



Pneumonia and Renal Replacement Therapy Are Risk Factors for Ceftazidime-Avibactam Treatment Failures and Resistance among Patients with Carbapenem-Resistant *Enterobacteriaceae* Infections

Ryan K. Shields,^{a,b} M. Hong Nguyen,^{a,b} Liang Chen,^c Ellen G. Press,^a Barry N. Kreiswirth,^c Cornelius J. Clancy^{a,b,d}

^aDepartment of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

^bXDR Pathogen Laboratory, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

^cPublic Health Research Institute Tuberculosis Center, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA

^dVA Pittsburgh Healthcare System, Pittsburgh, Pennsylvania, USA

ABSTRACT Ceftazidime-avibactam was used to treat 77 patients with carbapenem-resistant *Enterobacteriaceae* (CRE) infections at our center. Thirty- and 90-day survival rates were 81% and 69%, respectively; these rates were higher than those predicted by SAPS II and SOFA scores at the onset of infection. Clinical success was achieved for 55% of patients but differed by the site of infection. Success rates were lowest for pneumonia (36%) and higher for bacteremia (75%) and urinary tract infections (88%). By multivariate analysis, pneumonia ($P = 0.045$) and receipt of renal replacement therapy (RRT) ($P = 0.046$) were associated with clinical failure. Microbiologic failures occurred in 32% of patients and occurred more commonly among patients infected with KPC-3-producing CRE than among those infected with KPC-2-producing CRE ($P = 0.002$). Pneumonia was an independent predictor of microbiologic failure ($P = 0.007$). Ceftazidime-avibactam resistance emerged in 10% of patients, including 14% of those infected with *Klebsiella pneumoniae* and 32% of those with microbiologic failure. RRT was an independent predictor of the development of resistance ($P = 0.009$). Resistance was identified exclusively among *K. pneumoniae* bacteria harboring variant KPC-3 enzymes. Upon phylogenetic analysis of whole-genome sequences, resistant isolates from 87.5% (7/8) of patients clustered within a previously defined sequence type 258 (ST258) clade II sublineage; resistant isolates from one patient clustered independently from other ST258 clade II isolates. In conclusion, our report offers new insights into the utility and limitations of ceftazidime-avibactam across CRE infection types. Immediate priorities are to identify ceftazidime-avibactam dosing and therapeutic regimens that improve on the poor outcomes among patients with pneumonia and those receiving RRT.

KEYWORDS CRE, *Klebsiella pneumoniae*, ceftazidime-avibactam, failure, outcomes, pneumonia, renal replacement therapy

Ceftazidime-avibactam (Cef-Avi) has been commercially available in the United States for more than 2 years following Food and Drug Administration (FDA) approval for treatment of complicated urinary tract and intra-abdominal infections. Avibactam, a β -lactamase inhibitor, is active against class A (e.g., KPC), class C (e.g., AmpC), and certain class D (e.g., OXA-48) carbapenemases but not against class B metallo- β -lactamases (e.g., VIM, IMP, and NDM). Clinical experience with ceftazidime-avibactam against carbapenem-resistant *Enterobacteriaceae* (CRE) infections is accumulating, but important knowledge gaps remain, and real-world experience reported in

Received 12 December 2017 Returned for modification 21 December 2017 Accepted 23 February 2018

Accepted manuscript posted online 5 March 2018

Citation Shields RK, Nguyen MH, Chen L, Press EG, Kreiswirth BN, Clancy CJ. 2018. Pneumonia and renal replacement therapy are risk factors for ceftazidime-avibactam treatment failures and resistance among patients with carbapenem-resistant *Enterobacteriaceae* infections. *Antimicrob Agents Chemother* 62:e02497-17. <https://doi.org/10.1128/AAC.02497-17>.

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to M. Hong Nguyen, mhn5@pitt.edu.

TABLE 1 Characteristics of CRE isolates from patients treated with ceftazidime-avibactam

Pathogen (no. of isolates)	Median (range) MIC ($\mu\text{g/ml}$)				No. (%) of isolates with the following β -lactamase:				
	Ceftazidime-avibactam	Ceftazidime	Ertapenem ^a	Meropenem ^a	CTX-M	OXA ^b	SHV	TEM	KPC ^c
<i>K. pneumoniae</i> (60)	1 (0.12 to 2)	256 (32 to >512)	32 (0.06 to >64)	16 (0.06 to >64)	7 (12)	11 (18)	56 (93)	53 (88)	56 (93)
<i>E. coli</i> (9)	1 (0.25 to 2)	128 (2 to >512)	2 (0.06 to 64)	0.12 (0.06 to 4)	8 (89)	5 (56)	0 (0)	1 (11)	0 (0)
<i>E. cloacae</i> (5)	0.5 (0.5 to 8)	32 (0.5 to 128)	0.5 (0.06 to 8)	0.12 (0.06 to 0.5)	3 (60)	2 (40)	1 (20)	3 (60)	1 (20)
<i>E. aerogenes</i> (1)	0.5	128	4	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>K. oxytoca</i> (1)	0.5	128	8	0.25	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
<i>S. marcescens</i> (1)	1	128	1	0.25	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Total (77)	1 (0.12 to 8)	256 (0.5 to >512)	32 (0.06 to >64)	16 (0.06 to >64)	19 (25)	18 (23)	57 (74)	57 (74)	58 (75)

^aEighty-five percent (58/68) and 92% (55/60) of isolates that were not susceptible to ertapenem and meropenem, respectively, produced KPC. Non-KPC-producing isolates ($n = 19$) generally carried extended-spectrum CTX-M ($n = 15$) or AmpC ($n = 1$) variant β -lactamases; 3 isolates did not harbor KPC or ESBL.

^bNo isolate harbored an OXA-48 variant.

^cOf KPC-producing isolates, 62% (36/58) had KPC-3 and 38% (22/58) had KPC-2.

the literature is limited. We have published a study of 37 patients treated with ceftazidime-avibactam for a variety of CRE infections (1) and a subsequent study comparing patients treated with ceftazidime-avibactam to those receiving other treatment options for CRE bacteremia (2). In our experience, ceftazidime-avibactam was efficacious and well tolerated, but microbiologic failures occurred in 27% of patients and ceftazidime-avibactam resistance emerged in 8% (1). Ceftazidime-avibactam resistance arose in KPC-3-producing *Klebsiella pneumoniae* isolates of a novel sequence type (ST), ST258, clade II sublineage, which are phylogenetically distinct from previously characterized ST258 clade II isolates (3).

Important questions remain about the effectiveness of ceftazidime-avibactam across CRE infection types, the clinical and microbiologic features associated with poor treatment outcomes, and the emergence of resistance. The objectives of this study were to report our ongoing experience with ceftazidime-avibactam in the treatment of CRE infections, determine risk factors associated with treatment failure and the emergence of resistance, and describe resistance mechanisms and the genomics of resistant isolates.

RESULTS

Patients, microbiology, and treatment regimens. Seventy-seven consecutive patients treated with ≥ 3 days of ceftazidime-avibactam were evaluated. The median age was 62 years (range, 19 to 91); 61% (47/77) were men. The median Charlson Comorbidity Index score was 4 (range, 0 to 10). Twenty-six percent (20/77) of patients were transplant recipients (18 solid-organ and 2 bone marrow transplants). At the onset of infection, median SAPS II and SOFA scores were 41 (range, 8 to 81) and 5 (range, 0 to 20), respectively. Infections included pneumonia (43% [33/77]), primary bacteremia (26% [20/77]), urinary tract infection (UTI) (10% [8/77]), intra-abdominal infection (9% [7/77]), skin/soft tissue infection (8% [6/77]), and mediastinitis, subdural empyema/ventriculitis, and purulent tracheobronchitis (1% [1/77] each). Seventy-nine percent (26/33) and 21% (7/33) of pneumonia cases were ventilator and health care associated, respectively. Pyelonephritis was present in 50% (4/8) of UTIs; 25% (2/8) of UTIs resulted in secondary bacteremia.

K. pneumoniae was the predominant pathogen (78% [60/77]), followed by *Escherichia coli* (12% [9/77]), *Enterobacter cloacae* (6% [5/77]), and *Enterobacter aerogenes*, *Serratia marcescens*, and *Klebsiella oxytoca* (1 case each). All baseline CRE isolates were resistant to at least one carbapenem, as determined by the University of Pittsburgh Medical Center (UPMC) clinical microbiology laboratory, and susceptible to ceftazidime-avibactam, as determined using broth microdilution (Table 1) (median MIC, 1 $\mu\text{g/ml}$; range, 0.12 to 8 $\mu\text{g/ml}$). By broth microdilution methods, 96% (74/77), 88% (68/77), and 78% (60/77) of isolates were nonsusceptible to ceftazidime, ertapenem, and mero-

TABLE 2 Predicted versus actual mortality among patients treated with ceftazidime-avibactam for CRE infection

Scoring system	Score range	Predicted mortality (%) ^a	Actual mortality ^b
SAPS II	0–29	<10	21 (4/19)
	30–40	10–25	0 (0/18)
	41–52	26–50	15 (3/20)
	53–64	53–75	15 (2/13)
	≥65	>77	86 (6/7)
SOFA	0–6	<10	9.5 (4/42)
	7–9	15–20	21 (3/14)
	10–12	40–50	22 (2/9)
	13–14	50–60	0 (0/4)
	15–24	>80	75 (6/8)

^aDerived from previous publications validating the SAPS II (30) and SOFA (29) scores.

^bExpressed as a percentage (number of patients dying/total number of patients).

penem, respectively. Seventy-five percent (58/77) of isolates harbored a *bla*_{KPC} gene, including 93% (56/60) of *K. pneumoniae* isolates. Fifty-eight percent (35/60) and 35% (21/60) of *K. pneumoniae* isolates carried *bla*_{KPC-2} and *bla*_{KPC-3}, respectively. No isolates had genes encoding NDM, VIM, IMP, or OXA-48-like carbapenemases.

Ceftazidime-avibactam was administered as monotherapy or in combination regimens to 69% (53/77) or 31% (24/77) of patients, respectively. Combinations included intravenous (i.v.) ($n = 10$), inhaled ($n = 7$), or both i.v. and inhaled ($n = 2$) gentamicin, i.v. ($n = 1$) or intrathecal ($n = 1$) colistin, i.v. amikacin ($n = 1$), i.v. ciprofloxacin ($n = 1$), and i.v. tigecycline ($n = 1$). The median ceftazidime-avibactam treatment duration was 14 days (range, 4 to 71 days).

Treatment outcomes. Thirty- and 90-day survival rates were 81% (62/77) and 69% (54/72), respectively. Survival rates were higher than those predicted by SAPS II and SOFA scores, in particular for scores in the ranges of 30 to 64 for SAPS II and 10 to 14 for SOFA (Table 2). Clinical success was achieved for 55% (42/77) of patients and did not differ between those receiving monotherapy (56% [30/53]) and those receiving combination therapy (50% [12/24], $P = 0.62$). Failures were due to death ($n = 15$), recurrence ($n = 11$), or the absence of clinical improvement ($n = 9$). Success rates were 88% (7/8) for patients with urinary tract infections, 75% (15/20) for primary bacteremia, 67% (4/6) for skin/soft tissue infections, 43% (3/7) for intra-abdominal infections, 36% for pneumonia (12/33), and 33% (1/3) for other infections. Seventeen percent (7/42) of patients with 30-day clinical success had subsequent relapsing CRE infections at the same or contiguous infection sites within 90 days; the median time to relapse was 38 days (range, 34 to 84 days). By multivariate analysis, pneumonia (odds ratio [OR], 3.10; 95% confidence interval [CI], 1.03 to 9.34; $P = 0.045$) and receipt of renal replacement therapy (RRT) (OR, 4.78; 95% CI, 1.03 to 22.20; $P = 0.046$) were independent predictors of clinical failure (Table 3). The time to initiation of ceftazidime-avibactam treatment was not associated with clinical failure.

Microbiologic failures occurred in 32% (25/77) of patients, due to recurrent/ongoing infection ($n = 14$; within 30 days), relapsing infection ($n = 9$; within 31 to 90 days), or colonization ($n = 2$). The median time to microbiologic failure was 21 days (range, 7 to 84 days) from the initiation of ceftazidime-avibactam therapy. Failures were caused by *K. pneumoniae* ($n = 22$; 8 KPC-2- and 14 KPC-3-producing isolates) and *E. coli* ($n = 3$). For *K. pneumoniae* specifically, microbiologic failures were more common among KPC-3-producing isolates (67% [14/21]) than among KPC-2-producing isolates (23% [8/35]) ($P = 0.002$). Across all patients, pneumonia was the only independent predictor of microbiologic failure (OR, 2.71; 95% CI, 1.53 to 14.57; $P = 0.007$).

Ceftazidime-avibactam resistance and toxicity. Ceftazidime-avibactam resistance developed in 10% (8/77) of all patients and in 14% (8/59) of patients infected with *K. pneumoniae*. Thirty-two percent (8/25) of isolates associated with microbiologic failure were ceftazidime-avibactam resistant. Resistance emerged in 22% (8/37) and 0% (0/19)

TABLE 3 Risk factors for clinical failure of ceftazidime-avibactam therapy among patients with CRE infection

Risk factor ^a	Value ^b for patients with:		P value	Multivariate P value (OR, 95% CI) ^c
	Success (n = 42)	Failure (n = 35)		
Demographics and underlying conditions				
Male gender	29 (69)	18 (51)	0.16	
Age (yr)	64 (19–91)	59 (26–79)	0.22	
Solid-organ transplant recipient	9 (21)	9 (26)	0.79	
Charlson Comorbidity Index	4 (0–10)	5 (0–10)	0.86	
Severity of illness				
ICU at disease onset	17 (40)	27 (77)	0.001	Excluded
Renal replacement therapy	3 (7)	13 (37)	0.002	0.046 (4.78, 1.03–22.2)
SOFA score	5 (0–18)	8 (0–20)	0.0007	0.13
SAPS II score	37 (8–62)	43 (17–81)	0.04	0.98
Infection characteristics				
Infection type				
Pneumonia	12 (29)	21 (60)	0.01	0.045 (3.09, 1.03–9.34)
Primary bacteremia	15 (36)	5 (14)	0.04	
Urinary tract infection	7 (17)	1 (3)	0.07	
Intra-abdominal infection	3 (7)	4 (11)	0.70	
Skin/soft tissue infection	4 (10)	2 (6)	0.68	
Other	1 (2)	2 (6)	0.59	
CRE pathogen				
<i>K. pneumoniae</i>	34 (81)	25 (71)	0.42	
<i>E. coli</i>	5 (12)	4 (11)	1.00	
<i>E. cloacae</i>	2 (5)	3 (9)	0.65	
Other	2 (5)	2 (6)	1.00	
Presence of <i>bla</i> _{KPC}				
KPC-2	15 (36)	7 (20)	0.20	
KPC-3	19 (45)	17 (49)	0.82	
KPC negative	8 (19)	11 (31)	0.29	
Treatment characteristics				
Time to treatment initiation (h)	71 (15–162)	76 (3–168)	0.87	
Ceftazidime-avibactam monotherapy	30 (71)	23 (66)	0.63	

^aStatistically significant risk factors for clinical failure of ceftazidime-avibactam are shown in boldface. ICU, intensive care unit.

^bValues for categorical variables are numbers (percentages) of patients with the risk factor. Values for continuous variables are medians (ranges).

^cOR, odds ratio; CI, confidence interval.

of KPC-3- and KPC-2-producing *K. pneumoniae* infections, respectively ($P = 0.04$). Resistant isolates carried mutant *bla*_{KPC-3} encoding variant KPC-3 enzymes (Table 4; see also Table S1 in the supplemental material). Most mutations were within the KPC-3 Ω -loop; a variant with a tyrosine-for-aspartic acid substitution at Ambler amino acid position 179 (D179Y), alone or in combination with other mutations, was identified in 88% (7/8) of patients. Less commonly, mutations were identified outside the Ω -loop, including a glycine-for-valine substitution at amino acid 240 (V240G), which corresponds to KPC-8. Meropenem MICs against isolates carrying variant KPC-3 enzymes were decreased by ≥ 4 -fold (Table S1). Resistance developed following a median of 15 days (range, 7 to 31 days) of ceftazidime-avibactam therapy. Receipt of RRT was a strong independent predictor of the development of ceftazidime-avibactam resistance among patients with microbiologic failures (Table 5) (OR, 26.67; 95% CI, 2.24 to 317.1; $P = 0.009$). All cases of resistance were due to KPC-3, so this variable was excluded from multivariate analysis.

Overall, 21% (16/77) of patients required RRT at the time of treatment initiation. Among the remaining patients, 11% (7/61) developed acute kidney injury (AKI; defined by a 1.5 \times increase in serum creatinine levels from baseline [4]) within 7 days of treatment initiation, including one and two patients receiving concomitant colistin and aminoglycosides, respectively.

Phylogenetic analysis. Whole-genome sequences of baseline ($n = 8$) and postexposure ($n = 15$) *K. pneumoniae* isolates from patients in whom resistance emerged were compared to those of 22 ST258 clade II isolates from our center and others (Fig. 1). For

TABLE 4 Clinical and microbiologic characteristics of patients in whom ceftazidime-avibactam resistance developed

Pt ^a	Age (sex) ^b	Underlying disease ^c	CRE pathogen ^d	Type of initial infection	Baseline creatinine clearance (ml/min) ^e	Initial treatment ^f (days)	Clinical outcome at 30 days	Time to microbiologic failure (days)	Causes of microbiologic failure and outcome at 90 days	C-A MIC (μg/ml) for: Pretreatment isolate	Posttreatment isolate	KPC-3 variant of posttreatment isolate
1	49 (F)	Double lung transplant	ST258, KPC-3-producing CRKP	Pneumonia	51	C-A, 2.5 g i.v. q8h (10)	Failure	14	Reinfection: pneumonia, treated with C-A for an additional 14 days	2	1) 32 2) 256 ^g 256	1) D179Y T243M 2) D179Y T243M D179Y T243M
2	58 (F)	Morbid obesity s/p gastric sleeve surgery	ST258, KPC-3-producing CRKP	Intra-abdominal infection	CRRT	C-A, 0.94 g i.v. q12h (19)	Failure	41	Persistent pneumonia, treated with meropenem and gentamicin until death	4	1) 32 2) >256 ^g	1) V240G 2) D179Y
3	73 (M)	Esophageal cancer s/p esophagectomy	ST258, KPC-3-producing CRKP	Pneumonia	113	C-A, 2.5 g i.v. q8h (15)	Success	34 58	Respiratory colonization, not treated	2	1) 64 2) 128 ^g	1) D179Y 2) D179Y
4	67 (M)	Esophageal cancer s/p esophagectomy	ST258, KPC-3-producing CRKP	Pneumonia	CRRT	C-A, 1.25 g i.v. q8h (15); inhaled gentamicin (15)	Failure	25	Respiratory pneumonia, treated with meropenem and colistin for 14 days and survived	1	256	A177E D179Y
5	43 (F)	Double lung transplant	ST258, KPC-3-producing CRKP	Pneumonia	iHD	C-A, 0.94 g i.v. q48h (11)	Failure	74	Relapsing intra-abdominal infection, treated with C-A for an additional 15 days	2	64	D179Y
6	59 (M)	Coronary artery disease, diabetes mellitus	ST258, KPC-3-producing CRKP	Pneumonia	47	C-A, 2.5 g i.v. q8h (11)	Failure	7	Relapsing bacteremia, treated with meropenem for 14 days and survived	2	64	D179Y
7	60 (F)	Double lung transplant	ST258, KPC-3-producing CRKP	Pneumonia	iHD	C-A, 0.94 g i.v. q48h (7)	Failure	26 7	Persistent pneumonia, treated with doripenem and gentamicin for 14 days	Not applicable	1) 4 2) 32 ^g 1	1) Wild type 2) D179Y Wild type
8	68 (M)	Double lung transplant	ST258, KPC-3-producing CRKP	Pneumonia	CRRT	C-A, 2.5 g i.v. q8h (14); i.v. and inhaled gentamicin (12)	Failure	33 42	Respiratory colonization, not treated with C-A for an additional 18 days and survived	2	1) 2 2) 4 ^g 2	1) Wild type 2) Wild type Wild type
								61	Relapsing tracheobronchitis, treated with C-A and inhaled gentamicin for 17 days	Not applicable	64	D179Y
									Persistent tracheobronchitis, treated with meropenem and gentamicin for 11 days until death	Not applicable	64	D179Y

^aPt, patient.

^bM, male; F, female.

^cs/p, status post.

^dCRE, carbapenem-resistant *Enterobacteriaceae*; CRKP, carbapenem-resistant *K. pneumoniae*.

^eCRRT, continuous renal replacement therapy; iHD, intermittent hemodialysis.

^fC-A, ceftazidime-avibactam; q8h, every 8 h.

^gTwo morphologies isolated from the same biologic specimen.

TABLE 5 Risk factors for ceftazidime-avibactam resistance among patients with microbiologic failure

Risk factor ^a	Value ^b for patients with:		P value	Multivariate P value (OR, 95% CI) ^c
	Resistance (n = 8)	No resistance (n = 17)		
Demographics and underlying conditions				
Male gender	4 (50)	13 (76)	0.36	
Age (yr)	63 (43–73)	65 (29–79)	0.98	
Solid organ transplant recipient	4 (50)	3 (18)	0.16	0.31
Charlson Comorbidity Index	4.5 (2–7)	7 (0–10)	0.68	
Severity of illness				
ICU at disease onset	7 (88)	12 (67)	0.63	
Renal replacement therapy	5 (63)	1 (6)	0.006	0.009 (26.67, 2.24–317.1)
SOFA score	6.5 (2–14)	5 (2–19)	0.68	
SAPS II score	37.5 (23–75)	43 (17–78)	0.38	
Infection characteristics				
Infection type				
Pneumonia	7 (88)	8 (47)	0.09	0.23
Primary bacteremia	0 (0)	5 (29)	0.14	
Urinary tract infection	0 (0)	3 (18)	0.53	
Intra-abdominal infection	1 (13)	0 (0)	0.32	
Other	0 (0)	1 (6)	1.00	
CRE pathogen				
<i>K. pneumoniae</i>	8 (100)	14 (82)	0.53	
<i>E. coli</i>	0 (0)	3 (18)	0.53	
Presence of <i>bla</i> _{KPC}	8 (100)	14 (82)	0.53	
KPC-3	8 (100)	6 (35)	0.003	Excluded
Treatment characteristics				
Time to treatment initiation (h)	74 (3–214)	62 (46–114)	0.17	0.52
Duration of initial treatment (days)	13.5 (10–25)	14 (4–71)	0.38	
Ceftazidime-avibactam monotherapy	6 (75)	11 (65)	1.00	

^aStatistically significant risk factors for ceftazidime-avibactam resistance are shown in boldface. ICU, intensive care unit.

^bValues for categorical variables are numbers (percentages) of patients with the risk factor. Values for continuous variables are medians (ranges).

^cKPC-3 was excluded from multivariate analysis because all cases of resistance were due to KPC-3-producing isolates. OR, odds ratio; CI, confidence interval.

seven patients, ceftazidime-avibactam-susceptible baseline isolates and resistant post-exposure isolates clustered within the novel ST258 clade II sublineage that we reported previously (3). The 21 isolates from these patients were phylogenetically distinct from reference ST258 clade II *K. pneumoniae* isolates from our center and others in the United States. In contrast, baseline and postexposure isolates from patient 6 did not cluster within previously defined ST258 clade II lineages (Fig. 1) (3). Within individual patients, the median number of core genome single nucleotide polymorphisms (SNPs) between baseline and subsequent isolates was 20; however, the isolates from patient 6 differed from all other isolates by a median of 157 SNPs. Antibiotic resistance genes and plasmids carried by baseline and postexposure isolates are presented in Table S1 in the supplemental material.

DISCUSSION

This is the largest single-center study to date of patients treated with ceftazidime-avibactam. Our findings corroborate previous data from our center and others on clinical outcomes among patients receiving ceftazidime-avibactam for CRE infections (1, 2, 5–8), as well as preliminary data from our center on the rates and mechanisms of resistance (1, 3, 9, 10). The study also reports new findings, that pneumonia and RRT are independent risk factors for clinical failure of ceftazidime-avibactam treatment and independent risk factors for microbiologic treatment failure and the emergence of resistance, respectively. Our experience provides important insights into the clinical utility of ceftazidime-avibactam against CRE infections and the challenges and unanswered questions facing clinicians as they seek to use the agent most effectively.

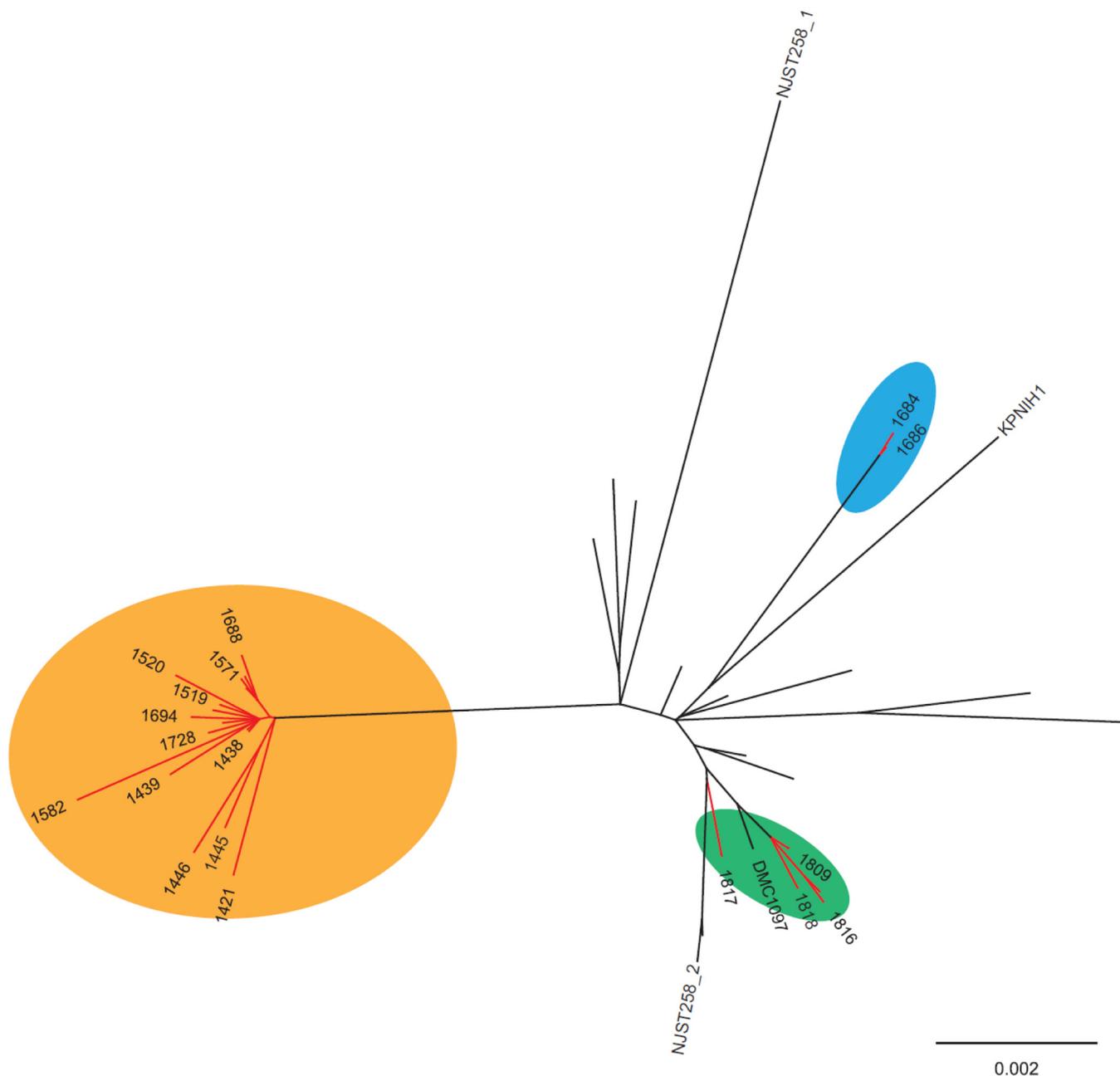


FIG 1 Phylogenetic comparison of ST258 clade II *K. pneumoniae* isolates from our center and others in the United States. The phylogenetic tree, based on core genome SNP analysis, was generated with the use of the maximum-likelihood optimality criterion. Branch lengths are proportional to the number of evolutionary changes, and all nodes had 100% bootstrap support. Twenty-three *K. pneumoniae* isolates from the present study and 22 isolates collected from hospitals in New York, New Jersey, Pennsylvania, Maryland, and Michigan in previous studies (41–43) were included. Isolates from our medical center are indicated by red lines. The colored ovals indicate the previously defined ST258 clade II sublineage associated with ceftazidime-avibactam resistance (orange) (3), the reference ST258 clade II lineage (green), and two distinct isolates from a single patient who developed ceftazidime-avibactam resistance (blue).

Overall 30-day survival and clinical success rates among our patients were 81% and 55%, respectively. Ceftazidime-avibactam treatment was not associated with excess mortality, compared to that predicted by SAPS II or SOFA scores (Table 2). In fact, among patients with middle-range SAPS II or SOFA scores, the mortality observed was less than that expected. Patients' outcomes differed strikingly by the type of infection. The clinical success rate for treating pneumonia, the most common infection in our cohort, was only 36%. In contrast, clinical success rates were 75%, 88%, and 67% for treating primary bacteremia, UTIs, and skin/soft tissue infections, respectively. By

Downloaded from <http://aac.asm.org/> on May 6, 2021 by guest

combining our data with those from other centers (5, 8), clinical success rates for ceftazidime-avibactam treatment of pneumonia and bacteremia are 43% (24/56) and 65% (45/69), respectively ($P = 0.02$). A previous study from our center reported that survival and clinical success rates were significantly higher if ceftazidime-avibactam, rather than other regimens, was used to treat carbapenem-resistant *K. pneumoniae* bacteremia (2). In a recent multicenter observational study of 137 patients with CRE infections, patients initially treated with ceftazidime-avibactam had lower rates of all-cause 30-day hospital mortality than patients initially treated with colistin (11). We reported previously that nephrotoxicity was significantly more likely with colistin- or aminoglycoside-containing regimens than with ceftazidime-avibactam (2). Here, AKI was documented in 11% of patients who were not on RRT at baseline; 43% of these patients were receiving colistin or an aminoglycoside with ceftazidime-avibactam. Therefore, a growing body of data demonstrates that ceftazidime-avibactam is a major advance in the treatment of CRE infections, offering an effective and well-tolerated alternative to salvage regimens.

It is not clear if ceftazidime-avibactam pharmacokinetics-pharmacodynamics contribute to worse outcomes among patients with CRE pneumonia. In healthy volunteers, both ceftazidime and avibactam demonstrated rapid penetration into the epithelial lining fluid (ELF), achieving average concentrations that were $\sim 30\%$ of those in plasma (12). These concentrations generally exceeded those that are effective in animal pneumonia models (13). However, there is no evidence as yet to support similar exposures among critically ill patients. Furthermore, the geometric mean maximum concentration [C_{\max}] of avibactam in ELF of healthy volunteers was 5.1 mg/liter, well below the concentration (8 mg/liter) that suppressed resistance amplification of a KPC-producing *K. pneumoniae* isolate in the hollow-fiber infection model (14). The importance of ELF avibactam exposures for patient outcomes or the development of resistance is unknown.

In a recently completed phase 3 randomized controlled trial, ceftazidime-avibactam was noninferior to meropenem among 527 clinically evaluable patients with nosocomial pneumonia. Clinical cure rates at the test-of-cure (TOC) visit were 77% (96/125) and 79% (103/131) for ceftazidime-avibactam and meropenem, respectively, among patients in the extended microbiologically evaluable population. Only six isolates in the study were CRE (five *K. pneumoniae* isolates and one *Serratia marcescens* isolate). The mortality rate among 56 patients with CRE pneumonia at our center and others who were treated with ceftazidime-avibactam is 39% (5, 8), which falls within the 34-to-41% range reported for 302 patients with CRE pneumonia in cohort studies prior to the availability of ceftazidime-avibactam (15–18). Further studies are needed to determine if poor pneumonia outcomes are due to underlying comorbid conditions and the severity of illness or to drug failure.

RRT was independently associated with ceftazidime-avibactam clinical failure and the emergence of resistance, suggesting that RRT may lead to inadequate drug exposures. Optimal dosing of ceftazidime-avibactam in patients with renal insufficiency, particularly those receiving continuous RRT (CRRT), is an ongoing challenge for clinicians. There are currently no ceftazidime-avibactam dosing recommendations in the setting of CRRT, and doses administered to patients differ considerably (1). A recent report described a patient on continuous venovenous hemofiltration (CVVH) who received 1.25 g ceftazidime-avibactam i.v. every 8 h and had adequate exposures throughout the dosing interval (19). The calculated mean CVVH extraction ratio percentages were 14.4% and 11.5% for ceftazidime and avibactam, respectively. Devising and validating ceftazidime-avibactam dosing strategies among patients requiring RRT, and CRRT specifically, are pressing priorities.

Ceftazidime-avibactam resistance emerged in 10% of patients, including 14% and 32% of those with KPC-producing *K. pneumoniae* infections and microbiologic treatment failures, respectively. The mechanisms of resistance were mutations in plasmid-borne bla_{KPC-3} , which resulted in variant KPC-3 enzymes. As reported previously, KPC-3 variants were generally associated with the restoration of carbapenem susceptibility

(Table S1 in the supplemental material) (3, 9, 10). The predominant mutation was D179Y within the KPC-3 Ω -loop, which forms the floor of the β -lactamase active site (3). In one patient, we identified a previously unreported KPC-3 variant (insertion of glutamic acid and leucine at amino acids 166 to 167 [166–167 EL ins]). Similar mutations have been selected during ceftazidime-avibactam exposure *in vitro* (20). It is not clear if a novel ST258 clade II *K. pneumoniae* sublineage at our center is particularly predisposed to KPC mutations and ceftazidime-avibactam resistance. Finally, clinicians should be aware that ceftazidime-avibactam resistant isolates may be reported as extended-spectrum- β -lactamase-producing, carbapenem-susceptible isolates rather than KPC-producing isolates (3, 10). Therefore, ceftazidime-avibactam susceptibility testing should be performed on all isolates recovered in cases of microbiologic treatment failure, regardless of the reported phenotype.

Combination therapy has been advocated as a strategy to improve clinical outcomes of CRE infections and prevent the emergence of further resistance (21–23). In a recent large retrospective multicenter study of CRE bacteremia that did not include treatment with ceftazidime-avibactam (24), combination therapy with active agents was not associated with an overall mortality rate lower than that for monotherapy with an active agent. However, combination therapy was associated with lower mortality in the subset of patients with higher severity-of-illness scores. Ceftazidime-avibactam combination therapy has not been linked with improved outcomes across retrospective cohort studies (1, 5, 8). In the present study, clinical success rates were nearly identical among patients receiving ceftazidime-avibactam monotherapy or combination therapy. In retrospective studies, it is impossible to rule out indication bias as an explanation for the lack of benefit with ceftazidime-avibactam combination regimens. Our data indicate that the administration of ceftazidime-avibactam was delayed by an average of 3 days from the time of culture collection. In the future, it will be important to determine if rapid CRE detection assays can shorten times to treatment with ceftazidime-avibactam (or other appropriate agents) and improve outcomes.

This study is limited by its single-center, retrospective design. We acknowledge that our results may not be representative of the experience at other institutions. Nevertheless, our report offers new insights into the utility and limitations of ceftazidime-avibactam across CRE infection types. Moving forward, priorities are to identify dosing and therapeutic regimens that improve upon the poor outcomes among patients with pneumonia and those receiving RRT. Ceftazidime-avibactam was approved by the FDA for the treatment of complicated UTIs and complicated intra-abdominal infections, but at many centers such as ours, its primary use will be in treating diverse CRE and other highly resistant bacterial infections. For this reason, it is important for clinicians to share their real-world experiences, so that the community can learn how to employ ceftazidime-avibactam most rationally. This mandate also holds for newly FDA approved agents, such as meropenem-vaborbactam, as they reach the clinic. Studies that characterize strain genomes, plasmid content, and resistance mechanisms will be necessary in order to refine CRE treatment paradigms.

MATERIALS AND METHODS

Study design and definitions. We conducted a retrospective study of patients with CRE infections who were treated with ceftazidime-avibactam for >48 h at the University of Pittsburgh Medical Center between April 2015 and April 2017. This study includes 38 patients who were previously reported from our center (1–3, 25).

CRE was defined by current Centers for Disease Control criteria as resistance to any carbapenem as determined by automated susceptibility testing methods (MicroScan; Beckman Coulter, Inc., Indianapolis, IN) in the clinical microbiology laboratory (26). A standard dosage of 2.5 g given intravenously (*i.v.*) every 8 h was used, with adjustments for renal impairment made according to manufacturer recommendations (27). Combination therapy was defined as concomitant administration (≥ 72 h) of another agent to which the CRE isolate was susceptible *in vitro*. Types of CRE infection were classified according to National Healthcare Safety Network (NHSN) criteria (28). Severity of illness was calculated using the simplified acute physiology (SAPS II) and sequential organ failure assessment (SOFA) scores (29, 30). Clinical success was defined as survival and absence of recurrence at 30 days following the onset of infection, resolution of signs and symptoms of infection, and the absence of microbiologic failure. Patient outcomes were determined by at least two independent investigators (from among R.K.S., M.H.N., and C.J.C.); in the

event of disagreement, cases were adjudicated by a third investigator. Microbiologic failure was defined as isolation of the same species following ≥ 7 days of ceftazidime-avibactam treatment. Recurring and relapsing infections were defined by microbiologic failure and concomitant signs of infection within 30 and 31 to 90 days of onset, respectively. Acute kidney injury (AKI) was defined by modified KDIGO guidelines as a $1.5\times$ increase in baseline serum creatinine levels within 7 days of treatment initiation (4).

CRE isolate characterization. MICs were measured for ceftazidime, ceftazidime-avibactam, ertapenem, and meropenem using reference Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods; avibactam was tested at a fixed concentration of $4\ \mu\text{g/ml}$ (31). Quality control (QC) was performed with *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853; all QC MICs were within the specified ranges (32). The presence or absence of β -lactamases was determined for all baseline CRE isolates as described previously (33, 34). In cases in which ceftazidime-avibactam resistance arose, baseline and postexposure isolates underwent whole-genome sequencing as described previously (3). In brief, core genome single nucleotide polymorphism (SNP) analysis was performed using BWA (35) and SAMtools (36), and a phylogenetic tree was generated using RAxML 8.0.0 (37) based on concatenated core SNPs. Twenty-two additional ST258 clade II genomes from a previous study were included for comparison (3). Sequences were assembled *de novo* using SPAdes 3.10.1 (38), followed by acquired resistance gene *in silico* mining using ResFinder (39). Plasmid replicon identification was performed using PlasmidFinder (40), as well as a BLASTN search against the complete sequenced plasmid database in GenBank.

Univariate comparisons between groups were made by the Fisher's exact test (for categorical variables) and Mann-Whitney U test (for continuous variables). Multivariate logistic regression was performed with backward selection procedures using covariates with a *P* value of <0.20 on univariate analysis. Significance was defined as a *P* value of ≤ 0.05 (two-tailed).

Accession number(s). The sequence data obtained in this study have been deposited in NCBI under BioProject number [PRJNA326665](https://doi.org/10.1093/bioinformatics/btq111).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.02497-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This study is supported by an investigator-initiated grant from Allergan awarded to R.K.S., and in part by National Institutes of Health (NIH) awards (K08AI114883 to R.K.S., R21AI117338 to L.C., R01AI090155 to B.N.K., R21AI111037 to C.J.C., and R21AI128338 to M.H.N.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

REFERENCES

- Shields RK, Potoski BA, Haidar G, Hao B, Doi Y, Chen L, Press EG, Kreiswirth BN, Clancy CJ, Nguyen MH. 2016. Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for carbapenem-resistant Enterobacteriaceae infections. *Clin Infect Dis* 63:1615–1618. <https://doi.org/10.1093/cid/ciw636>.
- Shields RK, Nguyen MH, Chen L, Press EG, Potoski BA, Marini RV, Doi Y, Kreiswirth BN, Clancy CJ. 2017. Ceftazidime-avibactam is superior to other treatment regimens against carbapenem-resistant *Klebsiella pneumoniae* bacteremia. *Antimicrob Agents Chemother* 61:e00883–17. <https://doi.org/10.1128/AAC.00883-17>.
- Shields RK, Chen L, Cheng S, Chavda KD, Press EG, Snyder A, Pandey R, Doi Y, Kreiswirth BN, Nguyen MH, Clancy CJ. 2017. Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla_{KPC-3} mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. *Antimicrob Agents Chemother* 61:e02097-16. <https://doi.org/10.1128/AAC.02097-16>.
- Palevsky PM, Liu KD, Brophy PD, Chawla LS, Parikh CR, Thakar CV, Tolwani AJ, Waikar SS, Weisbord SD. 2013. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am J Kidney Dis* 61:649–672. <https://doi.org/10.1053/j.ajkd.2013.02.349>.
- King M, Heil E, Kuriakose S, Bias T, Huang V, El-Beyrouy C, McCoy D, Hiles J, Richards L, Gardner J, Harrington N, Bason K, Gallagher J. 2017. Multicenter study of outcomes with ceftazidime-avibactam in patients with carbapenem-resistant Enterobacteriaceae infections. *Antimicrob Agents Chemother* 61:e00449-17. <https://doi.org/10.1128/AAC.00449-17>.
- Castón JJ, Lacort-Peralta I, Martín-Dávila P, Loeches B, Tabares S, Temkin L, Torre-Cisneros J, Paño-Pardo JR. 2017. Clinical efficacy of ceftazidime/avibactam versus other active agents for the treatment of bacteremia due to carbapenemase-producing Enterobacteriaceae in hematologic patients. *Int J Infect Dis* 59:118–123. <https://doi.org/10.1016/j.ijid.2017.03.021>.
- Krapp F, Grant JL, Sutton SH, Ozer EA, Barr VO. 2017. Treating complicated carbapenem-resistant Enterobacteriaceae infections with ceftazidime/avibactam: a retrospective study with molecular strain characterization. *Int J Antimicrob Agents* 49:770–773. <https://doi.org/10.1016/j.ijantimicag.2017.01.018>.
- Temkin E, Torre-Cisneros J, Beovic B, Benito N, Giannella M, Gilarranz R, Jeremiah C, Loeches B, Machuca I, Jimenez-Martin MJ, Martinez JA, Mora-Rillo M, Navas E, Osthoff M, Pozo JC, Ramos Ramos JC, Rodriguez M, Sanchez-Garcia M, Viale P, Wolff M, Carmeli Y. 2017. Ceftazidime-avibactam as salvage therapy for infections caused by carbapenem-resistant organisms. *Antimicrob Agents Chemother* 61:e01964-16. <https://doi.org/10.1128/AAC.01964-16>.
- Shields RK, Nguyen MH, Press EG, Chen L, Kreiswirth BN, Clancy CJ. 2017. *In vitro* selection of meropenem resistance among ceftazidime-avibactam-resistant, meropenem-susceptible *Klebsiella pneumoniae* isolates with variant KPC-3 carbapenemases. *Antimicrob Agents Chemother* 61:e00079-17. <https://doi.org/10.1128/AAC.00079-17>.
- Haidar G, Clancy CJ, Shields RK, Hao B, Cheng S, Nguyen MH. 2017. Mutations in bla_{KPC-3} that confer ceftazidime-avibactam resistance encode novel KPC-3 variants that function as extended-spectrum β -lactamases. *Antimicrob Agents Chemother* 61:e02534-16. <https://doi.org/10.1128/AAC.02534-16>.

11. van Duin D, Lok JJ, Earley M, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins RR, Doi Y, Kaye KS, Fowler VG, Jr, Paterson DL, Bonomo RA, Evans S; Antibacterial Resistance Leadership Group. 2018. Colistin versus ceftazidime-avibactam in the treatment of infections due to carbapenem-resistant Enterobacteriaceae. *Clin Infect Dis* 66:163–171. <https://doi.org/10.1093/cid/cix783>.
12. Nicolau DP, Siew L, Armstrong J, Li J, Edeki T, Learoyd M, Das S. 2015. Phase 1 study assessing the steady-state concentration of ceftazidime and avibactam in plasma and epithelial lining fluid following two dosing regimens. *J Antimicrob Chemother* 70:2862–2869. <https://doi.org/10.1093/jac/dkv170>.
13. Housman ST, Crandon JL, Nichols WW, Nicolau DP. 2014. Efficacies of ceftazidime-avibactam and ceftazidime against *Pseudomonas aeruginosa* in a murine lung infection model. *Antimicrob Agents Chemother* 58:1365–1371. <https://doi.org/10.1128/AAC.02161-13>.
14. Louie A, Castanheira M, Liu W, Grasso C, Jones RN, Williams G, Critchley I, Thye D, Brown D, Vanscoy B, Kulawy R, Drusano GL. 2012. Pharmacodynamics of beta-lactamase inhibition by NXL104 in combination with ceftaroline: examining organisms with multiple types of beta-lactamases. *Antimicrob Agents Chemother* 56:258–270. <https://doi.org/10.1128/AAC.05005-11>.
15. Tumbarello M, Trearichi EM, De Rosa FG, Giannella M, Giacobbe DR, Bassetti M, Losito AR, Bartoletti M, Del Bono V, Corcione S, Maiuro G, Tedeschi S, Celani L, Cardellino C, Spanu T, Marchese A, Ambretti S, Cauda R, Viscoli C, Viale P, Isgri S. 2015. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. *J Antimicrob Chemother* 70:2133–2143. <https://doi.org/10.1093/jac/dkv086>.
16. Alexander EL, Loutit J, Tumbarello M, Wunderink R, Felton T, Daikos G, Fusaro K, White D, Zhang S, Dudley MN. 2017. Carbapenem-resistant Enterobacteriaceae infections: results from a retrospective series and implications for the design of prospective clinical trials. *Open Forum Infect Dis* 4:ofx063. <https://doi.org/10.1093/ofid/ofx063>.
17. de Maio Carrilho CM, de Oliveira LM, Gaudereto J, Perozin JS, Urbano MR, Camargo CH, Grion CM, Levin AS, Costa SF. 2016. A prospective study of treatment of carbapenem-resistant Enterobacteriaceae infections and risk factors associated with outcome. *BMC Infect Dis* 16:629. <https://doi.org/10.1186/s12879-016-1979-z>.
18. Hauck C, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins RR, Scalera NM, Doi Y, Kaye KS, Evans S, Fowler VG, Jr, Bonomo RA, van Duin D, Antibacterial Resistance Leadership Group. 2016. Spectrum of excess mortality due to carbapenem-resistant *Klebsiella pneumoniae* infections. *Clin Microbiol Infect* 22:513–519. <https://doi.org/10.1016/j.cmi.2016.01.023>.
19. Wenzler E, Bunnell KL, Bleasdale SC, Benken S, Danziger LH, Rodvold KA. 2017. Pharmacokinetics and dialytic clearance of ceftazidime-avibactam in a critically ill patient on continuous venovenous hemofiltration. *Antimicrob Agents Chemother* 61:e00464-17. <https://doi.org/10.1128/AAC.00464-17>.
20. Livermore DM, Warner M, Jamrozny D, Mushtaq S, Nichols WW, Mustafa N, Woodford N. 2015. In vitro selection of ceftazidime-avibactam resistance in Enterobacteriaceae with KPC-3 carbapenemase. *Antimicrob Agents Chemother* 59:5324–5330. <https://doi.org/10.1128/AAC.00678-15>.
21. Daikos GL, Tsousi S, Tzouveleki LS, Anyfantis I, Psychogiou M, Argyropoulou A, Stefanou I, Sypsa V, Miriagou V, Nepka M, Georgiadou S, Markogiannakis A, Goukos D, Skoutelis A. 2014. Carbapenemase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. *Antimicrob Agents Chemother* 58:2322–2328. <https://doi.org/10.1128/AAC.02166-13>.
22. Tzouveleki LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. 2014. Treating infections caused by carbapenemase-producing Enterobacteriaceae. *Clin Microbiol Infect* 20:862–872. <https://doi.org/10.1111/1469-0691.12697>.
23. Tumbarello M, Viale P, Viscoli C, Trearichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M. 2012. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin Infect Dis* 55:943–950. <https://doi.org/10.1093/cid/cis588>.
24. Gutiérrez-Gutiérrez B, Salamanca E, de Cueto M, Hsueh PR, Viale P, Paño-Pardo JR, Venditti M, Tumbarello M, Daikos G, Cantón R, Doi Y, Tuon FF, Karaiskos I, Pérez-Nadales E, Schwaber MJ, Azap OK, Souli M, Roilides E, Pournaras S, Akova M, Pérez F, Bermejo J, Oliver A, Almela M, Lowman W, Almirante B, Bonomo RA, Carmeli Y, Paterson DL, Pascual A, Rodríguez-Baño J; REIPI/ESGBIS/INCREMENT Investigators. 2017. Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study. *Lancet Infect Dis* 17:726–734. [https://doi.org/10.1016/S1473-3099\(17\)30228-1](https://doi.org/10.1016/S1473-3099(17)30228-1).
25. Shields RK, Nguyen MH, Press EG, Chen L, Kreiswirth BN, Clancy CJ. 2017. Emergence of ceftazidime-avibactam resistance and restoration of carbapenem susceptibility in *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: a case report and review of literature. *Open Forum Infect Dis* 4:ofx101. <https://doi.org/10.1093/ofid/ofx101>.
26. Centers for Disease Control and Prevention. 29 June 2015, revision date. Healthcare-associated infections: FAQs about choosing and implementing a CRE definition. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.cdc.gov/hai/organisms/cre/definition.html>.
27. Forest Pharmaceuticals, Inc. 2016. Acyczac (ceftazidime-avibactam) prescribing information. Forest Pharmaceuticals, Inc., Cincinnati, OH.
28. Centers for Disease Control and Prevention, National Healthcare Safety Network. January 2018. CDC/NHSN surveillance definitions for specific types of infections. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.
29. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S. 1998. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on “sepsis-related problems” of the European Society of Intensive Care Medicine. *Crit Care Med* 26:1793–1800.
30. Le Gall JR, Lemeshow S, Saulnier F. 1993. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA* 270:2957–2963. <https://doi.org/10.1001/jama.1993.03510240069035>.
31. Shields RK, Clancy CJ, Hao B, Chen L, Press EG, Iovine NM, Kreiswirth BN, Nguyen MH. 2015. Effects of *Klebsiella pneumoniae* carbapenemase subtypes, extended-spectrum β -lactamases, and porin mutations on the in vitro activity of ceftazidime-avibactam against carbapenem-resistant *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 59:5793–5797. <https://doi.org/10.1128/AAC.00548-15>.
32. Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing (M100-S24). Clinical and Laboratory Standards Institute, Wayne, PA.
33. Clancy CJ, Chen L, Shields RK, Zhao Y, Cheng S, Chavda KD, Hao B, Hong JH, Doi Y, Kwak EJ, Silveira FP, Abdel-Massih R, Bogdanovich T, Humar A, Perlin DS, Kreiswirth BN, Nguyen MH. 2013. Epidemiology and molecular characterization of bacteremia due to carbapenem-resistant *Klebsiella pneumoniae* in transplant recipients. *Am J Transplant* 13:2619–2633. <https://doi.org/10.1111/ajt.12424>.
34. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J Antimicrob Chemother* 65:490–495. <https://doi.org/10.1093/jac/dkp498>.
35. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
36. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
37. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
38. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
39. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
40. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing.

- Antimicrob Agents Chemother 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
41. Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN. 2014. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. *Proc Natl Acad Sci U S A* 111: 4988–4993. <https://doi.org/10.1073/pnas.1321364111>.
42. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Henderson DK, Palmore TN, Segre JA. 2012. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Transl Med* 4: 148ra116. <https://doi.org/10.1126/scitranslmed.3004129>.
43. Wright MS, Perez F, Brinkac L, Jacobs MR, Kaye K, Cober E, van Duin D, Marshall SH, Hujer AM, Rudin SD, Hujer KM, Bonomo RA, Adams MD. 2014. Population structure of KPC-producing *Klebsiella pneumoniae* isolates from Midwestern U.S. hospitals. *Antimicrob Agents Chemother* 58:4961–4965. <https://doi.org/10.1128/AAC.00125-14>.