	0.015	7.588	70.406	0.108
Piperacillin	7.709	793.526	0.010	1.084	45.767	0.024
Cefazolin	65.877	110.746	0.595	27.126	287.930	0.094

^aKPC-14 differs from KPC-2 by a 2-amino-acid 242Gly and 243Thr deletion.

^bND, not detectable due to a low initial rate of hydrolysis.

ceftazidime-avibactam resistance is likely due to increased activity against ceftazidime rather than reduced inhibition against avibactam.

We further characterized and compared the kinetic parameters of KPC-14 and KPC-2. In brief, the sequences without the signal peptide (from *bla*_{KPC-14} and *bla*_{KPC-2}) were obtained by PCR amplification using primers NdeI-KPC-2-F(30–293) (5'-ACGCATATGG CGGAACCATTGCTAAAC-3') and XhoI-KPC-2-R-STOPdel (5'-TAACTCGAGCTGCCCGTTG ACGCCCAAT-3'), followed by insertion into plasmid pET28a in *E. coli* DH10B (Invitrogen). The KPC enzymes were then purified, and the steady-state kinetic parameters were determined as described previously (6, 7). The results showed that KPC-14 has a higher catalytic efficiency of ceftazidime and cefepime, but a lower hydrolysis activity of imipenem, than KPC-2 (Table 2). In addition, no meropenem hydrolysis could be detected with purified KPC-14 under current conditions (measurement made over 5 min). The hydrolytic profile of KPC-14 was similar to that in the previous report from Oueslati et al. (6) and was consistent with the MIC observations presented above. Moreover, a previous experiment determining the 50% inhibitory concentrations (IC₅₀) of β -lactamase inhibitors also suggested that the 2-amino-acid 242Gly and 243Thr deletion had no impact on the inhibition properties of avibactam (6).

Additional testing of BK13048 and the *bla*_{KPC-14} plasmid construct against other novel β -lactam- β -lactamase combinations, i.e., imipenem-relebactam and meropenem-vaborbactam (by disk diffusion assay), showed that they were susceptible to imipenem-relebactam (inhibition zone diameter, >28 mm for both strains) and meropenem-vaborbactam (inhibition zone diameter, >30 mm).

To better understand the genetic structure associated with the *bla*_{KPC-14} gene, comprehensive whole-genome sequencing was performed using a combination of the Oxford Nanopore MinION and Illumina HiSeq platforms, followed by hybrid assembly using Unicycler (8). The complete sequencing of BK13048 showed that it contains a 5,213,293-bp chromosome with an average G+C content of 57.6% and harbors 5,311 predicted open reading frames. In addition, it contains seven plasmids ranging from 5 kbp to 82 kbp. *In silico* multilocus sequencing typing (MLST) revealed that BK13048 belongs to sequence type (ST) 16 (9), which has been reported to cause nosocomial infections worldwide and is associated with *bla*_{NDM-1}-encoded carbapenemase and the presence of the extended-spectrum beta-lactamase (ESBL) gene *bla*_{CTX-M-15} (10). Analysis of acquired antimicrobial resistance (11) identified 14 antimicrobial resistance genes encoding resistance to β -lactams, aminoglycosides, fluoroquinolones, fosfomycin, sulfonamide, and trimethoprim (Table 3). In addition, *in silico* plasmid replicon typing (12) indicated that the seven plasmids belong to IncA/C, F, M, N, R, ColRNAI, and a novel incompatibility group (Table 3).

The *bla*_{KPC-14} gene is located on an IncN plasmid, pBK13048_KPC14 (Table 1). pBK13048_KPC14 is 50,635 bp long with an average G+C content of 53% and carries *bla*_{KPC-14} on the Tn4401b transposon (Fig. 1). Full plasmid sequence BLAST against NCBI GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that pBK13048_KPC14 is highly similar to plasmid pKm38_N from *Klebsiella oxytoca*, which was isolated in 1997

entiate KPC variants associated with carbapenem susceptibility and ceftazidime-avibactam resistance (16).

Taken together, we identified, completely sequenced, and characterized a novel bla_{KPC} variant from *K. pneumoniae* BK13048, designated bla_{KPC-14v}, that revealed an unexpected resistance to ceftazidime-avibactam. Comprehensive sequence analysis and assembly using both the Illumina and Oxford Nanopore platforms revealed the genetic changes in bla_{KPC-14} and its plasmid structure. In contrast to other ceftazidime-avibactam-resistant bla_{KPC} variants, the bla_{KPC-14} gene was not under ceftazidime-avibactam selection pressure, as evidenced by the fact that the isolation of BK13048 predated the U.S. introduction of this novel β -lactam and β -lactamase inhibitor combination in 2015.

Accession number(s). The complete nucleotide sequence of strain BK13048 has been deposited in GenBank as accession no. CP045015 to CP045022.

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