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MECHANISMS OF RESISTANCE

Patient-to-Patient Transmission of *Klebsiella pneumoniae* Carbapenemase Variants with Reduced Ceftazidime-Avibactam Susceptibility

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ABSTRACT We report patient-to-patient transmission of *Enterobacter hormaechei* isolates with reduced susceptibility to ceftazidime-avibactam due to production of KPC-40, a variant of KPC-3 with a two-amino-acid insertion in the Ω-loop region (L167_E168dup). The index patient had received a prolonged course of ceftazidime-avibactam therapy, whereas the second patient had not received the agent and still became colonized with the KPC-40-producing strain. The complex dynamics of KPC (*Klebsiella pneumoniae* carbapenemase) described here highlight several key diagnostic and therapeutic considerations.

KEYWORDS *Enterobacter cloacae* complex, avibactam, omega loop

KPC (*Klebsiella pneumoniae* carbapenemase)-producing *Enterobacteriales* strains are endemic in hospitals in certain regions of the United States. While *Klebsiella pneumoniae* is the predominant species, KPC is increasingly produced by other Gram-negative species due to the spread of KPC enzymes carried on mobile genetic elements (1, 2). Mortality from infections caused by these organisms has been high but has improved significantly with the advent of newer β-lactam–β-lactamase combinations, including ceftazidime-avibactam, which possess activity against KPC-producing *Enterobacteriales* (3). However, use of ceftazidime-avibactam has been associated with emergence of resistance in *K. pneumoniae* isolates, which usually occurs through mutations in the bla_{KPC} gene (4). Here, we report development of a novel KPC variant conferring ceftazidime-avibactam resistance in *Enterobacter hormaechei*, a common member of the *Enterobacter cloacae* complex, with subsequent patient-to-patient transmission.

The first patient (patient B) was a 69-year-old man who was admitted to the transplant intensive care unit of a hospital in the midwestern United States in 2016 for a liver transplant. Three months later, the patient was identified as a carrier of carbapenem-resistant *K. pneumoniae* (strain 01140-2) (5). He received two courses of ceftazidime-avibactam (14 and 33 days) in late 2016 and was transferred to a rehabilitation unit in early 2017. One week after transfer, carbapenem-resistant *E. hormaechei* (strain 04408-5) was identified from a rectal screening culture. On the same day, carbapenem-resistant *K. pneumoniae* (strain 04409-2) and *E. hormaechei* (strain 04409-1) isolates were identified from the rectal screening culture of a second patient (patient H) who was staying in the same rehabilitation unit. Patient H, a 63-year-old woman, did

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KPC-2       MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYR       60
KPC-3       MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYR       60
KPC-31      MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYR       60
KPC-25      MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYR       60
KPC-40      MSLYRRLVLLSCLSWPLAGFSATALTNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYR       60
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KPC-2       AEERFPLCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAE       120
KPC-3       AEERFPLCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAE       120
KPC-31      AEERFPLCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAE       120
KPC-25      AEERFPLCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAE       120
KPC-40      AEERFPLCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPISEKYLTTGMTVAE       120
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KPC-2       LSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFRLDRWELE--LNSAIPGDARD       178
KPC-3       LSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFRLDRWELE--LNSAIPGDARD       178
KPC-31      LSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFRLDRWELE--LNSAIPGDARY       178
KPC-25      LSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFRLDRWELELELNSAIPGDARD       180
KPC-40      LSAAAVQYSDNAAANLLLKELGGPAGLTAFMRSIGDTTFRLDRWELELELNSAIPGDARD       180
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KPC-2       TSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCG       238
KPC-3       TSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCG       238
KPC-31      TSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCG       238
KPC-25      TSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCG       240
KPC-40      TSSPRAVTESLQKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADWAVGDKTGTCG       240
            ************************************************************

KPC-2       VYGTANDYAVVWPTGRAPIVLAVYTRAPNKDDKHSEAVIAAAARLALEGLGVNGQ            293
KPC-3       VYGTANDYAVVWPTGRAPIVLAVYTRAPNKDDKYSEAVIAAAARLALEGLGVNGQ            293
KPC-31      VYGTANDYAVVWPTGRAPIVLAVYTRAPNKDDKYSEAVIAAAARLALEGLGVNGQ            293
KPC-25      VYGTANDYAVVWPTGRAPIVLAVYTRAPNKDDKHSEAVIAAAARLALEGLGVNGQ            295
KPC-40      VYGSANDYAVVWPTGRAPIVLAVYTRAPNKDDKYSEAVIAAAARLALEGLGVNGQ            295
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FIG 1 Alignment of relevant KPC variants. KPC-31 and KPC-40 confer reduced susceptibility to ceftazidime-avibactam and susceptibility to meropenem. The phenotype conferred by KPC-25 is not known. The signal peptide is underlined, and the Ω-loop region is boxed.

not develop infection and did not receive any antibiotic, including ceftazidime-avibactam, during her stay in the hospital. The two patients overlapped for 5 days in the rehabilitation unit.

The *K. pneumoniae* isolates from patients B and H were sequenced on the Illumina NextSeq platform (GenBank accession no. QRBQ00000000.1 and QRBO00000000.1). Comparative analysis showed that both isolates belonged to sequence type (ST) 258, and there were only two core genome single-nucleotide polymorphisms (SNPs) that separated the isolates from one another, as assessed by Snippy (https://github.com/tseemann/snippy). Comparative genome analysis of the *E. hormaechei* isolates from each patient showed that they both belonged to ST407 and had one core genome SNP that separated them from one another (GenBank accession no. QRBS00000000.1 and QRBR00000000.1). ST258 is the globally epidemic *K. pneumoniae* lineage that is often associated with KPC production (6), and *E. hormaechei* ST407 has been associated with production of the CTX-M-15 β-lactamase but not KPC (7). All four strains were bla_{KPC} positive by PCR (8). Examination of KPC sequences from the Illumina data showed that the *K. pneumoniae* isolate from patient B carried bla_{KPC} encoding KPC-31 (GenBank accession no. NG_055494.1), whereas the *K. pneumoniae* isolate from patient H carried bla_{KPC} encoding KPC-3. KPC-31 has an aspartic acid-to-tyrosine (D179Y) substitution at position 179 in the Ω-loop of KPC-3, which is known to confer resistance to ceftazidime-avibactam (Fig. 1) (4). The two *E. hormaechei* isolates, on the other hand, carried bla_{KPC} encoding KPC-40 (GenBank accession no. WP_115470049.1), which contains a duplication of leucine and glutamic acid residues between positions 168 and 169 (L167_E168dup), also located in the Ω-loop of the KPC enzyme. This duplication is seen in KPC-25 (GenBank accession no. NG_051167.1) as well, but its phenotype has not yet been reported. KPC-40 has a threonine-to-serine substitution at position 237 (T237S) located in the oxyanion hole compared with KPC-3. Previous site-saturation mutagenesis experiments suggested that this substitution specifically maintains kinetic activities of the KPC enzyme against β-lactams, including carbapenems (9).

MICs of representative antimicrobial agents were obtained by broth microdilution

TABLE 1 MICs of the KPC-producing strains

Antimicrobial agent	MIC (μg/ml) in:			
	Patient B for:		Patient H for:	
	K. pneumoniae 01140-2 (KPC-31, ST258)	*E. hormaechei* 04408-5 (KPC-40, ST407)	*K. pneumoniae* 04409-2 (KPC-31, ST258)	*E. hormaechei* 04409-1 (KPC-40, ST407)
Ceftazidime-avibactam[a]	64	16	4	8
Ertapenem	>4	4	>4	2
Imipenem	>8	≤1	>8	≤1
Meropenem	>8	≤1	>8	≤1
Cefotaxime	>32	>32	>32	>32
Ceftazidime	>16	>16	>16	>16
Cefepime	>16	8	>16	8
Aztreonam	>16	>16	>16	>16
Piperacillin-tazobactam	>64/4	>64/4	>64/4	>64/4
Ciprofloxacin	>2	≤0.25	>2	≤0.25
Levofloxacin	>8	≤1	>8	≤1
Gentamicin	≤1	≤1	≤1	≤1
Amikacin	16	≤4	16	≤4
Tobramycin	>8	≤1	>8	≤1
Doxycycline	>16	16	>16	16
Tigecycline	1	2	1	2
Colistin	≤0.25	≤0.25	≤0.25	≤0.25

[a]The breakpoint for ceftazidime-avibactam approved by the Clinical and Laboratory Standards Institute is ≤8/4 μg/ml for susceptible and ≥16/4 μg/ml for resistance.

using Sensititre GNX2F plates (Thermo). Ceftazidime-avibactam MICs were obtained by manual broth microdilution. The two *E. hormaechei* isolates producing KPC-40 showed reduced susceptibility to ceftazidime-avibactam and collateral susceptibility to carbapenems (Table 1).

To determine the impact of the L167_E168dup variant observed in KPC-40, isogenic mutants of KPC-3 and KPC-40 were constructed. The bla_{KPC} genes were amplified with primers KPC_P2_fwd_SpeI (5′-CCGACTAGTAAAATTCCAAACCCGAATGATCC-3′) and KPC_rev_EcoRI (5′-CCGGAATTCTTACTGCCCGTTGACGCCCAAT-3′) and cloned into pBCSK(-) (Agilent, Santa Clara, CA). *Escherichia coli* TOP10 cells were transformed with each construct, and transformants were selected using ampicillin 50 μg/ml and chloramphenicol 30 μg/ml. The bla_{KPC} sequences in the transformants were confirmed by Sanger sequencing. As expected, production of KPC-40 conferred reduced ceftazidime-avibactam susceptibility with an MIC of 64 μg/ml, compared with an MIC of 1 μg/ml observed with production of KPC-3 (the MIC of the recipient with an empty vector was 0.25 μg/ml). We also generated a transformant carrying bla_{KPC} that encoded L167_E168dup but not T237S. This transformant had a ceftazidime-avibactam MIC of 8 μg/ml, which confirmed the role of the L167_E168dup variant in reduced ceftazidime-avibactam susceptibility, although the concomitant T237S substitution was necessary for resistance. Certain disruptions of the Ω-loop are known to increase the flexibility of the structure, leading to improved binding of ceftazidime to the enzyme-active site (10).

The complete sequence of the plasmid carrying bla_{KPC-40} in *E. hormaechei* 04408-5 was determined by sequencing its genome on the MinION platform (Oxford Nanopore Technologies, Oxford Science Park, UK). Hybrid assembly of Illumina and MinION reads was performed using Unicycler v0.4.6 with default parameters (11). The resulting plasmid, p04408-5-KPC40, was 55,076 bp in size and carried the N replicon (GenBank accession no. MK862125). p04408-5-KPC40 was highly similar to pKm38_N (GenBank accession no. KY128483.1), a 69-kb N plasmid encoding KPC-2 and harbored by *Klebsiella michiganensis* strain Km97_38 isolated in 1997 (12). However, in addition to the differences in the bla_{KPC} gene, a 17-kb region upstream of bla_{KPC-2} containing resistance genes *aac(6′)-Ib*, *aadA1*, bla_{OXA-9}, bla_{TEM-1}, *strB*, *strA*, and *sul2* in pKm38_N was replaced by a 3.5-kb section containing a Tn*3*-family transposon Tn*5403*; and, as a result, p04408-5-KPC40 only carried bla_{KPC-40} and *dfrA14* resistance genes.

Likewise, the bla_{KPC} genes encoding KPC-3, KPC-31, and KPC-40 across the studied strains were all carried on N plasmids based on PCR of *E. coli* transformant strains,

suggesting a likely shared origin (5). We speculate that KPC-31 (*K. pneumoniae* strain 01140-2) and KPC-40 (*E. hormaechei* strain 04408-5) evolved from KPC-3 under selective pressure from prolonged ceftazidime-avibactam therapy in patient B, and the *E. hormaechei* strain was then transmitted to patient H (*E. hormaechei* strain 04409-1). As for the *K. pneumoniae* 04409-2 strain in patient H, it is equally plausible that KPC-31 reverted to KPC-3 in the absence of ceftazidime-avibactam exposure or that patient B harbored a mixed population of *K. pneumoniae* isolates producing both KPC-3 and KPC-31, the former of which was acquired by patient H.

The complex dynamics of KPC described here highlight several key diagnostic and therapeutic considerations at a time when active screening for KPC-producing *Enterobacteriales* isolates is increasingly utilized, and ceftazidime-avibactam is considered the standard of care for the treatment of infections caused by these organisms. Ceftazidime-avibactam susceptibility should always be tested when the agent is used, but the risk of resistance increases when KPC-producing strains are isolated from a patient with previous exposure to this agent or when such strains are expected yet reported as extended-spectrum β-lactamase producers (13). Furthermore, in the setting of possible hospital transmission, such organisms recovered from patients who have not been exposed to ceftazidime-avibactam should also be tested for susceptibility to this agent when its use is considered.

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