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Emergence of Ceftazidime-Avibactam Resistance and Restoration of Carbapenem Susceptibility in *Klebsiella pneumoniae* Carbapenemase-Producing *K pneumoniae*: A Case Report and Review of Literature

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We used meropenem to successfully treat a patient with bacteremia due to ceftazidime-avibactam-resistant, meropenem-susceptible *Klebsiella pneumoniae* that carried mutant bla_{KPC-3}. Meropenem was bactericidal against ceftazidime-avibactam-resistant *K pneumoniae* isolates in vitro. Nevertheless, the role of carbapenems in treating such infections remains uncertain, because meropenem resistance is selected readily during passage experiments.

Keywords. carbapenem-resistant *Enterobacteriaceae*; ceftazidime-avibactam resistance; *Klebsiella pneumoniae* carbapenemase; KPC mutations; sequence type 258 *Klebsiella pneumoniae*.

CASE REPORT

A 67-year-old man with esophageal cancer underwent esophagectomy, which was complicated by kidney injury necessitating continuous renal replacement therapy (CRRT), respiratory failure, and ventilator-associated pneumonia due to carbapenem-resistant *Klebsiella pneumoniae* ([CR-Kp] isolate 4-A). Ventilator-associated pneumonia was treated with ceftazidime-avibactam (1.25 grams intravenous every 8 hours [q8hrs]) and inhaled gentamicin (80 milligrams q12hrs) for 15 days. Ten days after treatment, he developed leukocytosis. Computerized tomography scan revealed an intra-abdominal abscess. Ceftazidime-avibactam was restarted empirically. Drainage culture grew meropenem-susceptible *K pneumoniae*, reported as extended-spectrum β-lactamase (ESBL)-producing (isolate 4-B) [1]. Ceftazidime-avibactam was continued for 15 days, the abscess was surgically drained, and the patient improved. Several weeks later, the patient developed meropenem-susceptible, ESBL-producing *K pneumoniae* bacteremia (isolate 4-C). He was treated successfully with meropenem 1 gram intravenous q12hrs (adjusted for creatinine clearance ~40 mL/min) for 18 days and subsequently discharged.

CHARACTERIZATION OF ISOLATES

Isolate 4-A was resistant to all β-lactams except ceftazidime-avibactam (Table 1). Isolates 4-B and 4-C were ceftazidime-avibactam-resistant, but meropenem-susceptible (Figure 1); ceftriaxone, cefepime, and aztreonam MICs were reduced ≥16-fold from baseline. Isolates underwent whole-genome sequencing, as described previously [2]. Isolate 4-A was multilocus sequence type (ST)-258, *K pneumoniae* carbapenemase-3 (KPC-3)-producing. Isolates 4-B and 4-C were ST258, with glutamic acid for alanine and tyrosine for aspartic acid substitutions at KPC-3 Ambler positions 177 (A177E) and 179 (D179Y), respectively. Isolates clustered within a novel phylogenetic sublineage of clade II ST258, which also includes isolates from 3 other patients at our center in whom ceftazidime-avibactam resistance emerged [2].

Time-kill assays were performed on longitudinal isolates from our 4 patients, using meropenem at 16 μg/mL (achievable serum concentration) [3], 4× minimum inhibitory concentration (MIC), and 8× MIC (Table 1; Figure 2). Meropenem (16 μg/mL) did not inhibit CR-Kp with wild-type bla_{KPC-3}, but it was bactericidal at 24 hours against bla_{KPC-3} mutants (4× MIC, 8× MIC, and 16 μg/mL log-kills: −3.18 to −5.98, −2.54 to −6.14, and −4.64 to −6.16 $\log_{10}$, respectively).

DISCUSSION

Ceftazidime-avibactam, a novel β-lactam/β-lactamase inhibitor, is US Food and Drug Administration-approved for treating complicated intra-abdominal infections (cIAI) and urinary tract infections (cUTI). The agent is active against carbapenem-resistant *Enterobacteriaceae* (CRE) expressing KPCs, but not metallo-β-lactamases. Ceftazidime-avibactam is likely to be widely used against off-label CRE infections. We used ceftazidime-avibactam to treat 37 CRE-infected patients (29 without cIAI or cUTI) [4]. Thirty-day clinical success and mortality rates were 59% (22 of 37) and 76% (28 of 37), respectively. In a multicenter series, 36 CRE-infected patients

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Table 1. Meropenem Time-Kill Results Against *Klebsiella pneumoniae* That Developed Ceftazidime-Avibactam Resistance[a]

			MIC (μg/mL)[b]			Log-Kill at 24 Hours in Presence of Meropenem[c]		
Isolate	ST	KPC Variant	Ceftazidime-Avibactam	Ceftazidime	Meropenem	4× MIC	8× MIC	16 μg/mL
1-A	**258**	**KPC-3**	**2 (S)**	**512**	**128**	[d]	[d]	**+3.53**
1-B	258	D179Y, T243M	256	>512	0.5 (S)	−4.97	−4.27	−4.97
1-C	258	D179Y, T243M	256	>512	0.25 (S)	−5.94	−4.94	−5.96
2-A	**258**	**KPC-3**	**4 (S)**	**256**	**32**	[d]	[d]	**+3.57**
2-B	258	V240G	32	>512	8	−6.14	−6.14	−6.14
2-C	258	D179Y	>256	>512	4	−4.64	−5.64	−4.64
3-A	**258**	**KPC-3**	**2 (S)**	**256**	**32**	[d]	[d]	**+3.52**
3-B	258	D179Y	128	512	0.25 (S)	−5.98	−5.98	−5.98
3-C	258	D179Y	64	512	0.125 (S)	−3.18	−3.18	−5.83
4-A	**258**	**KPC-3**	**1 (S)**	**256**	**16**	[d]	[d]	**+3.52**
4-B	258	A177E, D179Y	256	256	0.25 (S)	−3.70	−5.16	−6.16
4-C	258	A177E, D179Y	128	256	0.25 (S)	−3.26	−2.54	−5.96

Abbreviations: cfu, colony-forming units; CLSI, Clinical Laboratory Standards Institute; KPC, Klebsiella pneumoniae carbapenemase; MIC, minimum inhibitory concentration; S, susceptible based on CLSI interpretive criteria; ST, sequence type. Bolded rows represent the baseline isolate from each of the 4 patients.

[a]Time-kill assays were performed in duplicate for each isolate, using a 1×10^6 cfu/mL inoculum in cation-adjusted Mueller-Hinton broth. Bactericidal responses were defined as a ≥3-log decrease in cfu/mL from the starting inoculum at 24 hours.

[b]MICs were determined by broth microdilution according to reference methods (1). CLSI interpretative criteria were applied to define susceptibility as follows: ceftazidime-avibactam, ≤8 μg/mL; ceftazidime, ≤4 μg/mL; meropenem, ≤1 μg/mL.

[c]Difference in concentration ($\log_{10}$) compared with baseline (time 0). Data are shown for a representative replicate of each isolate.

[d]Not tested.

(20 without cIAI or cUTI) received compassionate-use ceftazidime-avibactam [5]. Clinical cure and in-hospital survival rates were 69% (25 of 36) and 61% (22 of 36), respectively. Results did not differ in either study if ceftazidime-avibactam was combined with another antibiotic. Outcomes were comparable to those reported previously for CRE-infected patients treated with ≥2 in vitro active agents [6]. Acute kidney injury was described in only 3 patients between studies, suggesting lower toxicity than with colistin or aminoglycosides.

Ceftazidime-avibactam resistance has emerged in ~10% of CR-Kp-infected patients treated at our center [2, 4]. Thus far, these are the only cases reported to develop during treatment. Resistance has been diagnosed after 10–19 days of drug exposure, exclusively in KPC-3-producing ST258 isolates. It is conferred by plasmid-borne $bla_{\text{KPC-3}}$ mutations, which reduce MICs of carbapenems (often restoring susceptibility) and other β-lactams [2, 7]. The D179Y variant, alone or in combination with other mutations, predominates in patients and after in vitro passage [8, 9], and manifests the strongest phenotypes [2, 7]. Most mutations have arisen within the KPC Ω-loop (positions 165–179), thereby enhancing ceftazidime affinity and possibly restricting avibactam binding [9]. The A177E mutation is newly reported. As in our case, *K pneumoniae* with mutant $bla_{\text{KPC-3}}$ may be identified as ESBL-producers (rather than KPC-producers) if carbapenemase screening is triggered by elevated carbapenem MICs. Failure to detect bla_{KPC} in a timely fashion may facilitate nosocomial dissemination.

Meropenem at clinically achievable concentrations was bactericidal against ceftazidime-avibactam-resistant *K pneumoniae* in vitro, and it eradicated isolate 4-C from our patient's bloodstream. However, meropenem resistance has emerged in ceftazidime-avibactam-resistant isolates from our patients during in vitro passage at subinhibitory meropenem concentrations [10]. In most passage experiments, resistance to ceftazidime-avibactam and other β-lactams was retained. Therefore, until more data are available, the role of carbapenems in treating patients is unclear. Combination regimens merit investigation. Of note, ceftazidime-avibactam dosing is not established for CRRT; our patient received half the conventional dose. Better pharmacokinetic data are needed in CRRT and other specialized populations and for various types of invasive infections.

CONCLUSIONS

In conclusion, clinicians should be alert for the inevitable emergence of more widespread ceftazidime-avibactam resistance. To best use and preserve ceftazidime-avibactam, resistant isolates should be studied for resistance mechanisms and genome and plasmid content.

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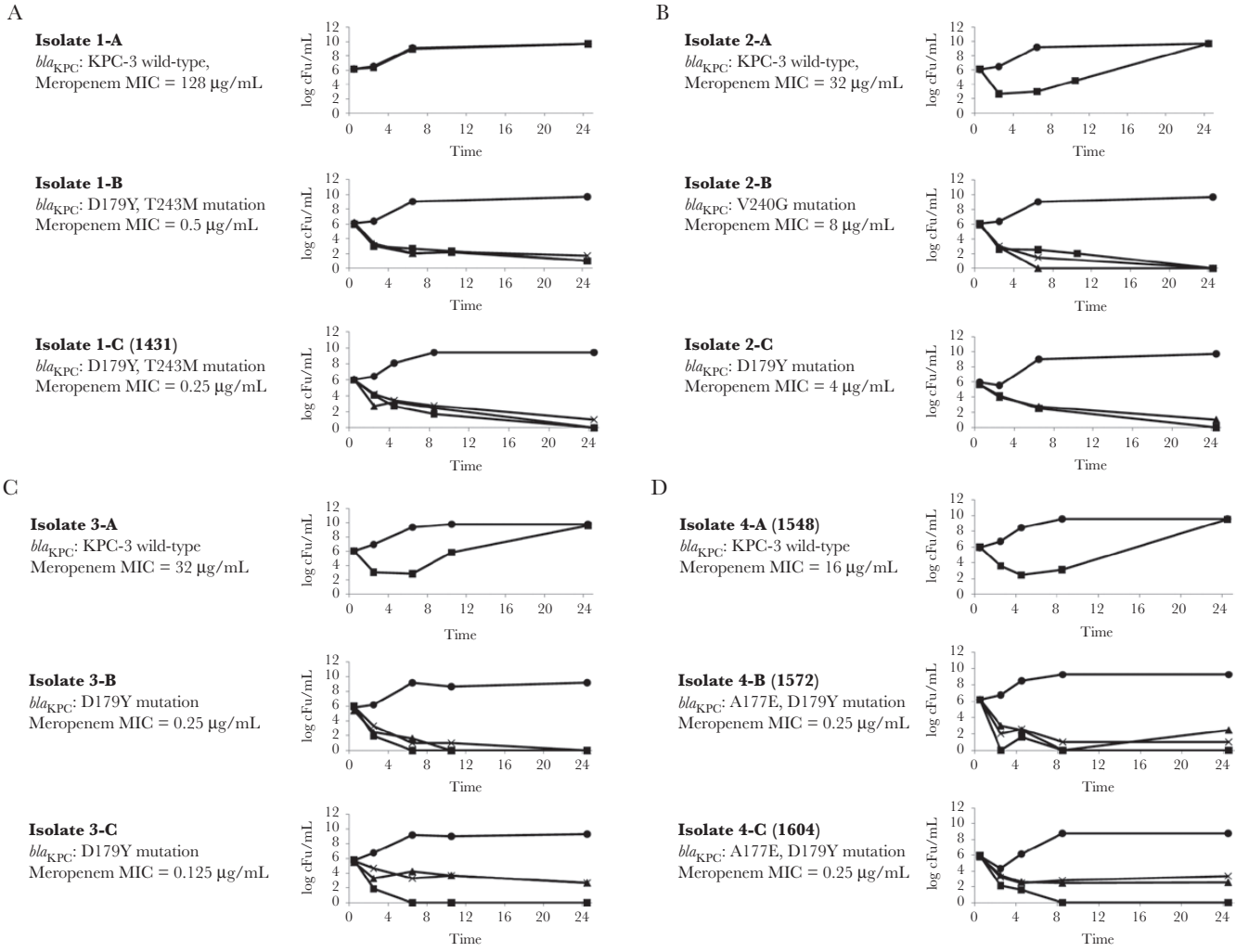

Figure 1. Time-kill responses of ceftazidime-avibactam-resistant *Klebsiella pneumonia* to meropenem. NOTE: Time-kill results in the presence of meropenem. Circle = control (no drug), triangle = 4× minimum inhibitory concentration (MIC), cross = 8× MIC, square = 16 µg/mL.

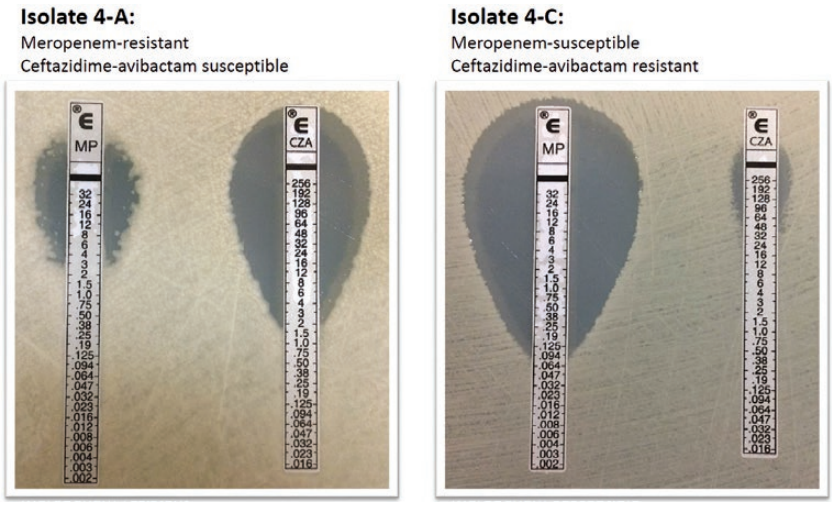

Figure 2. Restoration of meropenem susceptibility among ceftazidime-avibactam-resistant *Klebsiella pneumonia*. Etest results for meropenem and ceftazidime-avibactam against baseline isolate 4-A (left) and follow-up isolate 4-C (right).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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