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Low Frequency of Ceftazidime-Avibactam Resistance among *Enterobacteriaceae* Isolates Carrying bla_{KPC} Collected in U.S. Hospitals from 2012 to 2015

Mariana Castanheira, Rodrigo E. Mendes, Helio S. Sader

JMI Laboratories, North Liberty, Iowa, USA

ABSTRACT *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* isolates have been increasingly reported worldwide, and therapeutic options to treat infections caused by these organisms are limited. We evaluated the activity of ceftazidime-avibactam and comparators against 456 *Enterobacteriaceae* isolates carrying bla_{KPC} collected from 79 U.S. hospitals during 2012 to 2015. Overall, ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml; 99.3% susceptible) and tigecycline ($MIC_{50/90}$, 0.5/1 μg/ml; 98.9% susceptible at ≤2 μg/ml) were the most active agents. Only 80.5% and 59.0% of isolates were susceptible to colistin and amikacin, respectively. All three isolates (0.7%) displaying resistance to ceftazidime-avibactam (*K. pneumoniae*; MICs, ≥16 μg/ml) were evaluated using whole-genome sequencing analysis and relative quantification of expression levels of porins and efflux pump. Two isolates carried metallo-β-lactamase genes, bla_{NDM-1} or bla_{VIM-4}, among other β-lactam resistance mechanisms, and one displayed a premature stop codon in *ompK35* and decreased expression of *ompK36*. Ceftazidime-avibactam was active against 100.0 and 99.3% of isolates carrying bla_{KPC-3} (n = 221) and bla_{KPC-2} (n = 145), respectively. Isolates carrying bla_{KPC} were more commonly recovered from pneumonia (n = 155), urinary tract (n = 93), and skin/soft tissue (n = 74) infections. Ceftazidime-avibactam (97.8 to 100.0% susceptible) was consistently active against isolates from all infection sites. *K. pneumoniae* (83.3% of the collection) susceptibility rates were 99.2% for ceftazidime-avibactam, 98.9% for tigecycline, and 80.1% for colistin. Ceftazidime-avibactam susceptibility did not vary substantially when comparing isolates from intensive care unit (ICU) patients to those from non-ICU patients. Ceftazidime-avibactam was active against this large collection of isolates carrying bla_{KPC} and represents a valuable addition to the armamentarium currently available for the treatment of infections caused by KPC-producing *Enterobacteriaceae*.

KEYWORDS ceftazidime-avibactam, KPC, permeability

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Address correspondence to Mariana Castanheira, Mariana-castanheira@jmilabs.com.

Klebsiella pneumoniae carbapenemase (KPC)-producing isolates have been detected worldwide, and these isolates greatly concern health care professionals (1). Isolates carrying bla_{KPC} are mainly *Klebsiella pneumoniae*, but genes encoding KPC enzymes have been detected in several other members of the *Enterobacteriaceae* family, *Pseudomonas aeruginosa*, and *Acinetobacter* species. KPC enzymes hydrolyze virtually all β-lactams and are poorly inhibited by older β-lactamase inhibitors such as clavulanic acid and tazobactam (2).

Avibactam is a diazabicyclooctane β-lactamase inhibitor that demonstrates excellent inhibitory properties against class A β-lactamases, including KPC (3). For clinical development, avibactam was paired with ceftazidime, and this combination was ap-

proved by the U.S. Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections and in combination with metronidazole for complicated intra-abdominal infections (4).

In the United States, where isolates carrying bla_{KPC} were initially described (5), these organisms became endemic in a few hospitals, and recent data from the Centers for Disease Control and Prevention (CDC) demonstrate that these enzymes have been detected in all but two U.S. states (6). Studies demonstrated that ceftazidime-avibactam is very active against isolates carrying bla_{KPC} (7–10); however, the spread of isolates producing metallo-β-lactamases (MBLs) and recent reports of ceftazidime-avibactam-resistant *Enterobacteriaceae* isolates in U.S. hospitals (11–13) highlight the importance of monitoring and understanding the occurrence of these isolates.

In this study, we evaluated the activity of ceftazidime-avibactam and comparator agents tested against 456 *Enterobacteriaceae* isolates carrying bla_{KPC} detected among all carbapenem-resistant *Enterobacteriaceae* (CRE) isolates collected in 79 U.S. hospitals from 2012 to 2015. We also analyzed the susceptibility profiles of bla_{KPC}-harboring isolates against ceftazidime-avibactam and comparators stratified by the most common bacterial species, bla_{KPC} alleles, and infection types and those recovered from patients placed in intensive care units (ICUs). Furthermore, we evaluated additional β-lactam resistance mechanisms among three ceftazidime-avibactam-resistant isolates.

RESULTS AND DISCUSSION

Among 34,564 *Enterobacteriaceae* isolates collected during 2012 to 2015 in U.S. hospitals participating in the International Network for Optimal Resistance Monitoring (INFORM) surveillance program, 525 (1.4%) isolates displayed carbapenem resistance, and 456 of these isolates were positive for bla_{KPC} (1.2% of overall isolates; 87.0% of CRE). The most common organism carrying bla_{KPC} was *K. pneumoniae* ($n = 380$; 72.5% of the collection), but this carbapenemase-encoding gene was also detected among 35 *Enterobacter cloacae* species complex, 14 *Escherichia coli*, 11 *Klebsiella oxytoca*, 10 *Serratia marcescens*, 4 *Citrobacter freundii*, and 2 *Enterobacter aerogenes* isolates.

A total of 155 (34.0%) isolates harboring bla_{KPC} were collected from pneumonia in hospitalized patients, 93 (20.4%) from urinary tract infections (UTI), 74 (16.2%) from skin/soft tissue infections (SSSI), 50 (11.0%) from bloodstream infections (BSI), 18 (3.9%) from intra-abdominal infections (IAI), and 66 (14.5%) from other or unknown infection sites. These isolates were observed in all U.S. census regions, and the occurrence varied among regions; however, bla_{KPC}-positive isolates were considerably more frequent in the Mid-Atlantic census division (244 isolates; 5.4% of the *Enterobacteriaceae* isolates from the Mid-Atlantic) than in the remaining divisions (East North Central [$n = 79$; 1.1%], West South Central [$n = 43$; 1.1%], South Atlantic [$n = 42$, 0.9%], East South Central [$n = 15$, 0.5%], Mountain [$n = 15$, 0.5%], New England [$n = 7$, 0.3%], Pacific [$n = 9$, 0.2%], and West North Central [$n = 2$, 0.1%]).

Overall, ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml) (Table 1) was very active against isolates carrying bla_{KPC}, and this combination inhibited 99.3% of the isolates at the current U.S. FDA breakpoint. Only three isolates (0.7%) were nonsusceptible to ceftazidime-avibactam, and these isolates were all *K. pneumoniae* isolates displaying ceftazidime MIC values of 16 or >32 μg/ml (Tables 1 and 2).

One of two isolates displaying ceftazidime-avibactam MIC results of >32 μg/ml was recovered in New York during 2013 and carried bla_{KPC-2} and bla_{VIM-4} in addition to two narrow-spectrum β-lactamase-encoding genes (Table 2) (14). Additionally, this isolate displayed decreased expression of *ompK35* and *ompK37* and elevated expression of the efflux system AcrAB-TolC that was >15 times that of the baseline strain.

The second isolate displaying ceftazidime-avibactam MIC results of >32 μg/ml was recently described in a study comparing the outcomes of patients treated with ceftazidime-avibactam in a hospital in Texas (12). This isolate harbored six β-lactamase genes, including bla_{KPC-17}, bla_{NDM-1}, $bla_{CTX-M-55}$, and bla_{DHA-1}, and sequencing of outer membrane protein (OMP) genes demonstrated a nonsense mutation causing a premature stop codon in *ompK35* (Table 2).

TABLE 1 MIC distributions for ceftazidime-avibactam when tested against 456 KPC-producing *Enterobacteriaceae* isolates collected from 2012 to 2015 in U.S. hospitals

Organism group	No. of isolates tested	No. (cumulative %) of isolates inhibited at ceftazidime-avibactam MIC (μg/ml) of:													MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
		≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32		
KPC producers[a]	456	11 (2.4)	4 (3.3)	13 (6.1)	31 (12.9)	64 (27.0)	138 (57.2)	132 (86.2)	49 (96.9)	11 (99.3)	0 (99.3)	1 (99.6)	0 (99.6)	2 (100.0)	0.5	2
KPC allele																
bla_{KPC-2}	145	1 (0.7)	2 (2.1)	5 (5.5)	14 (15.2)	31 (36.6)	54 (73.8)	25 (91.0)	11 (98.6)	1 (99.3)	0 (99.3)	0 (99.3)	0 (99.3)	1 (100.0)	0.5	1
bla_{KPC-3}	221	3 (1.4)	2 (2.3)	6 (5.0)	9 (9.0)	23 (19.5)	63 (48.0)	84 (86.0)	24 (96.8)	7 (100.0)					1	2
Bacterial species																
Klebsiella pneumoniae	380	11 (2.9)	4 (3.9)	7 (5.8)	27 (12.9)	55 (27.4)	122 (59.5)	99 (85.5)	44 (97.1)	8 (99.2)	0 (99.2)	1 (99.5)	0 (99.5)	2 (100.0)	0.5	2
Enterobacter cloacae SC	35			1 (2.9)	0 (2.9)	3 (11.4)	5 (25.7)	22 (88.6)	2 (94.3)	2 (100.0)					1	2
Escherichia coli	14			3 (21.4)	3 (42.9)	6 (85.7)	1 (92.9)	0 (92.9)	1 (100.0)						0.25	0.5
Klebsiella oxytoca	11			1 (9.1)	1 (18.2)	0 (18.2)	1 (27.3)	6 (81.8)	1 (90.9)	1 (100.0)					1	2
Serratia marcescens	10						9 (90.0)	1 (100.0)							0.5	0.5
Infection type[b]																
Pneumonia	155	4 (2.6)	1 (3.2)	5 (6.5)	11 (13.5)	21 (27.1)	46 (56.8)	52 (90.3)	14 (99.4)	1 (100.0)					0.5	1
UTI	93	4 (4.3)	2 (6.5)	4 (10.8)	5 (16.1)	15 (32.3)	29 (63.4)	21 (86.0)	11 (97.8)	0 (97.8)	0 (97.8)	1 (98.9)	0 (98.9)	1 (100.0)	0.5	2
SSSI	74	1 (1.4)	1 (2.7)	2 (5.4)	6 (13.5)	10 (27.0)	19 (52.7)	24 (85.1)	6 (93.2)	5 (100.0)					0.5	2
BSI	50	1 (2.0)	0 (2.0)	1 (4.0)	4 (12.0)	9 (30.0)	16 (62.0)	7 (76.0)	9 (94.0)	2 (98.0)	0 (98.0)	0 (98.0)	0 (98.0)	1 (100.0)	0.5	2
IAI	18	1 (5.6)	0 (5.6)	0 (5.6)	1 (11.1)	3 (27.8)	7 (66.7)	5 (94.4)	1 (100.0)						0.5	1
ICU/non-ICU																
ICU	112	3 (2.7)	2 (4.5)	7 (10.7)	9 (18.8)	14 (31.2)	30 (58.0)	34 (88.4)	11 (98.2)	2 (100.0)					0.5	2
Non-ICU	174	6 (3.4)	2 (4.6)	3 (6.3)	13 (13.8)	29 (30.5)	50 (59.2)	50 (87.9)	14 (96.0)	6 (99.4)	0 (99.4)	1 (100.0)			0.5	2

[a]Isolates include *Citrobacter freundii* (4), *Enterobacter aerogenes* (2), *E. cloacae* species complex (SC) (35), *Escherichia coli* (14), *Klebsiella oxytoca* (11), *K. pneumoniae* (380), and *Serratia marcescens* (10).
[b]Pneumonia, pneumonia in hospitalized patients; UTI, urinary tract infections; SSSI, skin/soft tissue infections; BSI, bloodstream infections; IAI, intra-abdominal infections.

TABLE 2 Sequencing analysis and gene expression results for three KPC-producing *K. pneumoniae* isolates collected during 2012 to 2015 in U.S. hospitals that displayed nonsusceptible ceftazidime-avibactam MIC results

City, state, yr isolated	Ceftazidime-avibactam MIC (μg/ml)	β-Lactamases detected	Intrinsic β-lactam resistance genes		
			Gene product	Sequencing analysis[a]	Relative quantification (interpretation)[b]
New York, NY, 2013	>32	VIM-4, KPC-2, SHV-1, TEM-1	OmpK35	G211S, V241I	0.1 (decreased expression)
			OmpK36	R345H	6.6 (similar to baseline)
			OmpK37	R239K, 237TERY238 insertion, E244D, N274S, D275T, 274SSTNGG275 insertion, V277I, V295G, D350G	0.0 (decreased expression)
			AcrAB-TolC	NA	15.3 (elevated expression)
Philadelphia, PA, 2015	16	KPC-2, SHV-12, TEM-1	OmpK35	Internal stop codon	0.2 (similar to baseline)
			OmpK36	182A183 insertion, G191T, F200Y, H220N, N224L, 228S229 insertion, R229K, D231A, K232L, F267A, S268G, G269S, N270L, 272ESDSISG278 deletion, I312L, L320I, E349D, D351S, R354H, R355N, V358I	0.0 (decreased expression)
			OmpK37	25N26 insertion, N230G, M232Q, T233H, Q234Y, 236TERY237 insertion, R238K, E243D, D274T, 274SSTNGG275 insertion	326 (similar to baseline)
			AcrAB-TolC	NA	0.8 (similar to baseline)
Houston, TX, 2015	>32	NDM-1, KPC-17, CTX-M-55, TEM-1, DHA-1, SHV-122	OmpK35	Internal stop codon	0.5 (similar to baseline)
			OmpK36	134GD135 insertion, V358I	2.0 (similar to baseline)
			OmpK37	25N26 insertion, N230G, M232Q, T233H, Q234Y, 236TERY237 insertion, R238K, E243D, D274T, 274SSTNGG275 insertion	0.8 (similar to baseline)
			AcrAB-TolC	NA	4.4 (similar to baseline)

[a]Deletions, insertions, or stop codons that might significantly change protein function or alter gene expression are underlined. NA, not applicable.
[b]Changes from baseline expression are underlined.

The remaining isolate displaying resistance to ceftazidime-avibactam (MIC value of 16 μg/ml) carried bla_{KPC-2} and was recovered from Philadelphia, PA; it had decreased expression of *ompK36*, a premature stop codon in *ompK35*, and insertions in *ompK36* and *ompK37* (Table 2).

All three isolates displayed similar alterations in *ompK37* that included insertions in the L4 (236TERY237) and L5 (274SSTNGG275) regions. In the initial study describing OmpK37 (15), the L4 region was considered the least conserved among the isolates tested; thus, alterations in this region might have no significance. The same study also concluded that OmpK37 plays only a minor role in overall β-lactam resistance and no role in the entrance of cephalosporins into the cell due to the small size of the pore encoded by this gene (15); however, the role of this porin in the penetration of avibactam is unknown.

Four bla_{KPC} variants were detected in this study among 371 isolates for which amplicon sequencing for this carbapenemase gene was performed. The most common allele was bla_{KPC-3}, and it was detected among 221 isolates (59.6% of the isolates that had sequencing results available), followed by bla_{KPC-2}, which was detected among 145 (39.1%) isolates. The two remaining variants were bla_{KPC-4} and bla_{KPC-17}, which were detected in three and two isolates, respectively.

We compared the activities of ceftazidime-avibactam against isolates with the two most common bla_{KPC} alleles (Table 1). Ceftazidime-avibactam was 2-fold more active against isolates carrying bla_{KPC-2} ($MIC_{50/90}$, 0.5/1 μg/ml) (Table 1) than against isolates harboring bla_{KPC-3} ($MIC_{50/90}$, 1/2 μg/ml). KPC-2 and KPC-3 have only one amino acid difference; however, KPC-3 hydrolyzes ceftazidime 30 times more efficiently (k_{cat}, 3.0 s^{-1}) than KPC-2 (k_{cat}, 0.1 s^{-1}) (16), possibly explaining the higher MIC values. Nonetheless, all isolates carrying bla_{KPC-3} collected in U.S. hospitals during this 4-year study were inhibited by ceftazidime-avibactam at ≤4 μg/ml, and all were considered susceptible to this combination (Table 1).

TABLE 3 Susceptibility rates for KPC-producing isolates stratified by KPC variant, most common species, infection site, and ICU/non-ICU

Organism or group (no. of isolates)[b]	% susceptibility to[a]:							
	Ceftazidime-avibactam	Cefepime	Meropenem	Levofloxacin	Gentamicin	Amikacin	Tigecycline	Colistin
All KPC producers (456)	99.3	7.2	1.3	15.8	49.3	59.0	98.9	80.5
bla_{KPC-2} (145)	99.3	12.5	2.1	13.1	59.3	50.3	99.3	84.6
bla_{KPC-3} (221)	100.0	5.6	0.9	19.0	43.4	64.7	99.1	82.6
K. pneumoniae (380)	99.2	4.0	0.5	9.7	52.4	52.4	98.9	80.1
E. cloacae SC (35)	100.0	4.8	5.7	31.4	25.7	100.0	97.1	94.1
E. coli (14)	100.0	50.0	7.1	28.6	42.9	85.7	100.0	92.9
K. oxytoca (11)	100.0	25.0	0.0	81.8	18.2	90.9	100.0	90.9
S. marcescens (10)	100.0	25.0	0.0	70.0	70.0	80.0	100.0	10.0
Pneumonia (155)	100.0	9.1	1.3	22.6	54.2	63.9	100.0	76.5
UTI (93)	97.8	0.0	0.0	8.6	44.1	49.5	97.8	76.3
SSSI (74)	100.0	14.3	5.4	14.9	41.9	63.5	100.0	81.9
BSI (50)	98.0	7.7	0.0	16.0	58.0	60.0	100.0	79.6
IAI (18)	100.0	0.0	0.0	16.7	50.0	66.7	94.4	88.9
ICU (112)	100.0	19.4	5.4	24.1	50.9	61.6	100.0	77.8
Non-ICU (174)	99.4	17.8	6.3	16.7	53.4	61.5	97.7	82.1

[a]The susceptibility breakpoints used were from CLSI, except for tigecycline and ceftazidime-avibactam (U.S. FDA package inserts) and colistin (EUCAST website).
[b]Pneumonia, pneumonia in hospitalized patients; UTI, urinary tract infections; SSSI, skin/soft tissue infections; BSI, bloodstream infections; IAI, intra-abdominal infections.

Isolates carrying bla_{KPC-3} were also more resistant to other β-lactams than isolates harboring bla_{KPC-2}: cefepime and meropenem susceptibility rates were 5.6 versus 12.5% and 0.9 versus 2.1%, respectively (Table 3). KPC-3-producing isolates were also less susceptible to gentamicin (43.4% susceptible) than those carrying bla_{KPC-2} (59.3% susceptible). In contrast, KPC-2-producing isolates were less susceptible to levofloxacin (13.1 versus 19.0% susceptible) and amikacin (50.3 versus 64.7% susceptible) (Table 3) than KPC-3-producing isolates.

Ceftazidime-avibactam was active against 99.2% of the bla_{KPC}-carrying *K. pneumoniae* isolates at the U.S. FDA breakpoint (Table 1), and this compound inhibited all isolates belonging to other bacterial species at $\leq$4 μg/ml. *K. pneumoniae* isolates carrying bla_{KPC} exhibited high rates of resistance to all β-lactams tested, i.e., levofloxacin (87.9% resistant) and gentamicin and amikacin (47.6% nonsusceptible for both) (Table 3). A total of 98.9% and 80.1% of these isolates were susceptible to tigecycline and colistin, respectively.

The activities of ceftazidime-avibactam (97.8% to 100.0% susceptible) were consistent among infection types. Ceftazidime-avibactam susceptibility rates were slightly lower among UTI (97.8%; $MIC_{50/90}$, 0.5/2 μg/ml) and BSI (98.0%; $MIC_{50/90}$, 0.5/2 μg/ml) isolates, but all isolates from pneumonia ($MIC_{50/90}$, 0.5/1 μg/ml), SSSI ($MIC_{50/90}$, 0.5/2 μg/ml), and IAI ($MIC_{50/90}$, 0.5/1 μg/ml) sources were susceptible to this combination (Tables 1 and 3). The susceptibility rates when applying CLSI breakpoints for comparator agents varied among infection types and were 49.5% to 66.7% for amikacin, 41.9% to 58.0% for gentamicin, and 8.6% to 22.6% for levofloxacin (Table 3). Colistin susceptibility rates when applying EUCAST breakpoints varied from 76.3% to 88.9%, and isolates were very susceptible to tigecycline using the U.S. FDA breakpoint (94.4% to 100.0% susceptible) (Table 3). Overall, bla_{KPC}-carrying isolates recovered from UTI were more resistant to all antimicrobial agents tested than isolates from other infection sources (Table 3).

A total of 112 of the KPC-producing isolates were collected from ICU patients, and all isolates were susceptible to ceftazidime-avibactam (Table 1). Ceftazidime-avibactam inhibited 99.4% of the isolates from non-ICU patients at current breakpoints. Interestingly, non-ICU isolates were slightly more resistant to cefepime and levofloxacin than those from ICU patients (Table 3). Among selected comparator agents reported in Table 3, isolates from ICU patients were significantly less susceptible than non-ICU isolates only for colistin (77.8% versus 82.1%).

Ceftazidime-avibactam was very active against this contemporary (2012 to 2015) collection of isolates carrying bla_{KPC} from U.S. hospitals, regardless of the type of

infection, ICU/non-ICU provenance, bacterial species, or bla_{KPC} allele. Only three (0.7%) of 456 isolates displayed nonsusceptible ceftazidime-avibactam MIC results. Two of these isolates coproduced MBLs that are still considered uncommon in U.S. hospitals, but such isolates have been reported with increasing frequency.

According to the CDC CRE tracking system, isolates producing NDM enzymes have been reported in 25 states and VIM-producing *Enterobacteriaceae* isolates reported in at least seven states (6), but these numbers could be higher due to the lack of carbapenemase screening by smaller hospitals.

MBL-producing isolates still challenge patient treatment with clinically available antimicrobial agents, and none of the β-lactamase inhibitors clinically available or in late-stage development have inhibitory activity against these enzymes. The aztreonam-avibactam combination, currently in early clinical development stages, might be a possible choice for treating infections caused by MBL-producing isolates. MBLs do not hydrolyze monobactams such as aztreonam, and avibactam inhibits other β-lactamases present in these isolates (3). *In vitro* studies evaluating aztreonam-avibactam demonstrated that this combination was active against 94% to 100% of the MBL-producing isolates when applying the CLSI breakpoint of aztreonam alone for comparison purposes (9, 10).

Enterobacteriaceae isolates displaying elevated ceftazidime-avibactam MIC values that do not produce MBLs, such as one *K. pneumoniae* isolate detected as part of this study, have also been reported. Most of these isolates are laboratory-made strains that display amino acid alterations in the Ω loop of the KPC enzyme, and these avibactam-resistant KPC variants were generated by site-specific mutagenesis or single- and multistep mutation experiments (17–19). Although these studies highlight the potential emergence of resistance, clinical isolates harboring these variants have not been described, and experience shows that clavulanate- and/or tazobactam-resistant TEM and SHV enzymes are rare among clinical isolates (19).

Three reports of clinical *Enterobacteriaceae* isolates displaying ceftazidime-avibactam resistance have been published. The first was a *K. pneumoniae* isolate that carried bla_{KPC-3}, displayed a ceftazidime-avibactam MIC value of 32 μg/ml (11), and displayed OmpK35 alterations (R. M. Humphries, personal communication). The second study reported seven ceftazidime-avibactam-resistant isolates collected among 11 CRE isolates at a single Texas hospital (12). Six of these isolates carried bla_{NDM} genes, and one *K. oxytoca* isolate displaying a ceftazidime-avibactam MIC of 16 μg/ml did not carry carbapenemase genes (12). This isolate had an elevated expression of the AcrAB-TolC efflux system, which was 11.7 times greater than that for the reference strain, and amino acid alterations and deletions in OmpK36 (A192P, N229S, F268 deletion, and 272GDSDSI277 deletion [JMI Laboratories, unpublished data]).

The third study, recently published (13), reported three patients who had isolates displaying resistance to ceftazidime-avibactam during or after treatment with this antimicrobial agent among 37 consecutive patients who received ceftazidime-avibactam. The isolates that developed resistance during treatment carried bla_{KPC-3} and belonged to ST258, but the authors did not report changes in permeability for these isolates.

Resistance to ceftazidime-avibactam in clinical isolates seems to be caused by alterations in permeability, including decreased expression and/or mutations in porin genes and overexpression of efflux systems, and isolates carrying these alterations can be selected by use of other β-lactams or antimicrobial classes. A recent study by Pagès et al. suggested that OmpK35/OmpF, OmpK36/OmpC, and possibly other channels that were not identified are involved in the penetration of avibactam into the cell (20). Their results demonstrated that isolates producing β-lactamases, including narrow-spectrum and extended-spectrum β-lactamase (ESBL) enzymes, and additionally having one or both porins deleted still displayed ceftazidime-avibactam MIC values of $\leq$8 μg/ml. However, these authors did not evaluate isolates carrying bla_{KPC}. Further studies elucidating the role of porin loss or decreased expression in isolates producing a serine-carbapenemase are warranted.

Other published studies have reported the activity of ceftazidime-avibactam against isolates carrying bla_{KPC}; however, this study summarizes the activity of this combination against all isolates carrying bla_{KPC} detected among CREs recovered from 79 U.S. hospitals during a 4-year period. Our results with this comprehensive collection of bla_{KPC}-carrying isolates demonstrated that ceftazidime-avibactam is very active and an important option for the treatment of infections caused by these organisms.

Among comparator agents, tigecycline was the only agent that displayed susceptibility rates against bla_{KPC} isolates of greater than 90% (98.9% susceptible), while 80.5% of the isolates were susceptible to colistin (80.1% versus *K. pneumoniae* isolates harboring bla_{KPC}). Furthermore, with the exception of tigecycline, other comparator agents displayed variable activity against isolates collected from different infection types and with different bla_{KPC} alleles.

Knowing the type of carbapenemase and/or the profile of susceptibility to ceftazidime-avibactam for CRE isolates is important, especially in institutions where MBL-producing isolates have been identified. Many challenges might limit the availability of this information, including difficulties in detecting different carbapenemases and justifying the cost of these assays and the lack of U.S. FDA-cleared ceftazidime-avibactam susceptibility testing reagents (11). Resolving these issues and the development of alternative options to treat infections caused by MBL-producing organisms are urgently needed.

MATERIALS AND METHODS

Bacterial isolates. A total of 34,564 *Enterobacteriaceae* clinical isolates deemed to be causes of infection were collected from 79 U.S. hospitals during 2012 to 2015 as part of the International Network for Optimal Resistance Monitoring (INFORM) surveillance program. Hospitals were located in 37 states representing nine U.S. census divisions. Only one isolate per patient infection episode was included in the study. Species identification was confirmed when necessary by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonics (Billerica, MA, USA) MALDI Biotyper according to the manufacturer's instructions.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using reference broth microdilution methods against ceftazidime-avibactam (avibactam fixed at 4 μg/ml) and comparator antimicrobial agents as described by the Clinical and Laboratory Standards Institute (21). Categorical interpretations were those found in CLSI documents (22) or U.S. FDA package inserts for tigecycline (23) and ceftazidime-avibactam (4) and the EUCAST website (24). Quality control (QC) was performed using *E. coli* ATCC 25922 and 35218, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within published ranges as described in the CLSI documents.

KPC screening. *Enterobacteriaceae* isolates displaying a carbapenem-resistant phenotype (CRE), which was defined as any isolate displaying imipenem and/or meropenem MIC values of >2 μg/ml (CLSI criteria) (22), were screened for the presence of bla_{KPC}. *Proteus mirabilis* and indole-positive *Proteeae* were screened if meropenem MIC values were >2 μg/ml. KPC-encoding genes were screened using a microarray-based assay (Check-MDR CT101 kit; Check-Points, Wageningen, Netherlands) or using PCR methods as previously described (25). A total of 371 amplicons were sequenced and compared to reference sequences.

Characterization of ceftazidime-avibactam-nonsusceptible isolates. Total genomic DNA was extracted from bacterial cultures and prepared using the Nextera XT library construction protocol and index kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. Preparations were sequenced on a MiSeq (Illumina) instrument targeting a 30$\times$ coverage, and sequence reads were processed using the *de novo* assembler SPAdes 3.6.2 (26). Searches for β-lactamase and outer membrane protein genes were performed using a curated library (27–30) and applying criteria of $>$94% sequencing identity and 40% minimum length coverage. Additional analyzes of the resulting sequences were performed using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Expression of intrinsic genes encoding resistance to β-lactams was determined by quantitative real-time PCR (qRT-PCR) using DNA-free RNA preparations as previously described (31). Relative quantification of *acrA*, *ompK35*, *ompK36*, and *ompK37* transcripts was performed in triplicate by normalization to an endogenous reference gene (*gyrA*) using custom-designed primers showing efficiencies of $>$95.0%. Transcription levels were considered significantly different (increase for *acrA* and decrease for OMP genes) if at least a 10-fold difference compared to the reference strain *K. pneumoniae* ATCC 13883 was noted.

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