



# KPC-53, a KPC-3 Variant of Clinical Origin Associated with Reduced Susceptibility to Ceftazidime-Avibactam

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**ABSTRACT** This study reports on the characterization of a *Klebsiella pneumoniae* clinical isolate showing high-level resistance to ceftazidime-avibactam associated with the production of KPC-53, a KPC-3 variant exhibiting a Leu167Glu168 duplication in the  $\Omega$ -loop and a loss of carbapenemase activity. Whole-genome sequencing (WGS) revealed the presence of two copies of *bla*<sub>KPC-53</sub>, located on a pKpQIL-like plasmid and on a plasmid prophage of the *Siphoviridae* family, respectively. The present findings provide new insights into the mechanisms of resistance to ceftazidime-avibactam.

**KEYWORDS** CZA resistance, carbapenem-resistant *Enterobacterales*, mutant KPC carbapenemase, phagemid

Ceftazidime-avibactam (CZA) is a novel beta-lactam/beta-lactamase inhibitor combination recently introduced into clinical practice. The spectrum of activity includes some carbapenemase-producing *Enterobacterales* (CPE), such as KPC-producing strains of *Klebsiella pneumoniae* (KPC-Kp), which are of notable concern due to the high level of endemicity observed in several areas worldwide and the spectrum and severity of infections that they can cause (1).

Although resistance to CZA among KPC-Kp isolates was reported at very low rates in large surveillance studies (2, 3), acquired resistance to CZA in KPC-Kp by several mechanisms has been reported, including missense mutations or deletions in the  $\Omega$ -loop of the KPC  $\beta$ -lactamase and permeability defects (i.e., alterations in OmpK35/36 and upregulation of the AcrAB efflux system) coupled with an increased expression of KPC- or even SHV-type  $\beta$ -lactamases (2, 4, 5).

Here, we report on the identification of a novel *bla*<sub>KPC-3</sub> variant, named *bla*<sub>KPC-53</sub>, from a CZA-resistant KPC-Kp isolate belonging to sequence type 512 (ST512).

In November 2018, an Italian citizen (male, 69 years old) was admitted to the intensive care unit (ICU) of a neurorehabilitation hospital in Lombardy (Northern Italy) for an intracerebral hemorrhage. The patient came from home and had no recent history of contact with the health care system. Screening for CPE at admission, by culture of a rectal swab on MacConkey agar plus a meropenem disk (10  $\mu$ g) (6), was negative. During the initial ICU stay, the patient received a brief course of piperacillin-tazobactam (4.5 g three times a day [t.i.d.] for 5 days) for a febrile episode and a dose of cefazolin (2 g) as prophylaxis for a device insertion. After 22 days, the patient was febrile, and empirical antibiotic treatment with piperacillin-tazobactam (4.5 g t.i.d.) and gentamicin (3 mg/kg of body weight per day) was started. The urine culture (from a catheter) was positive for a *K. pneumoniae* isolate (LC-1825/18) showing a multidrug resistance (MDR) phenotype, including fluoroquinolones, expanded-spectrum cephalosporins, ertapenem, piperacillin-tazobactam, and CZA, by testing with the Phoenix

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**TABLE 1** MICs for *K. pneumoniae* LC-1825/18, *E. coli* DH10B(pIT-1825-FIIK1), and *E. coli* DH10B

Antibiotic	MIC ( $\mu\text{g/ml}$ ) (category) <sup>a</sup>		
	LC-1825/18	DH10B (pIT-1825-FIIK1)	DH10B
Ceftazidime	512 (R)	32 (R)	0.25 (S)
Ceftazidime-avibactam	64/4 (R)	8/4 (S)	0.25/4 (S)
Cefepime	16 (R)	1 (S)	$\leq 0.125$ (S)
Ertapenem	8 (R)	0.03 (S)	0.015 (S)
Meropenem	4 (I)	0.06 (S)	0.03 (S)
Meropenem-vaborbactam	2 (S)	$\leq 0.125$ (S)	$\leq 0.125$ (S)
Imipenem	2 (S)	0.5 (S)	0.25 (S)
Piperacillin-tazobactam	>128/4 (R)	$\leq 2/4$ (S)	$\leq 2/4$ (S)
Ciprofloxacin	>8 (R)	0.125 (S)	$\leq 0.0625$ (S)
Amikacin	8 (S)	16 (R)	$\leq 4$ (S)
Gentamicin	2 (S)	1 (S)	1 (S)
Trimethoprim-sulfamethoxazole	>8/152 (R)	4/76 (S)	$\leq 1/19$ (S)
Tigecycline	1 <sup>b</sup>	$\leq 0.125$ (S)	$\leq 0.125$ (S)
Fosfomicin	>128 (R)	ND	ND
Colistin	>8 (R)	$\leq 1$ (S)	$\leq 1$ (S)

<sup>a</sup>S, susceptible; R, resistant; I, susceptible with high exposure; ND, not determined.

<sup>b</sup>No EUCAST breakpoints (V10.0) for *K. pneumoniae* are available.

automated system (BD Diagnostics, Sparks, MD, USA). The production of a KPC-type enzyme was confirmed by a lateral flow immunochromatographic assay (LFIA) (Resist-5 O.O.K.N.V.; Coris BioConcept, Belgium) (7). Treatment was not modified, considering the result related to colonization of the urinary catheter. The patient died after 47 days of hospitalization due to a worsening of neurological conditions.

To investigate the mechanism responsible for CZA resistance, *K. pneumoniae* LC-1825/18 was subjected to further phenotypic and genotypic characterization. Antimicrobial susceptibility was determined by broth microdilution using custom plates (Merlin, Germany) and by agar dilution for fosfomicin (8), and data were interpreted according to the EUCAST V10.0 clinical breakpoints ([https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_tables/v\\_10.0\\_Breakpoint\\_Tables.pdf](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf)). Whole-genome sequencing (WGS) was performed using the Illumina (San Diego, CA, USA) MiSeq and the Oxford Nanopore Technologies (Oxford, UK) MinION platforms, as previously described (5). Gene transfer experiments were carried out as previously described (5).

The MDR profile of LC-1825/18 was confirmed, including also fosfomicin and colistin. The CZA MIC was 64/4  $\mu\text{g/ml}$ . The isolate was susceptible to amikacin, gentamicin, imipenem, meropenem (at increased exposure), and meropenem-vaborbactam (Table 1).

The hybrid genome assembly resulted in six complete circular molecules, including the chromosome and five extrachromosomal elements, namely, two large FII-FIB replicons, one X3 replicon, one small ColE replicon, and one 120-kb prophage plasmid related to the *Siphoviridae* family (Table 2).

Sequence analysis of the LC-1825/18 chromosome confirmed the identification as *K. pneumoniae sensu stricto* and revealed that the isolate belonged to ST512, a high-risk clone that during the past decade has greatly contributed to the global dissemination of KPC-Kp worldwide and, at present, represents one of the most common clonal lineages circulating in Italy (9). Further analysis showed that LC-1825/18 carried the chromosomal *bla*<sub>SHV-11</sub> gene, typical of this *K. pneumoniae* lineage, and a nonfunctional copy of the *mgrB* gene (inactivated by the insertion of an IS*Kpn26* element at nucleotide [nt] 74), which accounted for the colistin resistance phenotype (10). Investigation of the status of the major porins showed a truncated OmpK35 (AA89-STOP) and a mutated OmpK36 with a 2-amino-acid insertion (Gly134Asp135) previously demonstrated to result in a constricted porin channel contributing to a decreased susceptibility to CZA (11).

**TABLE 2** Genetic features of *K. pneumoniae* LC-1825/18

Genetic element	Size (bp)	Replicon(s)	Resistance trait(s)	GenBank accession no.	Closely related element (GenBank accession no., % query coverage, % identity) <sup>a</sup>
Chromosome	5,341,124		<i>bla</i> <sub>SHV-11</sub> <i>ompK35Δ</i> <i>mgrBΔ</i> <i>OmpK36</i> <sub>Gly134Asp135</sub> <i>GyrA</i> <sub>Ser83Ile</sub> <i>ParC</i> <sub>Ser80Ile</sub>	CP058325	
Plasmids					
pIT-1825-FIIK2	205,143	FII <sub>K2</sub> -FIB <sub>K</sub>	<i>dfrA12</i> <i>ant(3')-Ia</i> <i>aph(3')-Ia</i> <i>catA1</i> <i>mph(A)</i> <i>sul1</i>	CP058326	pAUSMDU00008079_01 (CP022692.2, 94, 100)
pIT-1825-FIIK1	120,302	FII <sub>K1</sub> -FIB	<i>bla</i> <sub>KPC-53</sub> <i>bla</i> <sub>TEM-1</sub> <i>aac(6')-Ib</i>	CP058327	pKpQIL-IT (JN233705.2, 84, 99.95)
pIT-1825-X3	43,380	X3	<i>bla</i> <sub>SHV-11</sub>	CP058328	plncX-SHV (JN247852.1, 100, 100)
ColE-1825	13,636	ColE	<i>aac(6')-Ib</i>	CP058329	ColE-LS6 (JX442973.1, 100, 99.99)
Bacteriophage					
vB_Kpn_1825-KPC53	119,621		<i>bla</i> <sub>KPC-53</sub>	CP058330	<i>Klebsiella</i> phage ST13-OXA48phi12.3 (MK422451.1, 69, 99.74) <i>Salmonella</i> phage SSU5 (JQ965645.1, 46, 78.16)

<sup>a</sup>BLAST results indicating the coverage (percent) of the query sequence and the corresponding identity (percent) at the nucleotide level.

Screening for acquired resistance determinants revealed genes for aminoglycoside-modifying enzymes; sulfonamide, trimethoprim, macrolide, and phenicol resistance; and several  $\beta$ -lactamases, including SHV-11, TEM-1, and a novel KPC-3 derivative that was named KPC-53 (GenBank accession number [MUT87649.1](#)). The acquired resistance determinants were variably distributed among the strain's accessory genome (Table 2). In particular, the *bla*<sub>KPC-53</sub> gene was associated with a typical *Tn4401a* transposon present in two copies, one carried by the pIT-1825-FIIK1 plasmid, which was highly similar to the archetypal pKpQIL plasmid (GenBank accession number [NC\\_014016](#)), and the other carried by the vB\_Kpn\_1825-KPC53 plasmid prophage (see Fig. S1 in the supplemental material). In the latter element, *Tn4401a* was inserted into the gene coding for the tail tip assembly protein L. The localization of *bla*<sub>KPC-53</sub> on a plasmid prophage related to those of the *Siphoviridae* family, circulating among *Enterobacterales*, provided new insights into the type of mobile genetic elements associated with this resistance gene and suggested a potential contribution of these elements to its dissemination (12, 13). Additional studies are needed to assess the horizontal transfer capabilities of phage vB\_Kpn\_1825-KPC53.

Compared to KPC-3, KPC-53 exhibits a 2-amino-acid duplication (Leu167Glu168) in the  $\Omega$ -loop, previously detected in KPC-25, a KPC-2 derivative (GenBank accession number [NG\\_051167.1](#)), and in KPC-40, a KPC-3 derivative which additionally contains a Thr237Ser substitution (14) (Fig. S2). While KPC-40 was shown to confer reduced susceptibility to CZA, the activity of KPC-25 has not been evaluated so far. Interestingly, unlike other KPC  $\Omega$ -loop mutants such as KPC-31 or KPC-33 (15, 16), KPC-53 production was detected by the LFIA, as also recently shown for a KPC-3 natural variant carrying a Leu167Glu168 deletion (17). These findings suggested that positions 167 and 168 in KPC are not critical for correct enzyme immunodetection by the LFIA.

Experiments to mobilize *bla*<sub>KPC-53</sub> to *Escherichia coli* by conjugation were unsuccessful, consistent with the presence of a deletion ( $\Delta$ *traMJYAL*) within the transfer operon of pIT-1825-FIIK1. The transfer of the latter plasmid was achieved by the electrotransformation of total DNA from LC-1825/18 into *E. coli* DH10B, using ceftazidime (4 mg/liter) for selection. PCR mapping experiments confirmed the presence of pIT-1825-FIIK1 in the transformants, and the identity of the *bla*<sub>KPC</sub> allelic variant was confirmed by Sanger sequencing. DH10B(pIT-1825-FIIK1) showed reduced susceptibility to CZA, with an MIC 32-fold higher than that of the isogenic DH10B host, while it retained susceptibility to carbapenems (with just a 2-fold MIC increase), cefepime, and piperacillin-tazobactam (Table 1), supporting a significant role of the Leu167Glu168 duplication in modifying the kinetic properties of the KPC enzyme. Therefore, the

relatively high carbapenem MICs of LC-1825/18 (2, 4, and 8  $\mu\text{g}/\text{ml}$  for imipenem, meropenem, and ertapenem, respectively) were likely contributed by the nonfunctional status of major porins rather than by a low residual activity of the mutated KPC enzyme.

Given the features of KPC-53, it is plausible that the increased *bla*<sub>KPC-53</sub> gene dosage coupled with porin alterations could confer high-level resistance to CZA in LC-1825/18, as previously observed in similar cases (4, 5). On the other hand, the reduced carbapenemase activity of KPC-53 could affect its detection by phenotypic tests for CPE, thus raising relevant implications for diagnostic and surveillance purposes. The present findings underscore the need for CZA susceptibility testing when its use is considered since the use of rapid molecular tests or LFIA might yield unreliable genotype-phenotype correlations if mutated genes are present (15).

Overall, the present results were consistent with those previously obtained by studies with a KPC-40 laboratory mutant corresponding to KPC-53 and with a KPC variant resembling KPC-53 from an uncharacterized *K. pneumoniae* strain of clinical origin, cloned into laboratory vectors (i.e., pBC-SK and pET30a) to evaluate the impact on CZA susceptibility (14, 18). In those cases, however, the influence that the high copy number of artificial plasmid vectors may have on the *bla*<sub>KPC</sub> gene dosage was not evaluated, and the possible contribution of the host genetic background (e.g., status of porins) was not discussed while investigating CZA resistance.

Interestingly, while it was previously speculated that KPC-40 might have evolved from KPC-3 under selective pressure from prolonged CZA therapy (14), no exposure to CZA was documented in this case, suggesting that the strain expressing KPC-53 might have been transmitted to the patient in the hospital setting. In fact, during the period between patient admission and the isolation of LC-1825/18, no additional KPC-Kp strains were isolated from inpatients of the same ICU, either from clinical or from surveillance specimens (all patients were routinely screened for CRE colonization at admission by culturing of rectal swabs on MacConkey agar plus a meropenem disk). However, we cannot exclude that the detection of colonization by KPC-Kp producing KPC-53 in other patients was missed due to the reduced carbapenemase activity of this enzyme, as previously observed with KPC-Kp producing KPC-31 (15). In this perspective, the use of culture media that do not include carbapenems as selective agents, such as SuperCAZ/AVI (19), could represent a valuable option to screen for the carriage of CZA-resistant KPC-Kp strains.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, PDF file, 0.3 MB.

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