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Authors

Humphries, Romney M
Yang, Shangxin
Hemarajata, Peera
et al.

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First Report of Ceftazidime-Avibactam Resistance in a KPC-3-Expressing *Klebsiella pneumoniae* Isolate

Romney M. Humphries,[a] Shangxin Yang,[a] Peera Hemarajata,[a] Kevin W. Ward,[a] Janet A. Hindler,[a] Shelley A. Miller,[a] Aric Gregson[b]

Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California, USA[a]; Department of Medicine, Division of Infectious Diseases, University of California, Los Angeles, Los Angeles, California, USA[b]

Ceftazidime-avibactam is the first antimicrobial approved by the U.S. FDA for the treatment of carbapenem-resistant *Enterobacteriaceae*. Avibactam, a non-β-lactam β-lactamase inhibitor, inactivates class A serine carbapenemases, including *Klebsiella pneumoniae* carbapenemase (KPC). We report a KPC-producing *K. pneumoniae* isolate resistant to ceftazidime-avibactam (MIC, 32/4 μg/ml) from a patient with no prior treatment with ceftazidime-avibactam.

The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) is a critical threat to public health. Carbapenemases identified in CPE are classified into three functional groups: class A serine carbapenemases (including *Klebsiella pneumoniae* carbapenemase [KPC]), class B metallo-β-lactamases (including New Delhi metallo-β-lactamase [NDM]), and class D β-lactamases (including the OXA-48-like enzymes) (1). In the United States, the CPE epidemic is driven almost exclusively by *K. pneumoniae* isolates that produce KPC (2). KPC efficiently hydrolyzes the carbapenems, as well as the extended-spectrum cephalosporins and aztreonam (1), and is resistant to the β-lactamase inhibitors clavulanic acid, tazobactam, and sulbactam (3). The KPC gene, bla_{KPC}, is located on a transmissible plasmid that also harbors resistance determinants for several other antimicrobial classes. Thus, isolates are often resistant to all agents commonly used for Gram-negative bacteria, with the exception of gentamicin or amikacin, for which variable susceptibility exists (4). Resistance to the polymyxins (colistin and polymyxin B) and tigecycline has been reported for these isolates (2), making the management of patients with serious infections caused by CPE exceedingly difficult.

In late 2014, the U.S. Food and Drug Administration (FDA) approved ceftazidime-avibactam (Avycaz; Actavis) for the treatment of complicated urinary tract infections and, when combined with metronidazole, complicated intra-abdominal infections caused by CPE and other multidrug-resistant Gram-negative bacteria. Avibactam is a novel non-β-lactam β-lactamase inhibitor that inactivates class A serine carbapenemases (including KPC). The MIC_{50} and MIC_{90} of ceftazidime-avibactam for KPC-producing *Enterobacteriaceae* were recently reported as 1/4 and 4/4 μg/ml, respectively, for a collection of 120 isolates recovered in 2013 from hospitals across the United States (5). Avibactam does not inhibit the activity of the class B β-lactamases and has only variable activity against the class D β-lactamases (6). No clinical isolates of KPC-producing *K. pneumoniae* that are resistant to ceftazidime-avibactam had been reported previously, despite extensive surveillance programs for this agent's potency (2, 5). Here, we report the first case of a KPC-producing *K. pneumoniae* isolate resistant to ceftazidime-avibactam. This isolate was recovered from a patient with no history of ceftazidime-avibactam therapy.

Case report. A 62-year-old woman with a past medical history of splenic vein thrombosis, status postsplenectomy, and locally advanced pancreatic cancer, status postchemotherapy and -radiation, underwent a pylorus-preserving pancreaticoduodenectomy at the Ronald Reagan UCLA Medical Center, Los Angeles, CA. She was discharged home 2 weeks after the operation. Seven days later, she presented to an outside hospital with vomiting and fever to 102°F. An abdominal computed tomography (CT) scan revealed rim-enhancing periductal fluid collections in the central liver and left hepatic lobe measuring up to 2 cm, suggestive of cholangitic abscesses, and inflammatory ascites of new onset. The patient was transferred back to the UCLA Medical Center for a higher level of care. On admission, she was started on vancomycin at 1 g intravenously (i.v.) every 12 h (q12h) and piperacillin-tazobactam at 3.375 g q8h infused over 4 h. The white blood cell count was 22,000 cells/μl, the hemoglobin level was 10.0 g/dl, and the lactate level was 19 mg/dl. Two of three blood cultures drawn on the day of admission grew *Citrobacter freundii* (resistant only to ampicillin and ampicillin-sulbactam [data not shown]) and carbapenem-resistant *K. pneumoniae*. At this time, antimicrobial therapy was changed to gentamicin at 7 mg/kg of body weight i.v. q24h and cefepime at 2 g q8h. Over the next 6 days, 3 additional blood cultures grew carbapenem-resistant *K. pneumoniae*. Gentamicin and cefepime were discontinued in favor of colistin at a 300-mg loading dose and then 150 mg q12h, meropenem at 2 g q8h infused over 4 h, and tigecycline at 100 mg q12h, when resistance to cefepime was confirmed. Altered mental status and worsening kidney injury, presumably due to the combined effects of gentamicin, colistin, and sepsis, required admission to the intensive care unit (ICU) and initiation of continuous renal replacement therapy for volume overload and metabolic acidosis. At this time, the Institutional Review Board and Actavis were contacted to obtain ceftazidime-avibactam for compassionate use. While no further blood cultures were positive for carbapenem-resistant *K.*

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Address correspondence to Romney M. Humphries, rhumphries@mednet.ucla.edu, or Janet A. Hindler, jfhindler@mednet.ucla.edu.

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TABLE 1 Antimicrobial susceptibility results for carbapenem-resistant *K. pneumoniae* isolates[a]

Antimicrobial(s)	MIC (μg/ml) (interpretation[b]) for *K. pneumoniae* isolate:		
	1	2	3
Amikacin	16 (S)	16 (S)	16 (S)
Ampicillin	>32 (R)	>32 (R)	>32 (R)
Ampicillin-sulbactam	>32 (R)	>32 (R)	>32 (R)
Aztreonam	>32 (R)	>32 (R)	>32 (R)
Cefepime	32 (R)	>32 (R)	>32 (R)
Ceftazidime	>32 (R)	>32 (R)	>32 (R)
Ceftazidime-avibactam	**4/4 (S)**	**32/4 (R)**	**8/4 (S)**
Ceftolozane-tazobactam	>256/4 (R)	>256/4 (R)	>256/4 (R)
Ceftriaxone	>32 (R)	>32 (R)	>32 (R)
Chloramphenicol	**≤4 (S)**	**>16 (R)**	**>16 (R)**
Ciprofloxacin	>2 (R)	>2 (R)	>2 (R)
Colistin	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Doxycycline	**2 (S)**	**16 (R)**	**16 (R)**
Ertapenem	>2 (R)	>2 (R)	>2 (R)
Gentamicin	1 (S)	2 (S)	2 (S)
Imipenem	8 (R)	128 (R)	128 (R)
Meropenem	16 (R)	128 (R)	128 (R)
Minocycline	**2 (S)**	**>16 (R)**	**>16 (R)**
Piperacillin-tazobactam	>256/4 (R)	>256/4 (R)	>256/4 (R)
Tigecycline	**1 (S)**	**8 (R)**	**8 (R)**
Tobramycin	10 (R)	>10 (R)	>10 (R)
Trimethoprim-sulfamethoxazole	**≤1/20 (S)**	**2/40 (S)**	**2/40 (S)**

[a] Carbapenem-resistant *K. pneumoniae* isolate 1 was obtained from blood on hospital days 1, 8, and 9, isolate 2 was obtained from blood on days 8 and 9, and isolate 3 was obtained from liver drain fluid on day 20 and sputum on day 22. Entries in bold are MICs that were different between the isolates.

[b] CLSI M100-S25 interpretive criteria were used in all cases, with the exception of ceftazidime-avibactam, ceftolozane-tazobactam, and tigecycline, for which FDA breakpoints (MIC breakpoints: ≤8/4 μg/ml, susceptible; ≥16/4 μg/ml, resistant; inhibition zone diameter breakpoints: ≥21 mm, susceptible; ≤20 mm, resistant) were used. Colistin MICs were interpreted according to EUCAST breakpoints. S, susceptible; R, resistant.

pneumoniae, carbapenem-resistant *K. pneumoniae* was isolated from the patient's hepatic drain fluid on hospital day 17.

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) standards (7), with MIC testing done on panels prepared in-house. Ceftazidime-avibactam was tested by both broth microdilution and disk diffusion, using research-use-only (RUO)-labeled reagents provided by AztraZeneca (London, United Kingdom). Ceftazidime-avibactam was tested using a constant concentration of avibactam (4 μg/ml). MIC results were interpreted according to CLSI M100-S25 breakpoints (8), with the exception of those for tigecycline and ceftazidime-avibactam, which were interpreted according to the FDA-cleared breakpoints. Colistin MICs were interpreted using the EUCAST *Enterobacteriaceae* breakpoint of <4 μg/ml to indicate susceptibility.

Results of susceptibility testing are presented in Table 1 and yielded three different susceptibility patterns for the carbapenem-resistant *K. pneumoniae* isolates. All three isolates were resistant to the extended-spectrum cephalosporins and aztreonam, the carbapenems, ampicillin-sulbactam, piperacillin-tazobactam, ciprofloxacin, and tobramycin. All three isolates were susceptible to amikacin, gentamicin, colistin and trimethoprim-sulfamethoxazole. Surprisingly, carbapenem-resistant *K. pneumoniae* isolate 2 was resistant to ceftazidime-avibactam by both the disk result (inhibition zone diameter, 19 mm) and the MIC (32/4 μg/ml), a finding confirmed by an outside reference laboratory (JMI, North Liberty, IA). In addition, carbapenem-resistant *K. pneumoniae* isolate 2 was resistant to chloramphenicol, doxycycline, minocycline, and tigecycline, whereas carbapenem-resistant *K. pneumoniae* isolate 1 was susceptible to these agents. Carbapenem-resistant *K. pneumoniae* isolate 3 was similarly resistant to chloramphenicol and the tetracyclines but susceptible to ceftazidime-avibactam. The isolates were tested for the presence of carbapenemases by using a laboratory-developed PCR assay that targets bla_{KPC}, bla_{NDM-1}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, and bla_{SME}. bla_{KPC} was the sole carbapenemase gene detected in the isolates. The KPC gene was sequenced, revealing 100% homology to bla_{KPC-3} (9).

We determined the MIC of meropenem-avibactam, which was tested in doubling dilutions of meropenem at concentrations of 0.06 to 32 μg/ml with a constant concentration of 4 μg/ml avibactam, for carbapenem-resistant *K. pneumoniae* isolate 2. The meropenem-avibactam MIC was 0.06/4 μg/ml, whereas the MIC of meropenem alone was >32 μg/ml. These data further support that the isolate does not also harbor a class B metallo-β-lactamase, as was reported elsewhere for a ceftazidime-avibactam-resistant isolate that expressed both bla_{KPC-2} and the metallo-β-lactamase gene bla_{VIM-4} (5). The addition of the efflux pump inhibitor phenyl-arginine β-naphthylamide at 40 μg/ml did not result in decrease in the ceftazidime-avibactam MIC for any of the patient's isolates, suggesting that efflux is not the mechanism for resistance in this isolate.

On hospital day 21, ceftazidime-avibactam (2.5 g q12 h) was added to meropenem at 1 g q8h, tigecycline at 100 mg q12h, and polymyxin B at 15,000 U/kg q12 h. The patient continued an ICU stay complicated by intermittent altered mental status, hypotension, continuous renal replacement therapy, and two episodes of respiratory failure, one of which was associated with carbapenem-resistant *K. pneumoniae* in the respiratory culture and multifocal pulmonary infiltrates. At this writing, she continues treatment for ongoing hepatic abscesses with i.v. trimethoprim-sulfamethoxazole at 10 mg/kg/day q12h and polymyxin B at 15,000 U/kg q12h, with no further isolation of carbapenem-resistant *K. pneumoniae*.

Discussion. CPE were identified by the U.S. Centers for Disease Control and Prevention as the highest priority for novel antimicrobial development in 2011. Despite this call to arms, ceftazidime-avibactam is the only antimicrobial approved by the U.S. FDA for the treatment of CPE. Importantly, ceftazidime-avibactam was approved via an expedited process based on phase II data as part of the Generating Antibiotics Incentives Now (GAIN) Act, and little clinical experience exists for the use of this antimicrobial for the treatment of CPE. In this report, we document the first case of ceftazidime-avibactam resistance in a KPC-producing isolate of *K. pneumoniae*, the most common CPE species in the United States. Importantly, this isolate was recovered in February 2015, prior to the widespread availability of ceftazidime-avibactam in the United States, and from a patient with no prior ceftazidime-avibactam treatment.

The resistance mechanism for ceftazidime-avibactam is under investigation for this isolate but does not appear to be related to mutation of bla_{KPC-3}. Avibactam-resistant variants of KPC-2 have been engineered through directed mutagenesis of sites critical to avibactam's activity. These mutations also abolished KPC-2's ability to hydrolyze ceftazidime, and as a result, isolates transformed with these engineered KPCs were susceptible to the ceftazidime compo-

nent of the combination (10). Similarly, mutations to the class A SHV and CTX-M-15 β-lactamases that confer resistance to avibactam have been documented, but again, these mutations abolished ceftazidime hydrolysis (11, 12). Nonetheless, it is theoretically possible that a combination of mutations to avibactam's target site could result in resistance both to avibactam and to ceftazidime as a result of ceftazidime hydrolysis. Efflux and porin mutations have been associated with ceftazidime-avibactam resistance in *Pseudomonas aeruginosa* and *Enterobacter cloacae* (13–15). The concomitant tetracycline resistance in our isolate suggested efflux as a plausible explanation for the resistance mechanism, since tetracycline resistance is commonly mediated by increased efflux in the *Enterobacteriaceae*; however, the addition of phenyl-arginine β-naphthylamide did not affect the ceftazidime-avibactam MIC.

A significant challenge to the clinical use of ceftazidime-avibactam is the current lack of FDA-cleared susceptibility tests for this antimicrobial, and it is unlikely that such tests will be available in the short term. RUO disks and Etest strips (bioMérieux, Durham, NC) are available by request from Allergan, but the reliability of these test methods is unknown. Furthermore, per FDA regulations, laboratories that choose to use RUO tests must sign a waiver indicating that they will not release results of testing for patient care. Because ceftazidime-avibactam resistance in KPC-expressing CPE was hitherto unreported, confirmation of the KPC gene has been suggested as a means to predict susceptibility to ceftazidime-avibactam among CRE. However, isolates resistant to ceftazidime-avibactam due to the production of both KPC and class B metallo-β-lactamase may be undetected if laboratories target only KPC by PCR (8). Furthermore, few laboratories test for carbapenemase production using phenotypic tests, which are no longer recommended as part of routine clinical testing by the CLSI (8). Regardless, our case clearly demonstrates that it is prudent to test for ceftazidime-avibactam susceptibility using MIC or disk diffusion tests, even for isolates that are positive for KPC and negative for metallo-β-lactamases. This is particularly important if ceftazidime-avibactam may be used as monotherapy for infections with KPC-producing CRE.

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