Multicenter Clinical and Molecular Epidemiological Analysis of Bacteremia Due to Carbapenem-Resistant *Enterobacteriaceae* (CRE) in the CRE Epicenter of the United States

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ABSTRACT Although the New York/New Jersey (NY/NJ) area is an epicenter for carbapenem-resistant *Enterobacteriaceae* (CRE), there are few multicenter studies of CRE from this region. We characterized patients with CRE bacteremia in 2013 at eight NY/NJ medical centers and determined the prevalence of carbapenem resistance among *Enterobacteriaceae* bloodstream isolates and CRE resistance mechanisms, genetic backgrounds, capsular types (*cps*), and antimicrobial susceptibilities. Of 121 patients with CRE bacteremia, 50% had cancer or had undergone transplantation. The prevalences of carbapenem resistance among *Klebsiella pneumoniae*, *Enterobacter* spp., and *Escherichia coli* bacteremias were 9.7%, 2.2%, and 0.1%, respectively. Ninety percent of CRE were *K. pneumoniae* and 92% produced *K. pneumoniae* carbapenemase (KPC-3, 48%; KPC-2, 44%). Two CRE produced NDM-1 and OXA-48 carbapenemases. Sequence type 258 (ST258) predominated among KPC-producing *K. pneumoniae* (KPC-Kp). The *wzi154* allele, corresponding to *cps*-2, was present in 93% of KPC-3-Kp, whereas KPC-2-Kp had greater *cps* diversity. Ninety-nine percent of CRE were ceftazidime-avibactam (CAZ-AVI)-susceptible, although 42% of KPC-3-Kp had an CAZ-AVI MIC of $\geq 4/4$ $\mu\text{g/ml}$. There was a median of 47 h from bacteremia onset until active antimicrobial therapy, 38% of patients had septic shock, and 49% died within 30 days. KPC-3-Kp bacteremia (adjusted odds ratio [aOR], 2.58; $P = 0.045$), cancer (aOR, 3.61, $P = 0.01$), and bacteremia onset in the intensive care unit (aOR, 3.79; $P = 0.03$) were independently associated with mortality. Active empirical ther-

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apy and combination therapy were not associated with survival. Despite a decade of experience with CRE, patients with CRE bacteremia have protracted delays in appropriate therapies and high mortality rates, highlighting the need for rapid diagnostics and evaluation of new therapeutics.

KEYWORDS carbapenem-resistant *Enterobacteriaceae*, clinical outcomes, molecular epidemiology, resistance mechanisms

Enterobacteriaceae cause 30% of health care-associated infections and until recently, were reliably susceptible to carbapenems (1, 2). Unfortunately, carbapenem-resistant *Enterobacteriaceae* (CRE) have now emerged worldwide as an urgent antibiotic resistance threat (3). CRE are not only carbapenem-resistant, but are resistant to most other antibiotic classes. Until recently, treatment options for infections caused by CRE were limited to agents such as polymyxins and tigecycline (4). Furthermore, the identification of CRE by culture typically takes 2 to 3 days, leading to delays in appropriate therapy, and patients infected with CRE have high mortality rates (5–8).

The emergence of CRE is largely due to the spread of mobile genes on plasmids that encode enzymes (carbapenemases) that hydrolyze carbapenems and other β -lactam agents (9). The New York/New Jersey area (NY/NJ) has been a global epicenter for CRE since the early 2000s, primarily due to the emergence of a sequence type of *Klebsiella pneumoniae* (ST258) that produces *K. pneumoniae* carbapenemase (KPC) (4, 10–13). Since then, NY/NJ has had the highest prevalence of CRE in the United States and is a major source of the worldwide spread of CRE (1, 11, 14). However, multicenter studies of the clinical and molecular epidemiology of CRE in this region have not been conducted. Therefore, we formed a consortium of NY/NJ medical centers to investigate the contemporary epidemiology of CRE bacteremia in this area where it is endemic.

(This work was presented, in part, at IDWeek 2015, San Diego, CA, 7 to 11 October 2015 [15]).

RESULTS

Prevalence of carbapenem resistance. The proportion of *K. pneumoniae* bloodstream infections ($n = 1133$) that were carbapenem-resistant was 9.7% (95% confidence interval [CI], 8.1% to 11.6%), with a range of 3.1 to 17.2% across study centers (Table 1). New York City (NYC) medical centers had a greater prevalence of carbapenem-resistant *K. pneumoniae* (CRKp) than centers outside NYC (11.9% versus 4.5%, respectively; $P < 0.001$). The proportions of bacteremias due to *Escherichia coli* ($n = 2550$) and *Enterobacter* spp. ($n = 279$) that were carbapenem-resistant were 0.1% (95% CI, 0% to 0.3%) and 2.2% (95% CI, 0.8% to 4.6%), respectively.

Characterization of CRE bloodstream isolates. One hundred twenty-one patients with CRE bacteremia were included in our analysis, including 109 (90%) episodes caused by *K. pneumoniae*, 5 (4%) by *Enterobacter* spp., 3 (2%) by *E. coli*, 1 each by *Klebsiella oxytoca*, *Morganella morganii*, and *Proteus mirabilis*, and 1 polymicrobial episode (*K. pneumoniae* and *Pantoea* [*Enterobacter*] *agglomerans*). Genotyping was performed on 106 isolates (Table 2). Carbapenemase genes were detected in 100 (94%) of these genotyped isolates and 98 (92%) of them were *bla*_{KPC}. We detected one *bla*_{OXA-48}-producing *K. pneumoniae* and one New Delhi metallo- β -lactamase (*bla*_{NDM-1}-producing *E. coli*). Among the carbapenemase-producing isolates, 85% possessed at least two additional β -lactamases, including 36% with an extended-spectrum β -lactamase (ESBL).

*bla*_{KPC-3} was identified in 48 (49%) of the 97 genotyped CRKp, and *bla*_{KPC-2} was identified in 44 (45%) of the CRKp. Of 92 KPC-producing *K. pneumoniae* (KPC-Kp), 77 (84%) were ST258, whereas only one of five non-KPC-producing CRKp was ST258. Other CRKp sequence types are presented in Fig. 1a. Fifty-one percent of ST258 KPC-2-Kp had the *wzi29* capsular polysaccharide gene allele (corresponding to ST258 clade I, *cps-1* group) (12, 16), with alleles *wzi83*, *wzi50*, and *wzi154* also being common (Fig. 1b).

TABLE 1 Prevalence of carbapenem resistance and resistance mechanisms among bloodstream isolates of *Klebsiella pneumoniae*, *Enterobacter* spp., and *Escherichia coli*, stratified by study site

Medical center ^a	No. of carbapenem-resistant bloodstream isolates/total no. of bloodstream isolates ^b (%)			No. of carbapenem resistance mechanisms ^c		
	<i>K. pneumoniae</i>	<i>Enterobacter</i> spp.	<i>E. coli</i>	KPC-2	KPC-3	Mechanism other than KPC
A	22/128 (17.2)	2/36 (5.6)	0/259	12	10	2
B	24/142 (16.9)	0/34	0/316	13	11	— ^d
C	18/201 (9.0)	0/48	1/353 (0.3)	7	11	1
D	7/110 (6.4)	1/30 (3.3)	1/175 (0.6)	2	4	3
E	24/220 (10.9)	1/46 (2.2)	0/559	9	4	—
F	5/77 (6.5)	0/22	1/182 (0.5)	1	2	2
G	5/162 (3.1)	1/40 (2.5)	0/471	2	4	—
H	5/93 (5.4)	1/23 (4.3)	0/235	1	5	—
Total	110/1133 (9.7)	6/279 (2.2)	3/2550 (0.1)	47	51	8

^aMedical centers F to H were located outside New York City. A subset of bacteremia episodes from Medical Centers A and B were included in other analyses (13, 54).

^bUsing only the first bloodstream isolate of each species per patient.

^cSome isolates from medical centers E and F were not available for genotypic analysis to determine the carbapenem resistance mechanism.

^d—, not available.

Conversely, the *wzi154* allele (corresponding to ST258 clade II, *cps-2* group) was identified in 39 (93%) of 42 ST258 KPC-3-*Kp*.

Mutations in *ompK35* and *ompK36* genes were present in 84% and 34% of CRKp, respectively (Table 2). All ST258 KPC-*Kp* possessed mutations in *ompK35*, whereas none of the non-ST258 KPC-*Kp* possessed mutations in either *ompK35* or *ompK36*. *K. pneumoniae* strains possessing *bla*_{KPC-3} were more likely to have a mutation in the *ompK36* gene than *K. pneumoniae* strains possessing *bla*_{KPC-2} (45% versus 23%, respectively; *P* = 0.03). CRKp isolates with mutations in *ompK35* or *ompK36* were more likely to have high-level carbapenem resistance than those without a porin gene mutation (96% versus 73%, respectively; *P* = 0.01).

Less than 3% of CRE isolates were susceptible to piperacillin-tazobactam and aztreonam and 13% were susceptible to cefepime. The proportions of isolates susceptible to non-β-lactam agents were as follows: tigecycline (98%), colistin (83%), polymyxin B (83%), doxycycline (62%), minocycline (60%), gentamicin (47%), amikacin (37%), trimethoprim-sulfamethoxazole (20%), ciprofloxacin (12%), levofloxacin (12%), and tobramycin (12%). None of the polymyxin-resistant CRE harbored *mcr-1*.

TABLE 2 β-Lactamases and *Klebsiella pneumoniae* outer membrane porin gene mutations among 106 genotyped CRE^a

CRE pathogen	No. of isolates (%)									
	KPC				SHV				<i>ompK35</i> mutation	<i>ompK36</i> mutation
	KPC-2	KPC-3	NDM	OXA-48 ^b	TEM non-ESBL ^c	non-ESBL	ESBL	CTX-M	AmpC	
<i>K. pneumoniae</i> (n = 97)	44 (45)	48 (49)		1 (1)	82 (85)	62 (64)	31 (32)	8 (8)		
KPC-2 (n = 44)	44 (100)	—			37 (84)	27 (61)	17 (39)	2 (5)		
KPC-3 (n = 48)		48 (100)			43 (90)	31 (65)	13 (27)	2 (4)		
Other (n = 5) ^d				1 (20)	2 (40)	4 (80)	1 (20)	4 (80)		
<i>Enterobacter</i> spp. (n = 5) ^e	1 (20)	3 (60)			4 (80)			1 (20)	4 (80)	N/A
<i>Escherichia coli</i> (n = 3) ^f	1 (33)		1 ^g (33)		1 (33)			2 (67)	1 (33)	N/A
<i>Morganella morganii</i> (n = 1)	1 (100)								1 (100)	N/A

^aCRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; N/A, not applicable.

^bThe OXA-48-producing *K. pneumoniae* also harbored SHV-11, CTX-M-15, and mutations in *ompK35* and *ompK36*.

^cNo ESBL TEM enzymes were identified.

^dOf the 4 *K. pneumoniae* that did not harbor a carbapenemase, 3 harbored CTX-M β-lactamases and mutations in *ompK35* or *ompK36* and 1 harbored wild-type SHV and TEM β-lactamases with an *ompK35* mutation.

^eOne *Enterobacter* sp. did not harbor a carbapenemase but harbored AmpC (ACT) and CTX-M β-lactamases.

^fOne *E. coli* did not harbor a carbapenemase but harbored a CTX-M β-lactamase.

^gThe NDM-1-producing *E. coli* also harbored CTX-M and AmpC (CMY) β-lactamases.

FIG 1 Multilocus sequence types (MLST) (a) and *wzi* gene alleles, stratified by MLST and KPC type (b), among carbapenem-resistant *Klebsiella pneumoniae*.

Of 104 isolates tested for susceptibility to ceftazidime-avibactam, 103 (99%) were susceptible based on the FDA breakpoint of $\leq 8/4$ $\mu\text{g/ml}$. The resistant isolate was an NDM-producing *E. coli* with a ceftazidime-avibactam MIC of >256 $\mu\text{g/ml}$. The ceftazidime-avibactam MIC₅₀ was greater for KPC-3-Kp (2 $\mu\text{g/ml}$) than for KPC-2-Kp (1 $\mu\text{g/ml}$), and the MIC was ≥ 4 $\mu\text{g/ml}$ for 42% of KPC-3-Kp compared with for 2% of KPC-2-Kp ($P < 0.001$; Table 3). Ceftazidime-avibactam MICs were also higher in isolates

TABLE 3 Ceftazidime-avibactam MICs among KPC-Kp, stratified by mutations in *ompK35* and *ompK36* outer membrane porin genes and KPC type^a

Organism/group (n)	No. (%) of isolates with ceftazidime-avibactam MICs ^b ($\mu\text{g/ml}$) of:						MIC ₅₀ ($\mu\text{g/ml}$)	MIC ₉₀ ($\mu\text{g/ml}$)
	0.25	0.5	1	2	4 ^c	8 ^d		
All KPC-Kp (n = 92)	4 (4)	2 (2)	27 (29)	38 (41)	20 (22)	1 (1)	2	4
Mutations in <i>ompK35</i> and <i>ompK36</i>								
Wild type (n = 15)	4 (27)	2 (13)	7 (47)	2 (13)	0	0	1	2
<i>ompK35</i> mutation only (n = 46) ^e	0	0	18 (39)	20 (43)	7 (15)	1 (2)	2	4
<i>ompK35</i> and <i>ompK36</i> mutations (n = 31) ^e	0	0	2 (6)	16 (52)	13 (42)	0	2	4
KPC-2 (n = 44) ^f								
Wild type (n = 9)	1 (11)	2 (22)	5 (56)	1 (11)	0	0	1	1
<i>ompK35</i> mutation only (n = 25)	0	0	14 (56)	10 (40)	1 (4)	0	1	2
<i>ompK35</i> and <i>ompK36</i> mutations (n = 10)	0	0	2 (20)	8 (80)	0	0	2	2
KPC-3 (n = 48) ^f								
Wild type (n = 6)	3 (50)	0	2 (33)	1 (33)	0	0	0.5	2
<i>ompK35</i> mutation only (n = 21)	0	0	4 (19)	10 (48)	6 (29)	1 (5)	2	4
<i>ompK35</i> and <i>ompK36</i> mutations (n = 21)	0	0	0	8 (38)	13 (62)	0	4	4

^aKPC-Kp, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.

^bA fixed concentration of 4 $\mu\text{g/ml}$ of avibactam was used to determine ceftazidime-avibactam MICs.

^cThe Clinical and Laboratory Standards Institute susceptible breakpoint for ceftazidime and *Enterobacteriaceae* is 4 $\mu\text{g/ml}$.

^dThe U.S. FDA susceptible breakpoint for ceftazidime-avibactam and *Enterobacteriaceae* is 8/4 $\mu\text{g/ml}$.

^eCeftazidime-avibactam MICs for KPC-Kp with *ompK35* and *ompK36* mutations were greater than those for KPC-Kp with *ompK35* mutations only ($P < 0.001$). MICs for KPC-Kp with *ompK35* mutations only were greater than those for KPC-Kp with wild-type genes ($P < 0.001$).

^fCeftazidime-avibactam MICs for KPC-3-Kp were greater than those for KPC-2-Kp ($P < 0.001$).

of *K. pneumoniae* with mutations in *ompK35* and *ompK36* than for those isolates that lacked porin gene mutations ($P < 0.001$; Table 3). KPC-3-*Kp* with *ompK35* and *ompK36* mutations were the least susceptible strains, with 62% of these isolates having a ceftazidime-avibactam MIC of 4 $\mu\text{g/ml}$. Ceftazidime-avibactam MICs were not correlated with the numbers of β -lactamase genes or the multilocus sequence types (MLSTs).

Characteristics of patients with CRE bacteremia. Patients with CRE bacteremia had a median age of 65 years (Table 4). Fifty percent of patients were receiving treatment for cancer or were transplant recipients. Other common comorbidities were diabetes (30%) and heart failure (17%). The most common bacteremia sources were intra-abdominal (42%) and urinary tract (21%) infections. Twenty-eight patients (23%) recently underwent endoscopic procedures, of which 71% were endoscopic retrograde cholangiopancreatography (ERCP). The median time from endoscopic procedure to CRE bacteremia was 7 days, and 24 (86%) of these patients did not have positive cultures for CRE before the procedure. Two-thirds of CRE bacteremia episodes were hospital-onset cases, and the majority of these cases occurred outside the intensive care unit (ICU). Overall, 17% of cases occurred in patients at a long-term-care facility, and 17% were community-onset cases. All but four community-onset cases had recent hospitalizations.

Treatments and outcomes. Only 16% of patients received active empirical therapy within 12 h of bacteremia onset (Table 4). Greater percentages of patients who received active empirical therapy presented with septic shock (53% versus 36%, respectively; $P = 0.16$) and required mechanical ventilation (47% versus 29%, respectively; $P = 0.10$) compared with patients who did not receive active empirical therapy, although these differences were not statistically significant. Twelve patients (10%) never received active therapy, of whom nine died before antimicrobial susceptibility testing results were available. Among the other patients, there was a median of 47 h (interquartile range [IQR], 17 to 70) from bacteremia onset until receipt of active therapy. Septic shock occurred within 2 days of bacteremia onset in 38% of patients.

The overall 14-day and 30-day mortality rates were 34% and 49%, respectively. Fifty percent of patients died during their index hospitalization. Among patients who died within 30 days, there was a median of 10 days from bacteremia onset until death. Patients with bacteremia due to *wzi154* CR*Kp* and KPC-3-*Kp* had greater 14-day (*wzi154*: 49% versus 22%, respectively; $P = 0.004$; KPC-3-*Kp*: 44% versus 24%, respectively; $P = 0.03$) and 30-day (*wzi154*: 63% versus 38%, respectively; $P = 0.01$; KPC-3-*Kp*: 58% versus 40%, respectively; $P = 0.055$) mortality rates than patients with other types of CRE bacteremia (Table 4). Neither the presence of high-level carbapenem resistance or *ompK35* and *ompK36* mutations was associated with increased mortality. Other baseline factors associated with increased 30-day mortality in univariate analysis were having a bacteremia source other than the urinary tract, having cancer, a bacteremia onset in the ICU, and receiving mechanical ventilation.

In multivariable analysis, KPC-3-*Kp* bacteremia (adjusted odds ratio [aOR], 2.58; 95% CI, 1.02 to 6.53; $P = 0.045$), cancer (aOR, 3.61; 95% CI, 1.30 to 10.02; $P = 0.01$), and bacteremia onset in the ICU (aOR, 3.79; 95% CI, 1.17 to 12.24; $P = 0.03$) were independently associated with increased 30-day mortality. Urinary tract source (aOR, 0.25; 95% CI, 0.07 to 0.99; $P = 0.049$) was independently associated with decreased mortality.

Definitive therapy. Ninety-eight (81%) patients were eligible for the definitive therapy analyses. Patients who received one active agent (monotherapy) had a 38% 30-day mortality rate, and this rate was similar among different antimicrobial therapies (Table 5). Forty percent of patients who received monotherapy also received a carbapenem. Patients who received ≥ 2 active agents (combination therapy) had a 51% mortality rate ($P = 0.19$ compared with that with monotherapy). Patients whose regimens included a carbapenem had a trend toward increased mortality compared with that in patients who did not receive a carbapenem (55% versus 37%, respectively; $P = 0.07$). However, after adjusting for age, infection source, cancer, location of

TABLE 4 Baseline characteristics of CRE bacteremia episodes and comparison of 30-day survivors to non-survivors

	No. (%) of episodes			
Variable ^a	Total (n = 121)	Survivors (n = 62)	Non-survivors (n = 59)	P value
Bacteremia characteristics				
CRE types				
<i>Klebsiella pneumoniae</i>	109 (90)	57 (92)	52 (88)	0.49
KPC- <i>K. pneumoniae</i> ^b	92 (87)	45 (82)	47 (92)	0.12
KPC-2- <i>K. pneumoniae</i>	44 (42)	25 (45)	19 (37)	0.39
KPC-3- <i>K. pneumoniae</i>	48 (45)	20 (36)	28 (55)	0.055
KPC and ESBL-producing <i>K. pneumoniae</i>	34 (32)	18 (33)	16 (31)	0.55
ST258 <i>K. pneumoniae</i> ^b	78 (74)	38 (69)	40 (78)	0.28
<i>ompK36</i> -mutant <i>K. pneumoniae</i> ^{b,c}	33 (31)	17 (31)	16 (31)	0.96
<i>Wzi</i> allele 154 ^b	43 (41)	16 (29)	27 (53)	0.01
<i>Enterobacter</i> species	5 (4)	2 (3)	3 (5)	0.68
<i>Escherichia coli</i>	3 (2)	2 (3)	1 (2)	1.00
Other ^d	4 (3)	1 (2)	3 (5)	0.36
High-level carbapenem resistance ^{b,e}	91 (86)	45 (82)	46 (90)	0.22
Polymicrobial bacteremia	31 (34)	15 (24)	16 (27)	0.71
Bacteremia source				
Intra-abdominal ^f	51 (42)	24 (39)	27 (46)	0.43
Urinary tract	25 (21)	20 (32)	5 (8)	0.001
Pneumonia	11 (9)	6 (10)	5 (8)	0.82
Vascular catheter	8 (7)	5 (8)	3 (5)	0.51
Skin and soft tissue	7 (6)	3 (5)	4 (7)	0.71
Unknown	19 (16)	4 (6)	15 (25)	0.004
Patient characteristics				
Age (years) (median [IQR])	65 (50–75)	64 (53–75)	66 (50–76)	0.94
Male sex	70 (58)	35 (57)	35 (59)	0.85
Comorbidities				
Cancer	48 (40)	19 (31)	29 (49)	0.04
Solid tumor	26 (22)	10 (16)	16 (27)	0.14
Hematologic malignancy	22 (18)	9 (14)	13 (22)	0.28
Neutropenic	18 (15)	6 (10)	12 (20)	0.10
Diabetes	36 (30)	21 (34)	15 (25)	0.31
Congestive heart failure	20 (17)	10 (16)	10 (17)	0.90
ESRD on dialysis	13 (11)	5 (8)	8 (14)	0.33
Transplant recipient	20 (17)	11 (18)	9 (15)	0.71
Solid organ transplant	14 (12)	8 (13)	6 (10)	0.64
Bone marrow transplant	6 (5)	3 (5)	3 (5)	1.00
Chronic lung disease	12 (10)	8 (13)	4 (7)	0.26
Cirrhosis	8 (7)	3 (5)	5 (8)	0.48
HIV infection	2 (2)	0	2 (3)	0.24
Healthcare exposures within 30 days				
Endoscopic procedures	28 (23)	10 (16)	18 (31)	0.06
Surgery	28 (23)	16 (26)	12 (20)	0.48
Onset of bacteremia				
Community onset	21 (17)	14 (23)	7 (12)	0.12
Hospitalization in last 3 mo	17 (14)	12 (19)	5 (8)	0.09
Long-term-care facility-onset	20 (17)	12 (19)	8 (14)	0.39
Hospital-onset	80 (66)	36 (58)	44 (75)	0.06
Onset in a non-ICU ward	49 (41)	26 (42)	23 (39)	0.74
Onset in an ICU	31 (26)	10 (16)	21 (36)	0.01
No. of days of hospitalization at bacteremia onset (median [IQR])	7 (1–23)	3 (1–21)	11 (1–25)	0.06
Mechanical ventilation at bacteremia onset	39 (32)	15 (24)	24 (41)	0.05
Empirical therapy				

(Continued on following page)

TABLE 4 (Continued)

Variable ^a	No. (%) of episodes			P value
	Total (n = 121)	Survivors (n = 62)	Non-survivors (n = 59)	
Timing of active therapy ^g				
Within 12 h of BSI onset	19 (16)	6 (10)	13 (22)	0.06
>12–24 h after BSI onset	9 (7)	4 (6)	5 (9)	0.74
>24–48 h after BSI onset	27 (22)	17 (27)	10 (17)	0.17
>48 h after BSI onset	54 (45)	32 (52)	22 (37)	0.18
Never received active therapy ^h	12 (10)	3 (5)	9 (15)	0.06

^aESBL, extended-spectrum β -lactamase; ESRD, end-stage renal disease; HIV, human immunodeficiency virus; ICU, intensive care unit; IQR, interquartile range; KPC, *Klebsiella pneumoniae* carbapenemase; ST, sequence type.

^bThe denominator for the percentages in these analyses is the 106 episodes of CRE bacteremia for which the bloodstream isolates underwent antimicrobial susceptibility testing by broth microdilution and molecular genotyping (55 survivors and 51 non-survivors).

^cAll *K. pneumoniae* with an *ompK36* gene mutation also had a mutation in the *ompK35*.

^dOther CRE were a polymicrobial infection with carbapenem-resistant *K. pneumoniae* and *Pantoea* (*Enterobacter*) *agglomerans*, *Klebsiella oxytoca*, *Morganella morganii*, and *Proteus mirabilis* (n = 1 each).

^eHigh-level carbapenem resistance was defined as having a meropenem MIC ≥ 16 μ g/ml.

^fIntra-abdominal sources included hepatobiliary infections and suspected gastrointestinal translocation of bacteria in neutropenic patients receiving chemotherapy.

^gActive therapy refers to treatment with any antimicrobial agent to which the CRE was susceptible *in vitro*.

^hNine of these patients died prior to the availability of antimicrobial susceptibility testing results, and three survived and cleared their bacteremia despite never receiving active therapy.

infection onset, and septic shock, there was no association between combination therapy (aOR, 1.56; 95% CI, 0.63 to 3.88; $P = 0.34$) or adjunctive carbapenem therapy (aOR, 1.79; 95% CI, 0.66 to 4.82; $P = 0.25$) and mortality.

DISCUSSION

This is the first study to report the clinical and molecular epidemiology of CRE across multiple medical centers in the NY/NJ area, the epicenter for these pathogens in the United States. We found that 9.7% of *K. pneumoniae* bloodstream isolates were carbapenem-resistant overall, including 11.9% of isolates in NYC centers. Our findings are consistent with those of Abdallah and colleagues, who found that 13% of *K. pneumoniae* isolates from multiple NYC medical centers during a similar time period possessed *bla*_{KPC} (17). In contrast, recent national surveillance studies reported carbapenem resistance in only 3 to 6% of *K. pneumoniae* (18–20). This study reaffirms that NY/NJ, and NYC in particular, is a high-prevalence location for CRKp. However, the prevalences of carbapenem resistance in *K. pneumoniae* in this study varied substantially by center, even among NYC centers (Table 1). In contrast to *K. pneumoniae*, we

TABLE 5 Comparisons of 30-day mortality rates after CRE bacteremia by a definitive antimicrobial regimen

Definitive antimicrobial regimen	30-day mortality rate (%)
Monotherapy ^a (n = 55)	38
Polymyxin (n = 25)	40
Polymyxin B (n = 18)	39
Colistin (n = 7)	43
Tigecycline (n = 17)	39
Aminoglycoside (n = 11)	43
With a carbapenem (n = 22)	50
Without a carbapenem (n = 33)	30
Combination therapy ^b (n = 43)	
Polymyxin-tigecycline (n = 18)	56
Aminoglycoside-tigecycline (n = 9)	33
With a carbapenem (n = 16)	62
Without a carbapenem (n = 27)	44
Regimens with carbapenems (n = 38)	55
Regimens without carbapenems (n = 60)	37

^aUse of only one active antimicrobial agent (an agent to which the CRE tested susceptible *in vitro*).

^bUse of at least two active antimicrobial agents.

found that carbapenem resistance in NY/NJ is less common among *Enterobacter* spp. and is rare in *E. coli*.

KPC-2 and KPC-3 were the most common carbapenemases in our study, and ST258 was the predominant KPC-*Kp*. Consistent with previous single-center reports (13, 21), nearly all ST258 KPC-3-*Kp* had the same capsular polysaccharide allele, *wzi154*. Strains with this allele have a capsule type (*cps*-2 group) that had not been described before the emergence of ST258 *K. pneumoniae* and these strains are classified within ST258 clade II (12). By contrast, there was marked heterogeneity in *wzi* alleles among ST258 KPC-2-*Kp* (13, 21).

Although KPC was the predominant carbapenemase, we also found an OXA-48-producing *K. pneumoniae* and an NDM-producing *E. coli*. Given that NDM- and OXA-48-producing CRE are highly prevalent in many other countries, continued vigilance to detect the emergence of these carbapenemases in the United States is essential (22).

Our extensive genetic investigation underscores the complexity of β -lactam resistance among *Enterobacteriaceae*. The vast majority of isolates not only produced a carbapenemase, but possessed two additional plasmid-mediated β -lactamases, including many with an ESBL. Furthermore, most CR*Kp* had mutations in outer membrane porin genes that were associated with high-level carbapenem resistance. These findings highlight that carbapenem resistance among CRE may not be due solely to carbapenemases, but also to additional β -lactamases and impaired antibiotic entry through outer membrane porins.

Patients with cancer and transplant recipients comprised 50% of patients with CRE bacteremia. Even when data from the hospital that was a cancer center was excluded, 46% of patients had one of these conditions. Furthermore, cancer was independently associated with a nearly 4-fold increase in the odds of mortality. The intrusion of CRE into neutropenic patients with hematologic malignancies has previously been highlighted as a major concern because these patients rely on immediate active antimicrobial therapy to combat Gram-negative bacterial infections (5). In our study, the majority of patients with cancer had solid tumors and did not have neutropenia. These patients with solid tumors had a 30-day mortality rate of 62%, a rate similar to that observed in patients with hematologic malignancies (59%). These findings illustrate that CRE bacteremia is associated with poor outcomes in patients with solid and hematologic malignancies.

CRE have typically been nosocomial pathogens, but the potential for extension of CRE into the community is a major concern. We found that CRE bacteremia commonly occurred in outpatients located in long-term-care facilities or with recent hospitalization. Thus, CRE prevention strategies should not be limited to hospital inpatient settings. We also found that 23% of patients had a recent endoscopy, typically ERCP, prior to CRE bacteremia, and most of these patients had not had a prior culture positive for CRE. Transmission of CRE from ERCP endoscopes was previously reported (23, 24). Due to the complexity of their design, high-level disinfection practices are required for sterilizing these endoscopes (25). The close temporal associations between endoscopy and bacteremia in this study suggest that ERCP endoscopes frequently contributed to CRE transmission and infection, and given the small number of published endoscopy-related CRE infections, this may be an underappreciated source of disease.

We observed a 49% 30-day mortality rate after CRE bacteremia. This figure is disappointingly similar to the mortality rate reported from patients with CRE bacteremia 10 years ago, when CRE were first widely recognized in NYC (6). Furthermore, the 38% rate of developing septic shock within 2 days and the median of 10 days from bacteremia onset until death suggest the majority of deaths were infection related. We also identified that bloodstream infections with *K. pneumoniae* strains possessing *bla*_{KPC-3} were independently associated with increased mortality compared with that from other types of CR*Kp* bacteremia in a multivariable analysis. Our data suggest that increased resistance may have contributed to the worse outcomes after KPC-3-*Kp* bacteremia, as KPC-3-*Kp* organisms were more likely to have altered OmpK36 porins and high-level carbapenem resistance. Other possible explanations are that *K. pneu*-

moniae strains harboring *bla*_{KPC-3} possess a more virulent capsular polysaccharide, as almost all strains had the *wzi154* allele, or they possess other virulence genes that are plasmid-encoded. However, *wzi154* ST258 KPC-3-*Kp* strains were previously shown to be less virulent than ST258 KPC-2-*Kp* strains in a larval model (21). Alternatively, host response factors play a role, and these factors might not be evident in currently tested larval models. Lastly, it is also possible that *wzi154* ST258 KPC-3-*Kp* organisms were transmitted among a sicker patient population, and the multivariable analysis was unable to adequately adjust for comorbid illness as a potential confounding variable.

We believe that the 2-day delay until receipt of CRE-active therapy likely contributed to these unacceptably poor outcomes, despite the fact that active empirical therapy was not associated with decreased mortality in this study. We believe that the association between active empirical therapy and mortality may have been confounded by the severity of illness, as patients who received CRE-active empirical therapy were numerically more likely to present with septic shock and require mechanical ventilation than those who did not receive active empirical therapy. Other large studies of CRE bacteremia and severe sepsis have demonstrated that inadequate empirical antimicrobial therapy is independently associated with increased mortality (8, 26–29). In addition to this potential confounder of illness severity, it is also possible that the limitations of the empirical CRE-active agents most commonly used in this study (polymyxins and tigecycline) diminished the benefit of providing active empirical therapy (30–32).

The 2-day delay in appropriate therapy highlights the limitations of traditional microbiologic techniques in identifying bloodstream pathogens. Two multiplex PCR systems have recently been approved in the United States that detect *bla*_{KPC} from blood culture broth within 2 h of culture positivity (33, 34). Routine use of these rapid diagnostic tools would likely decrease the delay in administering appropriate therapy for CRE bacteremia, which may improve patient outcomes.

In contrast to the results from previous large observational studies of bacteremia due to carbapenemase-producing *Enterobacteriaceae* (26, 35), we did not find a mortality benefit associated with combination therapy or use of a carbapenem in the definitive regimen in this study. In fact, our univariate analyses demonstrated a trend toward increased mortality with combination and concurrent carbapenem therapies. As observed for active empirical therapy, these analyses were likely confounded by the indication for therapy, whereby sicker patients received combination and concurrent carbapenem therapies, because these trends were not seen in multivariable analyses. One potential explanation for the differences in findings is that polymyxin B was primarily used in our cohort, whereas colistin was used in most of the previous studies. Polymyxin B achieves therapeutic concentrations more quickly than colistin and has more predictable pharmacokinetics (30). Thus, the additional benefit of a second active agent may be less with polymyxin B than with colistin. Second, isolates in our study expressed higher levels of carbapenem resistance (86% had meropenem MICs of ≥ 16 $\mu\text{g/ml}$) than in other studies (26, 35). The previously reported benefit of combining the carbapenem with an active agent was observed primarily with carbapenem MICs of ≤ 8 $\mu\text{g/ml}$.

The high mortality rates after CRE bacteremia may also reflect the limitations of polymyxins and tigecycline. Both agents have suboptimal clinical efficacies and unfavorable pharmacokinetic-pharmacodynamic profiles, and the polymyxins are highly nephrotoxic (30–32). Ceftazidime-avibactam was approved in the United States in 2015 as the first β -lactam/ β -lactamase inhibitor combination with *in vitro* activity against KPC-producing *Enterobacteriaceae*. Ninety-nine percent of CRE isolates in this study tested as susceptible to ceftazidime-avibactam compared with 83% susceptibility to the polymyxins. Given the excellent track record of β -lactam agents, ceftazidime-avibactam represents a promising treatment option for CRE infections. However, clinical trials of ceftazidime-avibactam primarily enrolled only patients with infections due to carbapenem-susceptible bacteria (36–38). In a single-center observational study of 37 patients with CRE infections who were treated with this agent, the 30-day mortality rate was only 24%. However, CRE infections recurred in 23% of patients and ceftazidime-

avibactam resistance developed in 8% of cases (39). Additional clinical data documenting the efficacy of this agent to treat CRE infections are urgently needed, particularly given its cost of nearly \$1,000 per day (40).

Although we rarely identified fully resistant isolates, the ceftazidime-avibactam MICs for 42% of KPC-3-*Kp* were within one doubling dilution of the FDA susceptible breakpoint. Our study suggests that OmpK36 mutations contribute to ceftazidime-avibactam resistance, as these mutations were strongly associated with elevated ceftazidime-avibactam MICs and were common in KPC-3-*Kp* (Table 3). These elevated MICs are particularly alarming in light of recent reports of clinical KPC-3-*Kp* isolates that are ceftazidime-avibactam-resistant and because these isolates predated the clinical use of ceftazidime-avibactam (39, 41). Furthermore, although not encountered in this study, KPC variants with mutations in the Ω -loop of the enzyme have been identified that increase ceftazidime-avibactam MICs (42, 43). Ongoing surveillance is critical for monitoring the emergence of ceftazidime-avibactam-resistant KPC-*Kp*. Importantly, ceftazidime-avibactam is not active against metallo- β -lactamase-producing *Enterobacteriaceae*, such as NDM-producers, and thus will be less useful in areas where these resistance mechanisms predominate.

This analysis has several limitations. The study was conducted in an area where CRE are endemic nosocomial pathogens, and the prevalence of resistance found here might not be applicable to other locations. Most centers in this study were large tertiary-care academic centers, and thus the proportions of oncology and transplant patients may be higher than what would be seen in community hospitals. We also did not analyze a control group of bacteremias due to carbapenem-susceptible *Enterobacteriaceae*, and thus we were unable to conduct a risk factor analysis. Finally, as in any observational study, our analysis of treatment regimens was subject to confounding biases.

In summary, we found that carbapenem resistance remains highly prevalent among *K. pneumoniae* bloodstream isolates in NY/NJ and that ST258 KPC-*Kp* remains the predominant type of CRE in this region. Despite more than a decade of experience with these pathogens, there were long delays until receipt of CRE-active therapies in patients with CRE bacteremia, during which time many patients developed septic shock, and nearly 50% of infected patients died. Rapid diagnostics and new antimicrobial agents with activity against CRE warrant evaluation as potential tools for decreasing the delay until active therapy and for improving outcomes.

MATERIALS AND METHODS

Study objectives and definitions. We conducted an observational cohort study of all patients with CRE bacteremia in 2013 at eight NY/NJ medical centers. Institutional review board approval was obtained from each institution, and only the first episode of bacteremia per patient was evaluated. Our study objectives were: (i) to determine the prevalence of carbapenem resistance among bloodstream isolates of *K. pneumoniae*, *Escherichia coli*, and *Enterobacter* spp. in NY/NJ; (ii) to characterize carbapenem resistance mechanisms, genetic backgrounds, capsular types, and antimicrobial susceptibilities of CRE bloodstream isolates, including susceptibilities to a newly approved agent, ceftazidime-avibactam; and (iii) to assess time to receipt of active antimicrobial therapy (an agent to which the CRE tested susceptible), 14- and 30-day mortality rates, and risk factors for 30-day mortality.

We defined carbapenem resistance according to the Centers for Disease Control and Prevention's CRE definition at the time of the study, namely as testing intermediate or resistant to imipenem, meropenem, or doripenem (MICs ≥ 2 μ g/ml), and testing resistant to third-generation cephalosporins (44, 45). High-level carbapenem resistance was defined as a meropenem MIC of ≥ 16 μ g/ml.

For each patient with CRE bacteremia, we recorded demographics, comorbidities, recent procedures, patient location at bacteremia onset, bacteremia source (determined by an infectious diseases physician), antimicrobial therapies, presence of septic shock (46), and 30-day mortality. We defined bacteremia onset as the time the first positive blood culture for CRE was collected. Hospital-onset cases were defined as occurring ≥ 3 days after hospitalization. Antibacterial agents initiated after the availability of antimicrobial susceptibility data were considered definitive therapy. This study predated the approval of ceftazidime-avibactam, and thus no patients received this agent.

Characterization of CRE isolates. A central laboratory performed antimicrobial susceptibility testing on CRE bloodstream isolates using Sensititre GNX2F broth microdilution panels (Thermo Fischer Scientific, Oakwood Village, OH) and ceftazidime-avibactam Etest strips (bioMérieux, Durham, NC) (45). The U.S. Food and Drug Administration (FDA) susceptible breakpoint of $\leq 8/4$ μ g/ml was applied for ceftazidime-avibactam. Results from local microbiology laboratories were used for isolates that were not available. Isolates for which polymyxin and tigecycline MICs were ≤ 2 μ g/ml were considered susceptible

to these agents. We used a multiplex real-time PCR to detect SHV, TEM, CTX-M, AmpC, KPC, NDM, VIM, IMP, and OXA-48-type β -lactamase genes, followed by gene sequencing of amplicons (47–49). Carbapenem-resistant *K. pneumoniae* (CRKp) underwent multilocus sequence typing (50), sequencing of outer membrane porin genes *ompK35* and *ompK36* (51), and sequencing of the *wzi* gene to determine capsular type (13, 21, 52). Additionally, PCR for the plasmid-mediated polymyxin resistance gene, *mcr-1*, was performed on all polymyxin-resistant CRE (53).

Statistical analysis. Characteristics of patients with 30-day survival were compared to those who died using chi-square or Fisher's exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables. *P* values of ≤ 0.05 (two-tailed) were considered statistically significant. A multivariable logistic regression model was constructed to identify baseline factors independently associated with 30-day mortality. All variables with *P* values ≤ 0.1 in univariate analysis were entered into the multivariable model, and a backward stepwise selection process was applied.

The definitive therapy analyses included patients who survived ≥ 3 days after bacteremia onset and received ≥ 24 h of an active antimicrobial agent. We compared 30-day mortality rates based on definitive antimicrobial regimens and constructed a multivariable logistic regression model to determine whether combination therapy or use of a carbapenem was independently associated with 30-day mortality. STATA, version 12.0 (StataCorp, College Station, TX) was used for statistical analyses.

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