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Ceftazidime-avibactam Activity Tested Against a Large Collection of Enterobacteriaceae Isolates Collected in United States (USA) Hospitals in the 2011-2013 Period, Including Organisms Producing KPC- and CTX-M-variants

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Abstract

Background: Increasing rates of multidrug-resistant (MDR) Enterobacteriaceae (ENT) challenges infection control and antimicrobial stewardship practices. We evaluated the activity of ceftazidime-avibactam (CAZ-AVI), a cephalosporin combined with a serine-β-lactamase (BL) inhibitor displaying activity against ENT, including those producing contemporary BLs.

Methods: 20,709 ENT isolates collected from 2011 to 2013 in 79 hospitals located in all nine USA Census regions were susceptibility (S) tested by CLSI broth microdilution against CAZ-AVI and comparators. CTX-M- and KPC-encoding genes were identified by a microarray based assay and/or reference PCR/sequencing.

Results: Overall CAZ-AVI inhibited 99.9% of isolates at ≤4 µg/mL (CLSI CAZ-S breakpoint) and was only less potent than meropenem (MIC_{90}, 0.25 and ≤0.06 µg/mL, respectively). Among 25 isolates displaying CAZ-AVI MICs at >4 µg/mL, 15 were indole-positive Proteae with MICs of 8-16 µg/mL and 3 *K. pneumoniae* (KPN) producing metallo-BLs (CAZ-AVI MIC, >32 µg/mL). Against the most prevalent bacterial species, CAZ-AVI inhibited all *E. coli* isolates, 99.9% of KPN and >99.9% of *E. cloacae* (ECL) at ≤4 µg/mL. CAZ-AVI $MIC_{50/90}$ for these species were 0.06/0.12, 0.12/0.25, 0.12/0.5 µg/mL, respectively (Table) whereas CAZ MIC_{90} values were 2, 32 and >32 µg/mL, respectively. All but one *P. mirabilis* were inhibited by CAZ-AVI at ≤0.5 µg/mL. 214 KPC-producers, 497 CTX-M-15-like and 102 CTX-M-14-like strains were identified and CAZ-AVI $MIC_{50/90}$ values for these strains were 0.5/2, 0.12/0.5 and 0.12/0.25 µg/mL, respectively. KPC-producers were very resistant to all comparators with CAZ-AVI, tigecycline ($MIC_{50/90}$, 0.5/1 µg/mL) and colistin ($MIC_{50/90}$, 0.5/2 µg/mL) being the only agents with acceptable coverage.

Conclusions: CAZ-AVI displayed high activity against contemporary ENT isolates, including those producing prevalent CTX-M-variants in the USA, and KPC-producers that are often MDR.

	CAZ-AVI MIC (µg/mL):	
Organisms/group (no. tested)	50%	90%
All (20,709)	0.12	0.25
E. coli (6,486)	0.06	0.12
K. pneumoniae (4,421)	0.12	0.25
E. cloacae (2,261)	0.12	0.5
P. mirabilis (1,626)	≤0.03	0.06
KPC-producers (214)	0.5	2
CTX-M-15-like-producers (497)	0.12	0.5
CTX-M-14-like-producers (102)	0.12	0.25

Introduction

Bacterial infections caused by Gram-negative bacterial pathogens producing β-lactamases have been recently documented throughout the United States (USA) and elevated prevalence of isolates producing CTX-M- and/or KPC-variants was observed. Carbapenem-resistant Enterobacteriaceae in the USA is mainly due to the presence of KPC-encoding genes and isolates harboring these enzymes have been reported to be multidrug resistant (MDR) and in certain instances, to be resistant to all clinically available antimicrobial agents, including polymyxins and tigecycline.

Avibactam is a non-β-lactam β-lactamase inhibitor of enzymes belonging to Ambler structural classes A, C, and some class D enzymes. When combined with a cephalosporin, avibactam was able to reduce the MIC values of β-lactamase-producing isolates, including those carrying bla_{KPC} from the resistant to susceptible ranges in the vast majority of tested isolates. Avibactam has been paired with ceftazidime and this cephalosporin/β-lactamase inhibitor combination currently is in various Phase II and III clinical trials for indications such as complicated intra-abdominal (cIAI) and complicated urinary tract infections (cUTI).

In this study, we evaluate the activity of ceftazidime-avibactam and comparator antimicrobial agents tested against 20,709 clinical Enterobacteriaceae isolates collected in USA hospitals during the 2011-2013 period. Among these isolates, 795 carried genes encoding KPC or CTX-M enzymes and were analyzed separately.

Methods

Bacterial isolates. A total of 20,709 Enterobacteriaceae clinical isolates were collected in 79 USA hospitals during 2011 (3,233 isolates), 2012 (8,640) and 2013 (8,836). These non-duplicate isolates considered clinically significant were recovered from bloodstream infections (3,315 isolates), pneumonia in hospitalized patients (3,486), skin/soft tissue infection (5,801), urinary tract infection (5,253), intra-abdominal infections (823), other respiratory tract infections (761) and other sites (1,270). Species identification was confirmed by standard biochemical tests and using the MALDI Biotyper (Bruker Daltonics, Billerica, Massachusetts, USA) according to the manufacturer instructions, where necessary.

Susceptibility testing. Broth microdilution test methods conducted according to the Clinical and Laboratory Standards Institute (CLSI) were performed to determine the antimicrobial susceptibility of ceftazidime-avibactam (inhibitor at fixed concentration of 4 µg/mL) and comparator agents. Validated minimum inhibitory concentration (MIC) panels were manufactured by ThermoFisher Scientific Inc. (Cleveland, Ohio, USA). Concurrent quality control (QC) testing was performed to assure proper test conditions and procedures. QC strains included: *Escherichia coli* ATCC 25922 and 35218 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within published ranges. Susceptibility percentages and validation of QC results were based on the CLSI guidelines (M100-S24) and susceptibility breakpoints were used to determine susceptibility/resistance rates (CLSI and EUCAST, 2014). *E. coli*, *Klebsiella* spp. and *P. mirabilis* isolates for which ceftriaxone or ceftazidime MIC were ≥2 µg/mL were considered to be phenotype-positive for ESBL production (CLSI, 2014).

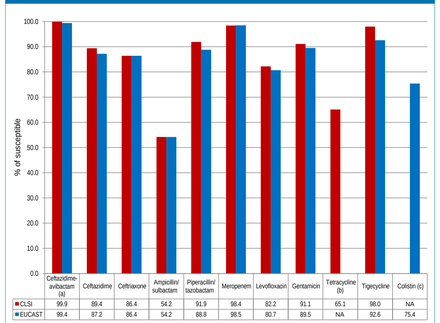
Results

- Overall, ceftazidime-avibactam inhibited 99.9 and 99.4% of the isolates at MIC values ≤4 µg/mL and ≤1 µg/mL that are the current susceptible breakpoints for ceftazidime according to the CLSI and EUCAST susceptibility criteria, respectively (Table 1).
- The highest ceftazidime-avibactam MIC among *E. coli* isolates (n=6,468) was 4 µg/mL (1 isolate). ESBL-phenotype strains were very susceptible ($MIC_{50,}$ 0.12 µg/mL and MIC_{90}, 0.25 µg/mL) and all non-ESBL strains were inhibited at ≤0.5 µg/mL by this compound (Table 1).
- Only three (0.1%, 4,421 overall) *K. pneumoniae* isolates had ceftazidime-avibactam MIC values >4 µg/mL (Table 1) and all produced metallo-β-lactamase enzymes: two NDM-1- and one KPC-2/VIM-4-producer. Ceftazidime-avibactam was active against non-ESBL *K. pneumoniae* (100.0% of strains inhibited at ≤1 µg/mL) and isolates displaying an ESBL-phenotype (meropenem susceptible; MIC_{50}, 0.25 µg/mL and MIC_{90}, 1 µg/mL; Table 1).
- Ceftazidime-avibactam exhibited potent activity against *P. mirabilis*, with a MIC_{90} of 0.06 µg/mL and only one isolate with MIC value at >32 µg/mL (Table 1).
- Ceftazidime-avibactam was highly active against *Enterobacter cloacae* (MIC_{50}, 0.12 µg/mL and MIC_{90}, 0.5 µg/mL, 100.0% inhibited at ≤4 µg/mL), including ceftazidime-non-susceptible strains (MIC_{50}, 0.5 µg/mL and MIC_{90}, 1 µg/mL; Table 1). One isolate had an MIC value of 32 µg/mL.
- Ceftazidime-avibactam was active against *E. aerogenes* ($MIC_{50/90}$, 0.12/0.25 µg/mL; Table 1), *Citrobacter koseri* ($MIC_{50/90}$, 0.06/0.12 µg/mL), *Citrobacter freundii* ($MIC_{50/90}$, 0.12/0.5 µg/mL) and *Serratia marcescens* ($MIC_{50/90}$, 0.12/0.5 µg/mL) isolates. Among these species, only one *E. aerogenes*, one *C. freundii and* three *S. marcescens* isolates had ceftazidime-avibactam MIC values at >4 µg/mL, one at 8 and four at 16 µg/mL (Table 1).
- Ceftazidime-avibactam inhibited 99.9, 100.0 and 94.4% of the *Morganella morganii, Proteus vulgaris* and *Providencia* spp. isolates at 1 µg/mL (Table 1).
- Using the current breakpoint criteria, meropenem (CLSI and EUCAST criteria) and tigecycline (US-FDA breakpoint only) along with ceftazidime-avibactam (applying ceftazidime alone breakpoints for comparison) were the only compounds to inhibit >95.0% of the isolates tested (Figure 1).
- Among isolates known to produce CTX-M-14-like, CTX-M-15-like and KPC enzymes, ceftazidime-avibactam inhibited all isolates at MIC values of 1, 2 and 4 µg/mL and ceftazidime alone inhibited 74.5, 15.3 and 1.9% of these isolates at the current CLSI breakpoint criteria (Figure 2).

Table 1. MIC distribution for ceftazidime-avibactam when tested against 20,709 Enterobacteriaceae clinical isolates collected during 2011-2013 in USA hospitals

Organism/group	No. of isolates tested	No. of isolates (cumulative %) inhibited at 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	20709	3166 (15.3)	6743 (47.8)	6960 (81.5)	2510 (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
Escherichia coli	6486	998 (15.4)	2808 (58.7)	2211 (92.8)	375 (98.6)	74 (99.7)	16 (99.9)	3 (>99.9)	1 (100.0)	--	--	--	--	0.06	0.12
non-ESBL-phenotype	5710	948 (16.6)	2680 (63.5)	1840 (95.8)	225 (99.7)	17 (100.0)	--	--	--	--	--	--	--	0.06	0.12
ESBL-phenotype	776	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
Klebsiella pneumoniae	4421	260 (5.9)	1566 (41.3)	1671 (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
non-ESBL-phenotype	3700	231 (6.2)	1531 (47.6)	1520 (88.7)	355 (98.3)	61 (99.9)	2 (100.0)	--	--	--	--	--	--	0.12	0.25
ESBL-phenotype	721	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-susceptible (MIC, ≤1 µg/mL)	4145	249 (6.0)	1559 (43.6)	1652 (83.5)	469 (94.8)	176 (99.0)	33 (99.8)	7 (100.0)	--	--	--	--	--	0.12	0.25
meropenem-non-susceptible (MIC, ≥2 µg/mL)	276	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
Klebsiella oxytoca	1159	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
non-ESBL-phenotype	1040	66 (6.3)	543 (58.6)	342 (91.4)	74 (98.6)	13 (99.8)	2 (100.0)	--	--	--	--	--	--	0.06	0.12
ESBL-phenotype	119	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
Proteus mirabilis	1626	1074 (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
non-ESBL-phenotype	1546	1050 (67.9)	458 (97.5)	32 (99.6)	5 (99.9)	1 (100.0)	--	--	--	--	--	--	--	≤0.03	0.06
ESBL-phenotype	80	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
Enterobacter cloacae	2261	38 (1.7)	114 (6.7)	1084 (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-susceptible (MIC, ≤4 µg/mL)	1788	31 (1.7)	110 (7.9)	1052 (66.7)	516 (95.6)	68 (99.4)	10 (99.9)	1 (100.0)	--	--	--	--	--	0.12	0.25
ceftazidime-non-susceptible (MIC, ≥8 µg/mL)	473	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
Enterobacter aerogenes	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-susceptible (MIC, ≤4 µg/mL)	666	31 (4.7)	262 (44.0)	295 (88.3)	61 (97.4)	17 (100.0)	--	--	--	--	--	--	--	0.12	0.25
ceftazidime-non-susceptible (MIC, ≥8 µg/mL)	165	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
Morganella 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
Citrobacter 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
Citrobacter 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
Serratia marcescens	1260	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
Proteus 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

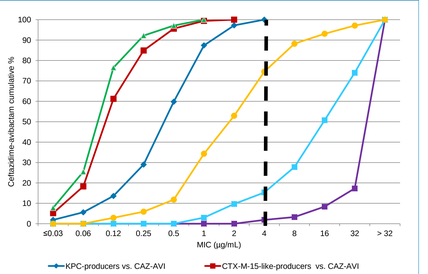
Figure 1. Activity of ceftazidime-avibactam and comparator antimicrobial agents when tested against 20,709 Enterobacteriaceae clinical isolates collected during 2011-2013 in USA hospitals



	Ceftazidime-avibactam (a)	Ceftazidime	Ceftriaxone	Ampicillin/sulbactam	Piperacillin/tazobactam	Meropenem	Levofloxacin	Gentamicin	Tetracycline (b)	Tigecycline	Colistin (c)
CLSI	99.9	89.4	86.4	54.2	91.9	98.4	82.2	91.1	65.1	98.0	NA
EUCAST	99.4	87.2	86.4	54.2	88.8	98.5	80.7	89.5	NA	92.6	75.4

a. Breakpoints for ceftazidime alone were applied for comparison purpose.
b. NA=Not applicable. EUCAST has not established tetracycline breakpoints for Enterobacteriaceae.
c. NA=Not applicable. CLSI has not established colistin breakpoints for Enterobacteriaceae.

Figure 2. Cumulative distribution of ceftazidime-avibactam (CAZ-AVI) and ceftazidime alone (CAZ) MIC values when tested against Enterobacteriaceae isolates producing KPC (n=214), CTX-M-15-like (n=479) and CTX-M-14-like (n=102). CLSI susceptible breakpoint for ceftazidime alone is represented in a black dotted line for comparison purposes



Conclusions

- Ceftazidime-avibactam demonstrated potent activity against Enterobacteriaceae strains including *E. coli*, *K. pneumoniae* and *P. mirabilis* displaying an ESBL-phenotype. This combination was also very active against *K. pneumoniae* strains showing decreased carbapenem susceptibility and ceftazidime-non-susceptible *Enterobacter* spp.
- The majority of isolates harboring KPC enzymes or genes encoding the most common types of CTX-M ESBLs in the USA were inhibited by ≤4 µg/mL of ceftazidime-avibactam (CLSI susceptible breakpoint for ceftazidime).
- Ceftazidime-avibactam will be an important addition to the armamentarium of antimicrobial agents used for the treatment of serious infections and those infections caused by MDR organisms.

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