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Outbreak of *Klebsiella pneumoniae* ST11 Resistant To Ceftazidime-Avibactam Producing KPC-31 and the Novel Variant KPC-115 during COVID-19 Pandemic in Argentina

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ABSTRACT We describe an outbreak of *Klebsiella pneumoniae* sequence type 11 (ST11) producing KPC variants resistant to ceftazidime-avibactam. Six patients hospitalized in the intensive care unit (mostly due to critical COVID pneumonia) presented infection or colonization by this bacterium. They had several comorbidities and required mechanical ventilation, central venous catheters, and urinary catheters. All 6 patients had a history of fecal colonization with KPC-producing *Enterobacterales* (KPC-E). Three of them had previous episodes of infection with ceftazidime-avibactam-susceptible KPC-producing *K. pneumoniae*, which were treated with ceftazidime-avibactam. Several phenotypic methods failed to detect carbapenemase production in these 6 ceftazidime-avibactam-resistant isolates, and they showed *in vitro* susceptibility to imipenem and meropenem. All of them rendered positive results for bla_{KPC} by PCR, and amplicon sequencing identified bla_{KPC-31} variant in 5 isolates and a novel variant, named $bla_{KPC-115}$, in the other. Moreover, matrix-assisted laser desorption ionization–time of flight mass spectrometry was able to detect KPC in all isolates. Ceftazidime-avibactam-resistant isolates, as well as those recovered from previous infection episodes (KPC-3-producing *K. pneumoniae*, ceftazidime-avibactam susceptible), displayed a unique pulse type and belonged to ST11. Based on whole-genome sequencing results of selected isolates, less than 7 single-nucleotide polymorphisms were identified among them, which was indicative of the presence of a unique clone. Both *in vivo* selection and horizontal transmission seemed to have occurred in our hospital. Detection of these strains is challenging for the laboratory. History of previous KPC-E infections or colonization and systematic testing for resistance to ceftazidime-avibactam might help raise awareness of this possibility.

IMPORTANCE *Klebsiella pneumoniae* is one of the main bacteria that cause infections in health care settings. This pathogen has developed a high level of resistance to many antibiotics. Some *K. pneumoniae* isolates can produce an enzyme known as carbapenemase KPC, making carbapenems (considered the last line for therapy) not effective to treat their infections. The combination ceftazidime-avibactam, approved by FDA in 2015, is useful to treat infections caused by KPC-producing *K. pneumoniae*. This study describes the emergence, in one hospital in Argentina, of *K. pneumoniae* isolates that produce KPC variants (KPC-31 and KPC-115) resistant to ceftazidime-avibactam. The ceftazidime-avibactam-resistant bacteria were isolated in inpatients, including some that previously received this combination as treatment. Transmission of this strain to other patients also occurred in the studied period. Detection of these bacteria is challenging for the laboratory. The knowledge and awareness of the emergence of this pathogen in our region are highly valuable.

Resistance to carbapenems in *Enterobacterales*, mainly associated with carbapenemase production, has significantly increased worldwide in the last decade (1, 2). Although carbapenemases include a wide range of β-lactamase families, KPC, NDM, and OXA are the most prevalent (2–5). Infections caused by carbapenemase-producing *Enterobacterales* (CPE) have limited antimicrobial options (1, 6). In recent years, new β-lactam/β-lactamase combinations, such as ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam, have been introduced into clinical use displaying excellent activity against class A carbapenemase producers (1, 7, 8). The Infectious Diseases Society of America has recently recommended these new combinations for the treatment of severe CPE infections (9). Ceftazidime-avibactam was the first of these new combinations to be introduced for the treatment of KPC-producing *Enterobacterales* (KPC-E). Not surprisingly, ceftazidime-avibactam resistance was described soon after its introduction in KPC-producing *Klebsiella pneumoniae* isolates (KPC-Kp) (10–12). Resistance to ceftazidime-avibactam in KPC-E, still reported at very low rates in different studies, may be mediated by several mechanisms, such as missense mutations or deletions in the Ω-loop of the carbapenemase or permeability defects coupled with augmented β-lactamase expression (12–15). At present, more than 100 variants of KPC carbapenemase have been reported, some of them associated with resistance to ceftazidime-avibactam.

In this study, we describe an outbreak in an intensive care unit (ICU) by *K. pneumoniae* sequence type 11 (ST11) producing KPC-31 and KPC-115. These bacteria displayed resistance to ceftazidime-avibactam, but *in vitro* susceptibility to meropenem or imipenem was observed. To our knowledge, neither KPC-31 nor the novel variant KPC-115 has been previously described in Argentina or even in Latin America.

RESULTS

The patients herein included underwent the usual long-term hospitalization at an ICU described worldwide for severe COVID pneumonia, followed by several hospital-acquired infections requiring different antimicrobial treatments. Fecal colonization and even infections by KPC-Kp occurred in these patients, and ceftazidime-avibactam was used as part of the treatment in 3 of them. Patients' main epidemiological characteristics, as well as antibiotic treatments and outcomes, are summarized in Table 1. Three patients had bacteremia (patients 3, 5, and 6), 1 urinary tract infection (patient 4), and 2 had no infection (patients 1 and 2) but colonization in the tracheal aspirate and central venous catheter tip.

The features of *K. pneumoniae* isolates, antimicrobial susceptibilities, and carbapenemase detection are outlined in Table 2. Six out of 15 isolates displayed resistance to ceftazidime-avibactam but susceptibility to imipenem and meropenem, while the remaining 9 were susceptible to ceftazidime-avibactam and resistant to imipenem and meropenem. All isolates were resistant to colistin, trimethoprim-sulfamethoxazole, and ciprofloxacin but susceptible to gentamicin, amikacin, and fosfomycin. MIC values for tigecycline were 2 to 4 mg/L (susceptible/intermediate). Ceftazidime-avibactam-resistant *K. pneumoniae* isolates rendered negative results for carbapenemase production in the double disc synergy test, BlueCarba assay, and modified Hodge test. Among the latest, lateral flow immunochromatographic assay (LFIA) was only positive in *K. pneumoniae* 5F. The matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based procedure was able to detect KPC in all isolates, including those resistant to ceftazidime-avibactam. Accordingly, multiplex PCR confirmed *bla*$_{KPC}$ presence and ruled out other common carbapenemase genes, such as *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, or *bla*$_{OXA-group-48}$. The 9 ceftazidime-avibactam-susceptible isolates presented *bla*$_{KPC-3}$. On the other hand, in the ceftazidime-avibactam-resistant isolates, *bla*$_{KPC-31}$ was detected in 5 isolates and a novel variant called *bla*$_{KPC-115}$ (GenBank accession number OM714909) in the remaining 1 isolate (Table 2). This new variant displayed a 6-nucleotide deletion translated into a 2-amino-acid deletion (absence of Asp169 and Ser170) and 1 substitution (Leu168Pro) compared to KPC-3.

TABLE 1 Patients' clinical features[a]

Patient	Gender/ age	Date of hospitalization	Severe COVID pneumonia	Positive rectal cultures for KPC-E	Infection with Kp-KPC in preceding mo	Previous use of CZA	Main treatment for the CZA-resistant episode	Outcome
1	M/53	03-21-2021	Y	Y	Y	Y	No atb. catheter removal	Discharge
2	M/47	05-27-2021	Y	Y	N	N	No atb. colonization	Discharge
3	F/71	05-28-2021	Y	Y	N	N	MEM + AMK	Discharge
4	M/46	05-22-2021	Y	Y	N	N	AMK	Discharge
5	F/36	05-21-2021	Y	Y	Y	Y	MEM + AMK	Death[b]
6	M/73	06-07-2021	N	Y	Y	Y	CZA + AMK, then MEM + AMK + FOF	Discharge

[a]F, female; M, male; Y, yes; N, no; CZA, ceftazidime-avibactam; No atb., no antibiotics were administered for these episodes because either removal of catheter or colonization was assumed; MEM, meropenem; AMK, amikacin; FOF, fosfomycin; KPC-E, KPC-producing *Enterobacterales*; KPC-Kp, KPC-producing *Klebsiella pneumoniae*. Both KPC-E colonization and KPC-Kp were considered in the preceding month of CZA-resistant isolation.
[b]Death occurred more than 30 days after CZA-resistant infection, being nonrelated to it.

All isolates displayed a unique pulse type and belonged to ST11, regardless of the KPC variant and ceftazidime-avibactam or carbapenem susceptibilities. In all isolates, the genetic context of bla_{KPC} belonged to Tn*4401a*. Based on WGS results, isolates 1A, 1C, 5B, and 5F belonged to ST11 and presented less than 7 single-nucleotide polymorphisms (SNPs) among them, corresponding to a unique clone. Moreover, isolates recovered from the same patient, 1A (ceftazidime-avibactam-susceptible) versus 1C (ceftazidime-avibactam-resistant), displayed 3 SNPs, while 5B (ceftazidime-avibactam-susceptible) versus 5F (ceftazidime-avibactam-resistant) displayed 4 SNPs.

In furtherance, the same acquired markers responsible for quinolone resistance [GyrA-83I, GyrA-87N, ParC-80I, *OqxA*, *OqxB*, *aac(6')-Ib-cr*], aminoglycosides [*aac(6')-Ib-cr*, *aadA2*, *aadA*, *aph3-Ia*], and sulfonamides (*sul1*, *sul3*) were detected in all genomes. With respect to colistin resistance, plasmid-encoded *mcr* markers were not detected in any isolate. Moreover, wild-type alleles were observed in *mgrB* and *pmrCAB* operons. However, PhoP displayed 1 substitution (Arg34Lys) and in PhoQ multiple substitutions were observed (Lys64Arg, Lys92Gln, Thr106Ala, Asp112Glu, Val139Ile, Phe163Leu, Ile198Val, Ser372Thr, Pro424Gln, Leu482Gln, and Glu487Gln). These findings will be the subject of future studies. The capsular *loci* belonged to *wzi* 39, K_Locus KL39, and O_Locus O3b. The Yersiniabactin coding gene in ICEKp10 (YbST: 22-2LV) and colibactin *clb3* (CbST: 13-2LV) were detected.

DISCUSSION

We report on an outbreak of KPC-Kp ST11 in the ICU of a private university hospital in Buenos Aires, Argentina, during the COVID pandemic. This episode was characterized by the emergence of ceftazidime-avibactam-resistant isolates. *In vivo* selection of these isolates was inferred in 3 patients (patients 1, 5, and 6) after ceftazidime-avibactam use. This resistant profile was mediated by modifications in KPC-3 (found in previous isolates in these patients), which resulted in KPC-31 and the novel variant KPC-115. Recently, Arcari et al. (16) described an interplay between KPC-3 and KPC-31 in patients with COVID that were treated with ceftazidime-avibactam, which was similar to the situation observed in our hospital. Additionally, some ceftazidime-avibactam-resistant isolates were recovered from patients where ceftazidime-avibactam was never used (patients 2, 3, and 4). We could therefore infer that interpatient transmission also occurred. These findings are consistent with previous reports (14).

Upon outbreak suspicion, the ICU was placed on epidemiologic alert to reinforce infection control measures for carbapenemases carriage and restrict the use of ceftazidime-avibactam. These actions succeeded in containing the outbreak, since no other KPC-producing bacteria resistant to ceftazidime-avibactam were detected after July 2021. Infection episodes were treated with antibiotics with apparent *in vitro* activity according to their MIC, such as meropenem and amikacin. No clinical or microbiological failures were noted, which is consistent with previous similar reports (14, 16–19). Patients colonized with ceftazidime-avibactam-resistant isolates (patients 1 and 2) received no antibiotic treatment for these instances.

The detection and report of these ceftazidime-avibactam-resistant KPC variants present a great challenge. As previously described, these isolates mostly appear "susceptible" to imipenem and meropenem, showing an extended-spectrum β-lactamase-like phenotype (20–22).

TABLE 2 Bacterial features, β-lactam susceptibilities, results of carbapenemase-detection methods, and KPC variants[a]

Patient	Isolate	2021 date (mo-day)	Source[b]	Antimicrobial susceptibility[c] (MIC in mg/L/interpretation)										KPC detection[d]			KPC-variant
				CZA	MEM	IPM	ETP	FEP	CAZ	CRO	TZP	SAM	FOX	DD-PBA, BCA, MHT	LFIA	MALDI-TOF MS	
1	1A	04-04	R	1/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	1B	04-04	U	1/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	1C	**04-15**	**C**	**>256/R**	**≤0.5/S**	**≤0.25/S**	**>1/R**	**16/R**	**>16/R**	**>4/R**	**16/S**	**16/I**	**≤4/S**	**-**	**-**	**+**	**31**
2	**2A**	**06-06**	**R**	**>256/R**	**≤0.5/S**	**≤0.25/S**	**>1/R**	**16/R**	**>16/R**	**>4/R**	**2/S**	**16/I**	**≤4/S**	**-**	**-**	**+**	**31**
3	**3A**	**06-08**	**B**	**>256/R**	**≤0.5/S**	**1/S**	**>1/R**	**16/R**	**>16/R**	**>4/R**	**8/S**	**16/I**	**≤4/S**	**-**	**-**	**+**	**31**
4	**4A**	**06-24**	**U**	**>256/R**	**≤0.5/S**	**≤0.25/S**	**>1/R**	**16/R**	**>16/R**	**>4/R**	**2/S**	**16/I**	**8/S**	**-**	**-**	**+**	**31**
5	5A	06-20	C	1/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	5B	06-25	R	1/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	5C	07-03	R	1/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	5D	07-03	C	1/S	8/R	8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	16/I	+	+	+	3
	5E	07-03	B	1/S	8/R	8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	16/I	+	+	+	3
	5F	**07-15**	**B**	**24/R**	**≤0.5/S**	**≤0.25/S**	**0.5/S**	**2/S**	**>16/R**	**>4/R**	**≤4/S**	**>16/R**	**≤4/S**	**-**	**+**	**+**	**115**
6	6A	06-19	R	2/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	6B	06-25	B	2/S	>32/R	>8/R	>1/R	>16/R	>16/R	>4/R	>64/R	>16/R	>16/R	+	+	+	3
	6C	**07-20**	**B**	**64/R**	**≤0.5/S**	**≤0.25/S**	**>1/R**	**16/R**	**>16/R**	**>4/R**	**8/S**	**>16/R**	**≤4/S**	**-**	**-**	**+**	**31**

[a]Ceftazidime-avibactam-resistant isolates are shown in bold.

[b]Sources: R, respiratory; U, urine; C, catheter; B, blood.

[c]Antimicrobials: CZA, ceftazidime-avibactam; MEM, meropenem; IPM, imipenem; ETP, ertapenem; FEP, cefepime; CAZ, ceftazidime; CRO, ceftriaxone; TZP, piperacillin-tazobactam; SAM, ampicillin-sulbactam; FOX, cefoxitin.

[d]KPC detection: DD-PBA, double disk diffusion with phenyl-boronic acid; BCA, BlueCarba assay; MHT, modified Hodge test; LFIA, lateral flow immunoassay; MALDI-TOF MS, use of this technology for detection of mature KPC protein.

Moreover, isolates showed resistance to ceftazidime-avibactam, a phenotype common for metalo-carbapenemase producers, which was ruled out due to negative results in both EDTA synergy test and multiplex PCR. A determined effort was made to elucidate the resistance mechanism due to atypical ertapenem nonsusceptibility and colistin resistance (mostly seen in KPC-Kp in our facility), as well as history of colonization or infection with KPC-Kp. In addition, and in accordance with previous studies, conventional phenotypic methods failed to detect the presence of KPC-31 carbapenemase (20–22), as well as the new variant KPC-115 (Table 2). Immunochromatographic detection carried out on ceftazidime-avibactam-resistant isolates allowed to detect KPC-115 but not KPC-31 producers (Table 2). Antonelli et al. (20) have already described failure of LFIA to detect KPC-31. This was also confirmed by other articles, although detection of other KPC variants showing ceftazidime-avibactam resistance was reported (17, 22). An exhaustive bibliographic revision of ceftazidime-avibactam-resistant KPC variants evidenced that there is no correlation among Ω-loop mutations and the LFIA detection. On the other hand, specific peak identification by MALDI-TOF MS contributed to the detection of all KPC variants, both KPC-31 and KPC-115 in those ceftazidime-avibactam-resistant isolates, as well as KPC-3 in ceftazidime-avibactam-susceptible isolates (Table 2). Therefore, this method, when available, could prove useful in clinical practice to directly identify different KPC variants (23). These results were further confirmed by PCR amplification and sequencing.

It is also important to comment that isolates producing KPC variants with ceftazidime-avibactam resistance might not grow in chromogenic media for detection of CPE fecal colonization because of their low carbapenem MIC. In fact, although not systematically investigated, some of our patients rendered rectal swabs negative for KPC producers at the time they were infected with KPC-31 (data not shown).

Although ceftazidime-avibactam-resistant *K. pneumoniae* isolates belonging to ST11 were previously reported in Argentina, they produced KPC-8 displaying enhanced catalytic efficiency toward ceftazidime (24). KPC-31 presents a single point mutation (Asp179Tyr) in the Ω-loop, assumed to be responsible for ceftazidime-avibactam resistance and restoration of the *in vitro* susceptibility to imipenem and meropenem (21). It was postulated that modifications in the Ω-loop drive to a higher affinity to ceftazidime, leading to ceftazidime-avibactam resistance, although with a shortfall in the hydrolytic efficiency on other *β*-lactams, such as carbapenems, cefotaxime, and piperacillin, among others. This hydrolytic profile may be partially observed in our results (Table 2) since MIC values for imipenem, meropenem, piperacillin-tazobactam, and cefoxitin for ceftazidime-avibactam-resistant isolates were lower than those for ceftazidime-avibactam-susceptible ones.

KPC-31 was described in some European countries and the United States (20, 21), while this report is the first to describe KPC-31 in Latin America.

KPC-115 displayed a 2-point deletion and 2 mutations compared to KPC-3, all located in the Ω-loop. Antinori et al. (25) also reported a KPC-3 variant with a 2-amino-acid deletion in the Ω-loop (glutamic acid and leucine in positions 167 and 168), near that observed in KPC-115. This KPC variant, as well as KPC-115, was resistant to ceftazidime-avibactam, susceptible to imipenem and meropenem, and rendered a positive result with LFIA (25). Although kinetic studies are required, these mutations are probably associated with an enhanced affinity toward ceftazidime, prevention of binding to avibactam, and reversion of carbapenem resistance, as previously reported for other variants (15, 26).

MATERIALS AND METHODS

Patient characteristics, bacterial isolates, and antimicrobial susceptibility. Ceftazidime-avibactam-resistant *K. pneumoniae* isolates were found in 6 patients hospitalized at the ICU of our institution in the city of Buenos Aires between April and July 2021. All these patients presented several comorbidities and required mechanical ventilation, urinary catheters, and central venous catheters, and 5 of them were admitted because of severe COVID pneumonia. Main clinical information was collected from medical records (Table 1). Fecal colonization with CPE was assessed by rectal swabs seeded onto CHROMAgar KPC medium (CHROMagar, France) followed by phenotypic and molecular carbapenemase characterization of suspected colonies. Isolates from fecal colonization were not included because they were not available for further investigation. All *K. pneumoniae* isolates (*n* = 15) recovered from clinical samples of these inpatients were included in this study. Isolates were identified by MALDI-TOF MS (Becton, Dickinson-Bruker Daltonics Biotyper, USA). Ceftazidime-avibactam MIC was determined by the epsilometric method (Liofilchem, Italy), colistin MIC by broth microdilution (SensiTest

Colistin, Liofilchem, Italy), fosfomycin MIC by agar dilution, and susceptibilities to other antimicrobials by Phoenix automatic system (Becton, Dickinson, USA). Susceptibility categories were interpreted according to EUCAST for colistin and fosfomycin, FDA for tigecycline, and CLSI for the remaining antibiotics (27–29).

Carbapenemase characterization. The presence of carbapenemases was analyzed by phenotypic procedures, including a double disc inhibition test using both phenyl-boronic acid and EDTA, as well as Blue-Carba assay, modified Hodge test, LFIA (NG-Test CARBA 5; NG Biotech, France), and a MALDI-TOF MS-based procedure to detect mature KPC protein (23).

Genotypic detection of carbapenemase coding genes was assessed by multiplex PCR for bla_{KPC}, $bla_{OXA-48\ group}$, bla_{NDM}, bla_{IMP}, and bla_{VIM} (30). Complete bla_{KPC} was amplified as previously described (31), and its genetic context was studied by a PCR mapping approach (32). Amplicons were sequenced at external facilities (Macrogen Inc., South Korea) and analyzed using the BLAST tool of the National Center for Biotechnology Information.

Molecular characterization of KPC-producing _K. pneumoniae_ isolates. Clonal relationship was analyzed by pulsed-field gel electrophoresis after digestion of genomic DNA with XbaI (XbaI-PFGE) (33). Additionally, WGS was performed in 4 selected isolates (1A, 1C, 5B, and 5F). Bacterial DNA was extracted using QIAamp DNA minikit (Qiagen, Germany) from overnight cultures and further underwent WGS with the Illumina MiSeq platform (Illumina Inc., USA), using a 2× 150-bp paired-end approach. _De novo_ assemblies of WGS data were generated using SPAdes software v3.13.3 (34). The ST was investigated utilizing the "_Klebsiella pneumoniae_" database PubMLST. Antimicrobial resistance genes were studied using ResFinder 4.0 (35), and K-type and virulence factors were studied using Kleborate v2.2.0 (36). Phylogeny was inferred using CSI Phylogeny 1.4 and visualized using FigTree 1.3.1 (37). SNPs were analyzed on the whole-genome by CSI Phylogeny 1.4 within the following parameters: select minimum depth at SNP position at 10×, minimum distance between SNPs at 10 bp, and minimum SNP quality score of 30 (37).

Data availability. The genomes of _K. pneumoniae_ 1A, 1C, 5B, and 5F have been deposited at GenBank under Bioproject PRJNA810071 and accession number SAMN26232644, SAMN26232645, SAMN26232646, and SAMN26232647, respectively.

The sequence of the novel $bla_{KPC-115}$ has been deposited at GenBank under accession number OM714909.1.

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