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Ceftazidime-Avibactam Activity Tested against *Enterobacteriaceae* Isolates from U.S. Hospitals (2011 to 2013) and Characterization of β-Lactamase-Producing Strains

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Ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.25 μg/ml) inhibited 99.9% (20,698/20,709) of *Enterobacteriaceae* isolates at ≤8 μg/ml. This compound was active against resistant subsets, including ceftazidime-nonsusceptible *Enterobacter cloacae* ($MIC_{50/90}$, 0.25/0.5 μg/ml) and extended-spectrum β-lactamase (ESBL) phenotype isolates. An ESBL phenotype was noted among 12.4% (1,696/13,692 isolates from targeted species) of the isolates, including 776 *Escherichia coli* (12.0% for this species; $MIC_{50/90}$, 0.12/0.25 μg/ml), 721 *Klebsiella pneumoniae* (16.3%; $MIC_{50/90}$, 0.12/0.25 μg/ml), 119 *Klebsiella oxytoca* (10.3%; $MIC_{50/90}$, 0.06/0.25 μg/ml), and 80 *Proteus mirabilis* (4.9%; $MIC_{50/90}$, 0.06/0.12 μg/ml) isolates. The most common enzymes detected among ESBL phenotype isolates from 2013 ($n = 743$) screened using a microarray-based assay were CTX-M-15-like ($n = 307$), KPC ($n = 120$), SHV ESBLs ($n = 118$), and CTX-M-14-like ($n = 110$). KPC producers were highly resistant to comparators, and ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml) and tigecycline ($MIC_{50/90}$, 0.5/1 μg/ml; 98.3% susceptible) were the most active agents against these strains. Meropenem ($MIC_{50/90}$, ≤0.06/≤0.06 μg/ml) and ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.25 μg/ml) were active against CTX-M-producing isolates. Other enzymes were also observed, and ceftazidime-avibactam displayed good activity against the isolates producing less common enzymes. Among 11 isolates displaying ceftazidime-avibactam MIC values of >8 μg/ml, three were *K. pneumoniae* strains producing metallo-β-lactamases (all ceftazidime-avibactam MICs, >32 μg/ml), with two NDM-1 producers and one *K. pneumoniae* strain carrying the bla_{KPC-2} and bla_{VIM-4} genes. Therapeutic options for isolates producing β-lactamases may be limited, and ceftazidime-avibactam, which displayed good activity against strains, including those producing KPC enzymes, merits further study in infections where such organisms occur.

Enterobacteriaceae species cause a variety of infection types and these organisms, including those producing extended-spectrum β-lactamases (ESBLs) and carbapenemases, have been implicated in severe health care-associated infections (HAIs) that are a leading cause of morbidity and mortality worldwide (1, 2). Among Gram-negative organisms associated with HAI in the United States, 15% of *Klebsiella pneumoniae* or *Klebsiella oxytoca* isolates and 2% of *Escherichia coli* isolates have been shown to be resistant to three or more antimicrobial classes and were categorized as multidrug resistant (MDR) (2). These isolates were the cause of central line-associated bloodstream infections, catheter-associated urinary tract infections, ventilator-associated pneumonia, and surgical site infections. Additionally, among hospitals reporting severe HAIs, 20% described the occurrence of carbapenem-resistant *Klebsiella* isolates that are usually MDR (2) and more recently, pandrug-resistant (PDR) isolates producing carbapenemases have been reported (3). Due to steadily increasing levels of antimicrobial resistance among *Enterobacteriaceae* isolates, therapeutic options are becoming scarcer, and antimicrobial agents with safety and efficacy challenges are often the last resource for treating patients with infections caused by these organisms. In a meta-analysis, patients with bacteremia due to ESBL-producing organisms had a statistically significant increased mortality and were more likely to have a delay in receiving appropriate antimicrobial treatment (3); thus, having reliable therapeutic options to treat infections caused by MDR and PDR *Enterobacteriaceae* isolates is extremely important.

Ceftazidime-avibactam is a combination of a cephalosporin and a diazabicyclooctane (DBO) β-lactamase inhibitor with prolonged deacylation rates (4). This non-β-lactam agent has good inhibitory properties against enzymes belonging to Ambler structural classes A and C, as well as some class D enzymes. Avibactam greatly improves (4- to 1,024-fold MIC reduction) the activity of ceftazidime versus most *Enterobacteriaceae* species that produce β-lactamase enzymes (5), including isolates producing KPC and CTX-M enzymes that are prevalent in the United States (6, 7) and many other geographic regions.

In this study, we evaluate the activities of ceftazidime-avibactam and comparator antimicrobial agents tested against 20,709 clinical *Enterobacteriaceae* isolates collected in U.S. hospitals during the period from 2011 to 2013. Among this collection, 743 isolates collected during 2013 were tested for common β-lactamase genes and were analyzed separately.

MATERIALS AND METHODS

Bacterial isolates. A total of 20,709 *Enterobacteriaceae* clinical isolates were collected in 79 U.S. hospitals during 2011 (3,233 isolates), 2012

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(8,640 isolates), and 2013 (8,836 isolates) as part of the International Network for Optimal Resistance Monitoring (INFORM) program. These nonduplicate consecutively collected isolates considered clinically significant were recovered from bloodstream infections (2,216 isolates), hospitalized patients with pneumonia (2,424 isolates), skin/soft tissue infections (3,493 isolates), urinary tract infections (2,686 isolates), intra-abdominal infections (110 isolates), and other sites (779 isolates). Species identification was confirmed by standard biochemical tests and using the matrix-assisted laser desorption ionization (MALDI) Biotyper (Bruker Daltonics, Billerica, MA) according to the manufacturer's instructions, where necessary.

Susceptibility testing. Broth microdilution testing methods using validated dry-form panels (Thermo Fisher Scientific, Inc., Cleveland, OH) were performed to determine the antimicrobial susceptibility of ceftazidime-avibactam (inhibitor at fixed concentration of 4 μg/ml; range tested, 0.015 to 32 μg/ml) and comparator agents (8). Comparator agents included ceftazidime (range tested, 0.015 to 32 μg/ml), ceftriaxone (0.06 to 8 μg/ml), ampicillin-sulbactam (0.25 to 32 μg/ml), piperacillin-tazobactam (0.5 to 64 μg/ml), meropenem (0.12 to 8 μg/ml), levofloxacin (0.12 to 4 μg/ml), gentamicin (1 to μg/ml), tigecycline (0.015 to 16 μg/ml), and colistin (0.5 to 8 μg/ml). Concurrent quality control (QC) testing was performed to ensure proper test conditions and procedures. QC strains included *Escherichia coli* ATCC 25922 and 35218 and *Pseudomonas aeruginosa* ATCC 27853, and all QC results were within published ranges. Susceptibility breakpoints were used to determine susceptibility/resistance rates according CLSI and EUCAST guidelines (9, 10). As indicated by pharmacokinetics/pharmacodynamics (PK/PD) target attainment simulations, a ceftazidime-avibactam-susceptible breakpoint of ≤8 μg/ml was applied for all *Enterobacteriaceae* species (11). *E. coli*, *Klebsiella* spp. and *Proteus mirabilis* isolates displaying the CLSI criteria for an ESBL phenotype (MIC of >1 μg/ml for aztreonam, ceftazidime, and/or ceftriaxone) (9) were grouped as ESBL phenotype. Ceftazidime-nonsusceptible isolates displayed MIC values of ≥8 μg/ml, which are intermediate or resistant MIC values according to the CLSI breakpoint criteria (9).

Screening for β-lactamases. All 743 isolates collected in U.S. hospitals during 2013 displaying the CLSI ESBL phenotypic criteria as described above were tested for β-lactamase-encoding genes using the microarray-based assay Check-MDR CT101 kit (Check-points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M groups 1, 2, 8 + 25, and 9, TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMY II, DHA, FOX, KPC, and NDM-1. The most common mutations that expand the spectrum of TEM and SHV enzymes are detected, and these mutations include 104K, 164S/C/H, or 123S for TEM and 138S, 238A, and 240K for SHV.

All isolates displaying a ceftazidime-avibactam MIC of >4 μg/ml were screened for the presence of metallo-β-lactamase and serine-carbapenemase-encoding gene families bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{GES}, bla_{IMI}, bla_{NMC-A}, and bla_{SME} by PCR as previously described (12). Amplicons were sequenced on both strands, and results were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Amino acid sequences were compared with those available through the internet using NCBI/BLAST. These isolates were also submitted to a simplified protein extraction, and the hydrolysis of the extracts was measured against ceftazidime, meropenem, and imipenem as previously described (13).

RESULTS AND DISCUSSION

Overall, 99.9% (20,698 of 20,709) of *Enterobacteriaceae* strains were inhibited at a ceftazidime-avibactam MIC of 8 μg/ml or less, which is the ceftazidime-avibactam-susceptible breakpoint supported by PK/PD target attainment simulation studies (Table 1) (9, 11). Ceftazidime-avibactam MIC_{50} and MIC_{90} values were only 0.12 and 0.25 μg/ml, respectively, for this collection of isolates, and this MIC_{90} value was only greater than the one for meropenem (MIC_{90}, ≤0.06 μg/ml) (Table 2) among the comparator agents. Several antimicrobial agents displayed good activity (>90% susceptibility) against *Enterobacteriaceae* isolates by applying the CLSI breakpoint criteria, and those with higher susceptibility rates were meropenem (98.4%), tigecycline (98.0%), piperacillin-tazobactam (91.9%), and gentamicin (91.1%) (Table 2).

The highest ceftazidime-avibactam MIC result when tested against *E. coli* isolates (n = 6,468) was 4 μg/ml (1 isolate), and 99.9% of the isolates were inhibited at ≤1 μg/ml (Table 1). Ceftazidime-avibactam (MIC_{50}, 0.06 μg/ml; MIC_{90}, 0.12 μg/ml) displayed very good activity against this bacterial species, being the only agent as potent as meropenem (MIC_{50}, ≤0.06 μg/ml; MIC_{90}, ≤0.06 μg/ml) (Table 2). Ceftazidime alone (91.7% susceptible), piperacillin-tazobactam (95.5%), meropenem (99.8%), and tigecycline (100.0%) (Table 2) were the comparators showing the highest susceptibility rates against these isolates using the CLSI breakpoints. Ceftazidime-avibactam also displayed good activity against ESBL phenotype isolates (12.0% [n = 776]; MIC_{50}, 0.12 μg/ml; MIC_{90}, 0.25 μg/ml) (Table 1).

A total of 5,580 *Klebsiella* isolates were tested, including 4,421 *K. pneumoniae* and 1,159 *K. oxytoca* isolates. Ceftazidime-avibactam inhibited all *K. oxytoca* isolates and all but three *K. pneumoniae* isolates at a MIC of ≤8 μg/ml (Table 1). *K. pneumoniae* isolates displayed elevated resistance rates to the comparator agents tested, and the highest susceptibility rates applying the CLSI breakpoint criteria were those of tigecycline (99.3% susceptible), meropenem (93.2%), and gentamicin (91.3%) (Table 2). The CLSI ESBL phenotypic criteria were observed among 721 (16.3%) *K. pneumoniae* isolates, and 276 (6.2%) displayed meropenem-nonsusceptible MIC results (CLSI breakpoint criteria). Ceftazidime-avibactam displayed acceptable activity against these resistant subsets ($MIC_{50/90}$, 0.25/1 and 0.5/2 μg/ml, respectively) (Table 1). *K. oxytoca* isolates were more susceptible to comparator agents than *K. pneumoniae*, and various agents were active against ≥90.0% of isolates (Table 2). A total of 119 *K. oxytoca* isolates exhibited the ESBL phenotype, and ceftazidime-avibactam was active against these isolates (MIC_{50}, 0.25 μg/ml; MIC_{90}, 1 μg/ml) (Table 1).

Ceftazidime-avibactam exhibited potent activity against *P. mirabilis* (1,626 isolates), with a MIC_{90} of 0.06 μg/ml and 99.9% of strains (1,625 of 1,626) inhibited at ≤0.5 μg/ml (Table 1). This cephalosporin–β-lactamase inhibitor combination was also very active against ESBL phenotype isolates ($MIC_{50/90}$, 0.06/0.12 μg/ml) (Table 1). Only one isolate had a ceftazidime-avibactam MIC of >0.5 μg/ml (MIC, >32 μg/ml), and this isolate was highly resistant to all β-lactam agents tested (data not shown). Ceftazidime alone, piperacillin-tazobactam, and meropenem were very active against *P. mirabilis* isolates, inhibiting >98.0% of the isolates at the current CLSI breakpoints (Table 2).

Ceftazidime-avibactam was highly active against *Enterobacter cloacae* (2,261 isolates; MIC_{50} of 0.12 μg/ml and MIC_{90} of 0.5 μg/ml, with only one isolate not inhibited at ≤8 μg/ml), including ceftazidime-nonsusceptible strains (20.9%; MIC_{50}, 0.12 μg/ml; MIC_{90}, 0.5 μg/ml). The highest ceftazidime-avibactam MIC value among *E. cloacae* isolates was 32 μg/ml, representing one strain isolated from a urinary tract infection in a medical center located in New York City. This isolate was also resistant to meropenem (MIC, 8 μg/ml) and was negative for all carbapenemase-encoding genes tested, and no hydrolytic activity against carbapenems or ceftazidime was noted for this isolate. Ceftazidime-avibactam

TABLE 1 Frequency distribution of *Enterobacteriaceae* isolates collected in U.S. hospitals during 2011 to 2013 and tested against ceftazidime-avibactam

Organism/group (*n*)	No. (%) of isolates with ceftazidime-avibactam MIC (μg/ml) of:												MIC (μg/ml)	
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32	50%	90%
All *Enterobacteriaceae* (20,709)	3,166 (15.3)	6,743 (47.8)	6,960 (81.5)	2,510 (93.6)	932 (98.1)	267 (99.4)	81 (99.8)	25 (99.9)	14 (99.9)	6 (>99.9)	1 (>99.9)	4 (100.0)	0.12	0.25
E. coli														
All (6,486)	998 (15.4)	2,808 (58.7)	2,211 (92.8)	375 (98.6)	74 (99.7)	16 (99.9)	3 (100.0)	1 (100.0)					0.06	0.12
ESBL phenotype (776)[a]	50 (6.4)	128 (22.9)	371 (70.7)	150 (90.1)	57 (97.4)	16 (99.5)	3 (99.9)	1 (100.0)					0.12	0.25
K. pneumoniae														
All (4,421)	260 (5.9)	1,566 (41.3)	1,671 (79.1)	510 (90.6)	267 (96.7)	99 (98.9)	40 (99.8)	5 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	3 (100.0)	0.12	0.25
ESBL phenotype (721)[a]	29 (4.0)	35 (8.9)	151 (29.8)	155 (51.3)	206 (79.9)	97 (93.3)	40 (98.9)	5 (99.6)	0 (99.6)	0 (99.6)	0 (99.6)	3 (100.0)	0.25	1
Meropenem nonsusceptible (276)[b]	11 (4.0)	7 (6.5)	19 (13.4)	41 (28.3)	91 (61.2)	66 (85.1)	33 (97.1)	5 (98.9)	0 (98.9)	0 (98.9)	0 (98.9)	3 (100.0)	0.5	2
K. oxytoca														
All (1,159)	68 (5.9)	551 (53.4)	386 (86.7)	98 (95.2)	41 (98.7)	13 (99.8)	0 (99.8)	2 (100.0)					0.06	0.25
ESBL phenotype (119)[a]	2 (1.7)	8 (8.4)	44 (45.4)	24 (65.5)	28 (89.1)	11 (98.3)	0 (98.3)	2 (100.0)					0.25	1
P. mirabilis														
All (1,626)	1,074 (66.1)	498 (96.7)	42 (99.3)	9 (99.8)	2 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	0 (99.9)	1 (100.0)	≤0.03	0.06
ESBL phenotype (80)[a]	24 (30.0)	40 (80.0)	10 (92.5)	4 (97.5)	1 (98.8)	0 (98.8)	0 (98.8)	0 (98.8)	0 (98.8)	0 (98.8)	0 (98.8)	1 (100.0)	0.06	0.12
E. cloacae														
All (2,261)	38 (1.7)	114 (6.7)	1,084 (54.7)	644 (83.1)	275 (95.3)	82 (98.9)	16 (99.6)	7 (>99.9)	0 (>99.9)	0 (>99.9)	1 (100.0)		0.12	0.5
Ceftazidime nonsusceptible (473)[c]	7 (1.5)	4 (2.3)	32 (9.1)	128 (36.2)	207 (79.9)	72 (95.1)	15 (98.3)	7 (99.8)	0 (99.8)	0 (99.8)	1 (100.0)		0.5	1
E. aerogenes														
All (831)	37 (4.5)	266 (36.5)	347 (78.2)	133 (94.2)	40 (99.0)	5 (99.6)	0 (99.6)	2 (99.9)	0 (99.9)	1 (100.0)			0.12	0.25
Ceftazidime nonsusceptible (165)[c]	6 (3.6)	4 (6.1)	52 (37.6)	72 (81.2)	23 (95.2)	5 (98.2)	0 (98.2)	2 (99.4)	0 (99.4)	1 (100.0)			0.25	0.5
M. morganii (776)	369 (47.6)	261 (81.2)	85 (92.1)	37 (96.9)	15 (98.8)	8 (99.9)	0 (99.9)	0 (99.9)	1 (100.0)				0.06	0.12
C. koseri (503)	38 (7.6)	273 (61.8)	144 (90.5)	36 (97.6)	7 (99.0)	4 (99.8)	1 (100.0)						0.06	0.12
C. freundii (547)	7 (1.3)	86 (17.0)	256 (63.8)	132 (87.9)	46 (96.3)	15 (99.1)	3 (99.6)	1 (99.8)	0 (99.8)	1 (100.0)			0.12	0.5
S. marcescens (1,260)	6 (0.5)	83 (7.1)	583 (53.3)	433 (87.7)	127 (97.8)	16 (99.0)	8 (99.7)	1 (99.8)	1 (99.8)	2 (100.0)			0.12	0.5
P. vulgaris (301)	146 (48.5)	130 (91.7)	21 (98.7)	1 (99.0)	3 (100.0)								0.06	0.06
Providencia spp. (538)	125 (23.2)	107 (43.1)	130 (67.3)	102 (86.2)	35 (92.8)	9 (94.4)	10 (96.3)	6 (97.4)	12 (99.6)	2 (100.0)			0.12	0.5
KPC producers, 2013 only (120)	10 (8.3)	1 (9.2)	6 (14.2)	11 (23.3)	18 (38.3)	34 (66.7)	26 (88.3)	11 (97.5)	0 (97.5)	0 (97.5)	0 (97.5)	1 (100.0)	1	4
CTX-M-15-like-producers, 2013 only (284)	9 (3.2)	11 (7.0)	32 (18.3)	123 (61.6)	70 (86.3)	33 (97.9)	5 (99.6)	1 (100.0)					0.25	1
CTX-M-14-like-producers, 2013 only (107)	8 (7.5)	6 (13.1)	28 (39.3)	49 (85.0)	14 (98.1)	2 (100.0)							0.25	0.5

[a] CLSI criteria for an ESBL phenotype for *E. coli*, *Klebsiella* spp., and *P. mirabilis* were applied (MIC of >1 μg/ml for aztreonam, ceftazidime, and/or ceftriaxone).
[b] Meropenem-nonsusceptible isolates were categorized according to the CLSI criteria (MIC, ≥2 μg/ml) (8, 9).
[c] Cefazidime-nonsusceptible isolates were categorized according to the CLSI criteria (MIC, ≥8 μg/ml) (8, 9).

TABLE 2 Activities of ceftazidime-avibactam and comparator antimicrobial agents tested against *Enterobacteriaceae* isolates collected during 2011 to 2013 in U.S. hospitals[a]

Isolates (*n*) and antimicrobial agent(s)	MIC (μg/ml)			% S/I/R by criteria[b]	
	50%	90%	Range	CLSI	EUCAST
All *Enterobacteriacea* (20,709)					
Ceftazidime-avibactam[c]	0.12	0.25	≤0.03 to >32	99.9[c]	
Ceftazidime	0.12	8	≤0.015 to >32	89.4/1.3/9.3	87.2/2.2/10.6
Ceftriaxone	≤0.06	>8	≤0.06 to >8	86.4/1.1/12.5	86.4/1.1/12.5
Ampicillin-sulbactam	8	>32	≤0.25 to >32	54.2/17.4/28.4	54.2/0.0/45.8
Piperacillin-tazobactam	2	16	≤0.5 to >64	91.9/3.1/5.0	88.8/3.1/8.1
Meropenem	≤0.06	≤0.06	≤0.06 to >8	98.4/0.1/1.5	98.5/0.5/1.0
Levofloxacin	≤0.12	>4	≤0.12 to >4	82.2/2.0/15.8	80.7/1.5/17.8
Gentamicin	≤1	4	≤1 to >8	91.1/1.3/7.6	89.5/1.6/8.9
Tigecycline	0.25	1	≤0.03 to >4	98.0/1.9/0.1	92.6/5.4/2.0
Colistin	0.5	>8	≤0.06 to >8	−/−/−	75.4/0.0/24.6
E. coli (6,486)					
Ceftazidime-avibactam[c]	0.06	0.12	≤0.03 to 4	100.0[c]	
Ceftazidime	0.12	2	≤0.015 to >32	91.8/1.5/6.7	89.1/2.7/8.2
Ceftriaxone	≤0.06	>8	≤0.06 to >8	88.8/0.2/11.0	88.8/0.2/11.0
Ampicillin-sulbactam	8	>32	≤0.25 to >32	50.8/21.3/27.9	50.8/0.0/49.2
Piperacillin-tazobactam	2	8	≤0.5 to >64	95.2/2.2/2.6	92.9/2.3/4.8
Meropenem	≤0.06	≤0.06	≤0.06 to 8	99.8/<0.1/0.1	99.9/0.1/0.0
Levofloxacin	≤0.12	>4	≤0.12 to >4	69.9/0.7/29.4	69.5/0.4/30.1
Gentamicin	≤1	>8	≤1 to >8	87.6/0.5/11.9	86.8/0.8/12.4
Tigecycline	0.12	0.12	≤0.03 to 1	100.0/0.0/0.0	100.0/0.0/0.0
Colistin	0.5	0.5	≤0.06 to 8	−/−/−	99.6/0.0/0.4
K. pneumoniae (4,421)					
Ceftazidime-avibactam[c]	0.12	0.25	≤0.03 to >32	99.9[c]	
Ceftazidime	0.12	32	≤0.015 to >32	85.4/1.1/13.5	84.0/1.4/14.6
Ceftriaxone	≤0.06	>8	≤0.06 to >8	85.0/0.4/14.6	85.0/0.4/14.6
Ampicillin-sulbactam	8	>32	≤0.25 to >32	73.7/6.9/19.4	73.7/0.0/26.3
Piperacillin-tazobactam	4	>64	≤0.5 to >64	87.4/2.3/10.3	81.5/5.9/12.6
Meropenem	≤0.06	≤0.06	≤0.06 to >8	93.8/0.2/6.0	94.0/1.5/4.5
Levofloxacin	≤0.12	>4	≤0.12 to >4	86.5/1.3/12.2	85.6/0.9/13.5
Gentamicin	≤1	2	≤1 to >8	91.5/1.7/6.8	90.3/1.2/8.5
Tigecycline	0.25	1	0.015 to >4	99.0/0.9/0.1	95.1/3.9/1.0
Colistin	0.5	1	0.12 to >8	−/−/−	96.9/0.0/3.1
K. oxytoca (1,159)					
Ceftazidime-avibactam[c]	0.06	0.25	≤0.03 to 4	100.0[c]	
Ceftazidime	0.12	0.5	0.03 to >32	97.4/0.2/2.4	95.3/2.1/2.6
Ceftriaxone	≤0.06	2	≤0.06 to >8	90.0/1.1/8.9	90.0/1.1/8.9
Ampicillin-sulbactam	8	32	0.5 to >32	63.2/25.1/11.7	63.2/0.0/36.8
Piperacillin-tazobactam	2	8	≤0.5 to >64	91.7/0.5/7.8	90.1/1.6/8.3
Meropenem	≤0.06	≤0.06	≤0.06 to >8	99.5/0.2/0.3	99.7/0.2/0.1
Levofloxacin	≤0.12	0.25	≤0.12 to >4	97.0/0.6/2.4	95.6/1.4/3.0
Gentamicin	≤1	≤1	≤1 to >8	96.8/1.0/2.2	96.4/0.4/3.2
Tigecycline	0.12	0.5	0.06 to 4	99.9/0.1/0.0	98.4/1.5/0.1
Colistin	0.5	0.5	0.25 to 4	−/−/−	99.8/0.0/0.2
P. mirabilis (1,626)					
Ceftazidime-avibactam[c]	≤0.03	0.06	≤0.03 to >32	99.9[c]	
Ceftazidime	0.06	0.12	≤0.015 to >32	99.1/0.7/0.2	97.2/1.9/0.9
Ceftriaxone	≤0.06	≤0.06	≤0.06 to >8	95.7/0.7/3.6	95.7/0.7/3.6
Ampicillin-sulbactam	1	16	≤0.25 to >32	88.7/6.9/4.4	88.7/0.0/11.3
Piperacillin-tazobactam	≤0.5	1	≤0.5 to >64	99.8/0.1/0.1	99.7/0.1/0.2
Meropenem	≤0.06	0.12	≤0.06 to 2	99.9/0.1/0.0	100.0/0.0/0.0
Levofloxacin	≤0.12	>4	≤0.12 to >4	75.2/5.1/19.7	70.7/4.5/24.8
Gentamicin	≤1	8	≤1 to >8	89.6/2.7/7.7	86.3/3.3/10.4
Tigecycline	2	4	0.12 to >4	84.8/14.7/0.5	47.6/37.2/15.2
Colistin	>8	>8	0.5 to >8	−/−/−	0.6/0.0/99.4
E. cloacae (2,261)					
Ceftazidime-avibactam[c]	0.12	0.5	≤0.03 to 32	>99.9[c]	
Ceftazidime	0.25	>32	0.03 to >32	79.1/1.1/19.8	76.8/2.3/20.9
Ceftriaxone	0.25	>8	≤0.06 to >8	75.0/2.1/22.9	75.0/2.1/22.9

(Continued on following page)

TABLE 2 (Continued)

Isolates (*n*) and antimicrobial agent(s)	MIC (μg/ml)			% S/I/R by criteria[b]	
	50%	90%	Range	CLSI	EUCAST
Ampicillin-sulbactam	16	>32	≤0.25 to >32	29.9/22.8/47.3	29.9/0.0/70.1
Piperacillin-tazobactam	2	64	≤0.5 to >64	83.8/8.0/8.2	80.4/3.4/16.2
Meropenem	≤0.06	≤0.06	≤0.06 to >8	99.2/0.2/0.6	99.4/0.4/0.2
Levofloxacin	≤0.12	0.5	≤0.12 to >4	94.7/1.5/3.8	93.6/1.1/5.3
Gentamicin	≤1	≤1	≤1 to >8	95.3/0.6/4.1	95.0/0.3/4.7
Tigecycline	0.25	0.5	0.06 to 4	98.6/1.4/0.0	95.1/3.5/1.4
Colistin	0.5	>8	0.12 to >8	−/−/−	79.9/0.0/20.1
S. marcescens (1,260)					
Ceftazidime-avibactam[c]	0.12	0.5	≤0.03 to 16	99.8[c]	
Ceftazidime	0.25	0.5	0.03 to >32	97.1/0.4/2.5	96.2/0.9/2.9
Ceftriaxone	0.25	2	≤0.06 to >8	89.5/2.2/8.3	89.5/2.2/8.3
Ampicillin-sulbactam	32	>32	1 to >32	7.9/15.2/76.9	7.9/0.0/92.1
Piperacillin-tazobactam	2	8	≤0.5 to >64	96.1/2.6/1.3	94.3/1.8/3.9
Meropenem	≤0.06	≤0.06	≤0.06 to >8	99.0/0.3/0.7	99.3/0.3/0.4
Levofloxacin	≤0.12	1	≤0.12 to >4	95.7/2.2/2.1	92.7/3.0/4.3
Gentamicin	≤1	≤1	≤1 to >8	97.6/0.6/1.8	96.7/0.9/2.4
Tigecycline	0.5	1	0.12 to >4	98.9/0.9/0.2	94.9/4.0/1.1
Colistin	>8	>8	0.25 to >8	−/−/−	6.0/0.0/94.0
KPC producers (120)[d]					
Ceftazidime-avibactam[c]	0.25	1	≤0.015 to >32	97.5[c]	
Ceftazidime	>32	>32	4 to >32	2.5/2.5/95.0	0.0/2.5/97.5
Ceftriaxone	>8	>8	8 to >8	0.0/0.0/100.0	0.0/0.0/100.0
Aztreonam	>16	>16	16 to >16	0.0/0.0/100.0	0.0/0.0/100.0
Ampicillin-sulbactam	>32	>32	32 to >32	0.0/0.0/100.0	0.0/0.0/100.0
Piperacillin-tazobactam	>64	>64	64 to >64	0.0/2.5/97.5	0.0/0.0/100.0
Meropenem	>8	>8	1 to >8	1.7/5.8/92.5	7.5/28.3/64.2
Levofloxacin	>4	>4	≤0.12 to >4	10.8/1.7/87.5	9.2/1.6/89.2
Gentamicin	4	>8	≤1 to >8	51.7/11.6/36.7	45.0/6.7/48.3
Tigecycline	0.5	1	0.06 to 4	98.3/1.7/0.0	91.7/6.6/1.7
Colistin	0.5	8	0.25 to >8	−/−/−	83.1/0.0/16.9
CTX-M-15-like (284)[d,e]					
Ceftazidime-avibactam[c]	0.25	0.5	≤0.015 to 4	100.0[c]	
Ceftazidime	16	>32	0.25 to >32	12.6/12.7/74.7	2.8/9.8/87.4
Ceftriaxone	>8	>8	4 to >8	0.0/0.0/100.0	0.0/0.0/100.0
Aztreonam	>16	>16	0.25 to >16	8.1/4.2/87.7	2.1/6.0/91.9
Ampicillin-sulbactam	32	>32	4 to >32	7.7/14.1/78.2	7.7/0.0/92.3
Piperacillin-tazobactam	8	>64	≤0.5 to >64	74.9/14.5/10.6	56.9/18.0/25.1
Meropenem	≤0.06	≤0.06	≤0.06 to 8	97.9/0.3/1.8	98.2/1.8/0.0
Levofloxacin	>4	>4	≤0.12 to >4	14.0/3.9/82.1	12.3/1.7/86.0
Gentamicin	>8	>8	≤1 to >8	48.1/1.0/50.9	47.0/1.1/51.9
Tigecycline	0.12	0.5	0.03 to 4	99.3/0.7/0.0	95.4/3.8/0.7
Colistin	0.5	0.5	0.12 to >8	−/−/−	96.1/0.0/3.9
CTX-M-14-like (107)[d,f]					
Ceftazidime-avibactam[c]	0.25	0.5	≤0.015 to 0.5	100.0[c]	
Ceftazidime	2	8	0.06 to >32	83.0/12.3/4.7	41.5/41.5/17.0
Ceftriaxone	>8	>8	>8	0.0/0.0/100.0	0.0/0.0/100.0
Aztreonam	8	>16	≤0.12 to >16	48.1/27.4/24.5	11.3/36.8/51.9
Ampicillin-sulbactam	16	>32	4 to >32	33.0/22.7/44.3	33.0/0.0/67.0
Piperacillin-tazobactam	2	8	≤0.5 to >64	96.2/1.0/2.8	94.3/1.9/3.8
Meropenem	≤0.06	≤0.06	≤0.06 to 0.12	100.0/0.0/0.0	100.0/0.0/0.0
Levofloxacin	>4	>4	≤0.12 to >4	15.1/3.8/81.1	14.2/0.9/84.9
Gentamicin	≤1	>8	≤1 to >8	65.1/1.9/33.0	64.2/0.9/34.9
Tigecycline	0.12	0.25	0.06 to 4	99.1/0.9/0.0	96.2/2.9/0.9
Colistin	0.5	1	0.12 to >8	−/−/−	94.3/0.0/5.7

[a] The most common species and β-lactamase-producing isolates (2013 only) were analyzed separately.
[b] Percentages of isolates susceptible (S), intermediate (I), and resistant (R) according to 2014 CLSI (9) and EUCAST (10) criteria. −, negative.
[c] A susceptible breakpoint of ≤8 μg/ml was applied as indicated by PK/PD target attainment simulation studies (11).
[d] The KPC- and CTX-M-producing isolates analyzed were collected during 2013 only.
[e] Isolates also carrying genes encoding KPC or CTX-M-14-like enzymes were excluded from this analysis.
[f] Isolates also carrying genes encoding KPC or CTX-M-15-like enzymes were excluded from this analysis.

MIC values were slightly lower among *Enterobacter aerogenes* isolates ($MIC_{50/90}$, 0.12/0.25 μg/ml) than *E. cloacae* isolates.

Only two *Serratia marcescens* isolates had ceftazidime-avibactam MIC values of >8 μg/ml (99.8% of isolates [1,258 of 1,260] inhibited at ≤8 μg/ml; $MIC_{50/90}$, 0.12/0.5 μg/ml). The two isolates displaying a ceftazidime-avibactam MIC of 16 μg/ml displayed negative results for all carbapenemases tested and lack of hydrolysis for ceftazidime.

The ceftazidime-avibactam MIC_{90} was 0.12 μg/ml against *Citrobacter koseri* isolates, and 100.0% of the isolates tested ($n = 503$) were inhibited at ≤2 μg/ml (Table 1). Ceftazidime-avibactam MIC values were slightly higher among *Citrobacter freundii* isolates ($MIC_{50/90}$, 0.12/0.5 μg/ml) compared to *C. koseri* isolates ($MIC_{50/90}$, 0.06/0.12 μg/ml) (Table 1). One isolate had a ceftazidime-avibactam MIC value of 16 μg/ml, and this isolate carried no carbapenemase genes targeted and displayed no hydrolysis against ceftazidime.

Ceftazidime-avibactam exhibited potent activity against *Proteus vulgaris*, with a MIC_{90} of 0.06 μg/ml and the highest MIC at 0.5 μg/ml (Table 1). The activity of this β-lactam–β-lactamase inhibitor combination was also elevated against *Morganella morganii* isolates, and the MIC_{50} and MIC_{90} values were 0.06 and 0.12 μg/ml, respectively. All but one isolate were inhibited at a ceftazidime-avibactam MIC of ≤1 μg/ml.

A total of 99.6% of the *Providencia* isolates were inhibited at a ceftazidime-avibactam MIC of ≤8 μg/ml (Table 1), and this combination displayed MIC_{50} and MIC_{90} values of 0.12 and 0.5 μg/ml, respectively, when tested against these isolates. All 14 isolates displaying elevated ceftazidime-avibactam MIC values of 8 to 16 μg/ml were negative when screened for carbapenemase-encoding genes and displayed no hydrolytic activity against ceftazidime.

Occurrence of β-lactamase-producing isolates in 2013 and activity of ceftazidime-avibactam and comparators. Among *Enterobacteriaceae* isolates from 2013 ($n = 8,836$), the CLSI epidemiological screening criteria for the ESBL phenotype (9) were observed among 743 isolates (12.5% of 5,943 isolates belonging to targeted species), including 368 *E. coli* isolates (12.3% of the overall samples for this species), 298 *K. pneumoniae* isolates (17.0%), 38 *K. oxytoca* isolates (8.0%), and 39 *P. mirabilis* isolates (5.7%). These isolates were screened against several common β-lactamase-encoding genes.

A total of 307 isolates were positive for CTX-M group 1 (here called "CTX-M-15-like" and including CTX-M-1, CTX-M-15, and CTX-M-3 among others), and this was the most prevalent β-lactamase detected. The CTX-M-15-like enzyme was detected among all four organisms. This β-lactamase was observed alone in 130 strains or in 14 combinations with 1 to 4 other β-lactamase-encoding genes/families, including KPC (10 isolates) (Table 3). Isolates harboring CTX-M-15-like β-lactamase-encoding genes without carbapenemases or other CTX-M groups ($n = 284$) displayed elevated MIC values for cephalosporins, but ceftazidime-avibactam was very active against these isolates ($MIC_{50/90}$, 0.06/0.25 and 0.12/0.5 μg/ml, respectively). Meropenem ($MIC_{50/90}$, ≤0.06/≤0.06 μg/ml), tigecycline ($MIC_{50/90}$, 0.12/0.5 μg/ml), colistin ($MIC_{50/90}$, 0.5/0.5 μg/ml), and ceftazidime-avibactam were the most active agents tested against these strains (Table 3).

KPC serine-carbapenemases were very prevalent, being observed among 120 isolates, most of them *K. pneumoniae* (113 isolates) (Table 3), but also including *E. coli* (5 isolates) and *K. oxytoca* (2 isolates). KPC-encoding genes were detected alone or in the presence of narrow-spectrum enzymes (SHV and/or TEM) in 62 isolates, and combinations with transferable cephalosporinases, CTX-M and SHV ESBL were also noted (Table 3). KPC producers were very resistant to all agents tested (Table 2), and ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 μg/ml) and tigecycline ($MIC_{50/90}$, 0.5/1 μg/ml) were the most active antimicrobial agents against these isolates. Colistin ($MIC_{50/90}$, 0.5/8 μg/ml) displayed activity against 83.1% of the KPC-producing isolates according to EUCAST breakpoints. One KPC-producing isolate had ceftazidime-avibactam MIC result of >32 μg/ml, and further investigations demonstrated that this *K. pneumoniae* isolate produced KPC-2 and VIM-4. This isolate, belonging to sequence type 258 (ST258), also carried genes encoding CMY-2 and narrow-spectrum TEM and SHV and had overexpression of the efflux pump AcrAB-TolC and reduced expression of porin OmpK35- and OmpK37-encoding genes (M. Castanheira, L. M. Deshpande, J. C. Mills, R. N. Jones, S. G. Jenkins, and A. N. Schuetz, submitted for publication).

CTX-M group 9 enzymes and the CTX-M-14-like β-lactamase, the most common variant detected in U.S. hospitals within this group (6), were detected among 110 isolates: 94 *E. coli*, 10 *K. pneumoniae*, and 6 *P. mirabilis* isolates (Table 3). The CTX-M-14-like enzyme was the only β-lactamase detected among 57 isolates, and in 39 isolates, a combination of this enzyme and the TEM WT was observed (Table 3). Ceftazidime, piperacillin-tazobactam, meropenem, and tigecycline inhibited >80% (83.0 to 100.0%) of the CTX-M-14-like enzyme-producing isolates according to the CLSI breakpoint criteria, and colistin inhibited 94.3% of the isolates when the EUCAST breakpoint was applied. Ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.25 μg/ml; highest MIC, 0.5 μg/ml) displayed good activity against these strains (Table 2).

SHV enzymes with an extended spectrum of activity (SHV ESBLs) were detected among 118 isolates, and the vast majority of isolates (109/118) were *K. pneumoniae* (Table 3). SHV ESBL enzymes were found in all bacterial species, and SHV-12, SHV-5, SHV-7, SHV-2, and SHV-30 (in this order), are the most common SHV ESBL types reported in these U.S. hospitals (6). SHV ESBLs were detected in various combinations with other β-lactamases (Table 3). For the 61 isolates in which an SHV ESBL was the only extended-spectrum enzyme detected, meropenem ($MIC_{50/90}$, ≤0.06/≤0.06 μg/ml), ceftazidime-avibactam ($MIC_{50/90}$, 0.25/0.5 μg/ml) (Table 1), and tigecycline ($MIC_{50/90}$, 0.25/1 μg/ml) were the most active agents (data not shown).

Transferable cephalosporinases (plasmidic AmpCs) were detected among 62 isolates, and 54 strains displayed a positive result for the CMY II probe (here, "CMY-2-like"; 39 *E. coli*, 7 *K. pneumoniae* and 8 *P. mirabilis* isolates) (Table 3). All CMY-2-like enzyme-positive isolates that did not carry carbapenemases or CTX-M enzymes ($n = 48$) were susceptible to meropenem and tigecycline (100.0% susceptible according to CLSI breakpoint criteria). Ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.5 μg/ml) (Table 1), meropenem ($MIC_{50/90}$, ≤0.06/0.12 μg/ml) (data not shown), and tigecycline ($MIC_{50/90}$, 0.12/1 μg/ml) were the most active compounds against these isolates.

Other β-lactamases were also detected in smaller numbers, as follows: FOX (6 isolates), TEM ESBL (12 isolates), and one *K. pneumoniae* isolate each producing DHA and ACT/MIR. Additionally, narrow-spectrum enzymes of the SHV WT (all *K. pneumoniae* isolates [a ubiquitous enzyme in this species]) and TEM WT were detected among 284 and 348 isolates, respectively

TABLE 3 Enzymes alone and in combinations detected among isolates collected during 2013 in U.S. hospitals[a]

β-Lactamase(s) and enzyme(s)	No. of isolates producing enzyme(s) shown				
	Total	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*
Carbapenemases					
KPC	2	2			
KPC, CMY II, SHV WT, TEM WT	2		2		
KPC, CTX-M group 1, CMY II, SHV WT, TEM WT	1		1		
KPC, CTX-M group 1, SHV ESBL, SHV WT, TEM WT	1		1		
KPC, CTX-M group 1, SHV WT	5		5		
KPC, CTX-M group 1, SHV WT, TEM WT	3		3		
KPC, FOX, TEM WT	1			1	
KPC, SHV ESBL	1		1		
KPC, SHV ESBL, SHV WT	2		2		
KPC, SHV ESBL, SHV WT, TEM WT	42		42		
KPC, SHV WT	10		10		
KPC, SHV WT, TEM WT	44		44		
KPC, TEM WT	6	3	2	1	
ESBLs					
CTX-M group 1	130	127	1	1	1
CTX-M group 1, CMY II	1	1			
CTX-M group 1, CTX-M group 9	1	1			
CTX-M group 1, CTX-M group 9, TEM WT	2	2			
CTX-M group 1, SHV ESBL	3		3		
CTX-M group 1, SHV ESBL, SHV WT	1		1		
CTX-M group 1, SHV ESBL, SHV WT, TEM WT	3		3		
CTX-M group 1, SHV ESBL, TEM WT	1				1
CTX-M group 1, SHV WT	22		22		
CTX-M group 1, SHV WT, TEM WT	55		55		
CTX-M group 1, TEM WT	78	67		3	8
CTX-M group 9	57	55			2
CTX-M group 9, SHV ESBL, SHV WT, TEM WT	1		1		
CTX-M group 9, SHV WT	4		4		
CTX-M group 9, SHV WT, TEM WT	5		5		
CTX-M group 9, TEM ESBL	1	1			
CTX-M group 9, TEM WT	39	35			4
SHV ESBL	8		5	3	
SHV ESBL, SHV WT	38		38		
SHV ESBL, SHV WT, TEM WT	11		11		
SHV ESBL, TEM WT	4	2		2	
TEM ESBL	11	7		1	3
Transferable AmpC					
ACT/MIR, TEM WT	1		1		
CMY II, SHV ESBL, SHV WT, TEM WT	1		1		
CMY II, SHV ESBL, TEM WT	1	1			
CMY II, SHV WT	3		3		
CMY II, TEM WT	25	20			5
CMY II	20	17			3
DHA, SHV WT	1		1		
FOX	1				1
FOX, SHV WT	3		3		
FOX, TEM WT	1				1

(Continued on following page)

TABLE 3 (Continued)

β-Lactamase(s) and enzyme(s)	No. of isolates producing enzyme(s) shown				
	Total	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*
Narrow-spectrum enzymes					
SHV WT	24		24		
SHV WT, TEM WT	2		2		
TEM WT	18	12			6
Negative results	46	15	1	26	4

[a] Combinations were grouped according to the most relevant enzyme present.

(Table 3). Forty-six strains had negative results for all β-lactamases tested. The majority of these β-lactamase-negative isolates were *K. oxytoca* isolates that might hyperproduce OXY (K1) and were not evaluated in this study, but *E. coli*, *K. pneumoniae*, and *P. mirabilis* isolates displaying borderline MIC values (≤2 μg/ml) for the β-lactams used for the ESBL screening criteria were also noted. Among these isolates displaying negative β-lactamase screening results, one *P. mirabilis* isolate had a ceftazidime-avibactam MIC of >32 μg/ml; this strain was isolated from an intraabdominal infection in a medical center located in West Roxbury, MA. Screening for all carbapenemases, including metallo-β-lactamases followed up by ceftazidime hydrolysis displayed negative results for the presence of β-lactamases (data not shown).

Enterobacteriaceae isolates are an important cause of infections, and production of β-lactamases, including ESBLs, transferable cephalosporinases, and carbapenemases among these organisms has become a matter of great concern (1, 2, 14) since in most instances these resistance determinants are disseminated by plasmids and other mobile genetic elements carrying resistance genes for other antimicrobial classes.

A high prevalence of CTX-M- and KPC-producing isolates has been noticed in certain areas of the United States, but ESBL phenotype rates and the occurrence of isolates producing these enzymes are very heterogeneous (6, 7). However, due to the constant dissemination of these isolates and transfer of patients among hospitals, treatment options for severe infections caused by these MDR organisms need to be available.

According to recent publications, ceftazidime-avibactam is expected to play a role in the empirical monotherapy of invasive infections suspected to be caused by resistant *Enterobacteriaceae* pathogens and also potentially as therapy of KPC-producing *Enterobacteriaceae* infection (14, 15). The *in vitro* results from this study support this prediction, since ceftazidime-avibactam demonstrated very good activity against most isolates in this large collection of *Enterobacteriaceae* isolates recovered over 3 years in over 70 hospitals throughout the country. Additionally, as demonstrated for isolates collected in 2012 (7), ceftazidime-avibactam was very active against recent isolates producing the most common β-lactamases detected in U.S. hospitals, including CTX-M and KPC variants. The potent Gram-negative spectrum of activity of ceftazidime-avibactam, including activity against resistant organisms, demonstrates that it warrants further study in difficult-to-treat serious infections where resistant Gram-negative bacteria may occur.

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