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Activity of Ceftazidime-Avibactam Tested Against Enterobacteriaceae Isolates with Characterized β-Lactamase Encoding Genes Collected from United States Hospitals During 2012

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Abstract

Background: Ceftazidime (CAZ)-avibactam (AVI), currently being evaluated in Phase 3 clinical trials for indications including complicated urinary tract and intra-abdominal infections, has been reported to have good in vitro activity against isolates producing β-lactamases (BLs) from Ambler class A, C and some D. We evaluated the activity of this cephalosporin-BL inhibitor combination and comparator agents tested against 701 CLSI ESBL-phenotype isolates screened for genes encoding ESBLs, KPC, NDM and plasmidic (p) AmpC BLs.

Methods: 701 clinical isolates, n (% BL-producing), including 328 *E. coli* (11.9%), 296 *K. pneumoniae* (KPN; 16.0%), 44 *K. oxytoca* (KOX; 10.0%) and 33 *P. mirabilis* (4.8%), were collected from 73 hospitals located in nine USA Census regions. Isolates were susceptibility tested by reference broth microdilution methods and evaluated using Check-MDR CT101 microarray.

Results: ESBLs, carbapenemases and/or pAmpCs were detected among 631 (90.0%) isolates. 118 isolates (112 KPN) carried *bla*$_{KPC}$. 13 KPC-producers also carried CTX-M Group (G) 1 (which includes CTX-M-15). KPC-producers were very resistant (R) to all agents tested, except for CAZ-AVI (Table; MIC$_{50/90}$, 0.5/2 μg/mL) and tigecycline (TIG; MIC$_{50/90}$, 0.5/1 μg/mL). Among 290 isolates carrying CTX-M G1 alone or with 1-4 other BLs, CAZ-AVI was very active (MIC$_{50/90}$, 0.12/0.5 μg/mL) being slightly less potent than meropenem and TIG (MIC$_{50/90}$, ≤0.06/≤0.06 and 0.12/1 μg/mL, respectively). CAZ-AVI was very active against CTX-M G9-producers (70 isolates; MIC$_{50/90}$, 0.12/0.25 μg/mL). CMY II and SHV ESBL alone were detected among 54 and 83 isolates, respectively; and CAZ-AVI had good coverage against these isolates (MIC$_{50/90}$, 0.25/1 and 0.12/0.5 μg/mL, respectively). CTX-M G9-, CMY II- and SHV ESBL-producing strains were not as R to comparators as KPC- and CTX-M G1-producers. Other BLs detected were FOX (10 isolates), TEM ESBL (9), DHA (7), CTX-M G2 (3), NDM-1 (2; Colorado) and CTX-M G8+25 (1). Elevated CAZ-AVI MIC (>4 μg/mL) were only detected in the two NDM-producers.

Conclusions: CAZ-AVI demonstrated good activity against contemporary isolates producing the most prevalent BLs detected in USA hospitals. CAZ-AVI appears to be a promising addition to the armamentarium to treat multidrug-R gram-negative pathogens.

β-lactamase group[a]	MIC$_{50/90}$ in μg/mL				
	CAZ-AVI	PIP/TAZ	CAZ	MER	LEV
KPC (118)	0.5/2	>64/>64	>32/>32	>8/>8	>4/>4
CTX-M-14-like (70)	0.12/0.25	2/8	2/16	≤0.06/≤0.06	>4/>4
CTX-M-15-like (290)	0.12/0.5	16/>64	16/>32	≤0.06/≤0.06	>4/>4
SHV ESBL (83)	0.25/1	>64/>64	>32/>32	≤0.06/0.12	>4/>4
CMY-2-like (54)	0.12/0.5	4/>64	16/>32	≤0.06/0.12	>4/>4

a. Groups do not include isolates that harbor other enzymes listed. CAZ-AVI=ceftazidime-avibactam; CAZ=ceftazidime; PIP/TAZ=piperacillin/tazobactam; MER=meropenem; LEV=levofloxacin.

Introduction

Genes encoding β-lactamases are usually carried in plasmids or genetic structures that harbor additional genes defining resistance to other antimicrobial classes and conferring the isolates "multi-drug resistant (MDR) phenotypes". Very few antimicrobial treatment options seem to provide an adequate coverage for these β-lactamase-producing isolates, especially for those strains producing KPC or metallo-β-lactamases. Isolates producing these carbapenemases are resistant to most or all clinically available β-lactam agents and are usually refractory to fluoroquinolones, aminoglycosides and in some instances to colistin and tigecycline.

β-lactam/β-lactamase inhibitor combinations have been used in clinical practice for several decades; however, older inhibitors such as tazobactam, sulbactam and clavulanate are generally not active against isolates producing various contemporary β-lactamases. Avibactam is a novel non-β-lactam β-lactamase inhibitor of Ambler structural classes A, C, and some class D enzymes. When combined with a cephalosporin, avibactam has been able to reduce the MIC values against β-lactamase-producing isolates, including those carrying *bla*$_{KPC}$, from the resistant category to susceptible MIC ranges for the vast majority of tested isolates. Ceftazidime, like other cephalosporins, has limited activity against isolates producing broad-spectrum β-lactamases; but, ceftazidime activity can be restored when combined with avibactam.

In this study, we evaluated the activity of ceftazidime-avibactam tested against 701 contemporary Enterobacteriaceae isolates collected in United States (USA) hospitals during 2012 that displayed an ESBL-phenotype according to the Clinical and Laboratory Standards Institute (CLSI) criteria and that were screened for the presence of contemporary β-lactamases.

Methods

Bacterial isolates. A total of 5,739 isolates of *Escherichia coli* (n=2,767), *Klebsiella* spp. (n=2,289; 1,847 *K. pneumoniae* and 442 *K. oxytoca*) and *Proteus mirabilis* (n=683) were consecutively collected from 72 USA hospitals during 2012. Only one isolate per patient was included in the study from the following sites of infection: bloodstream (n=991); pneumonia from hospitalized patients (n=1,047); intra-abdominal (n=285); skin and skin structure (n=1,565); urinary tract (n=1,558); and other or unknown sources (n=293). Species identification was confirmed when necessary by Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) by following manufacturer instructions.

Antimicrobial susceptibility testing. All isolates were susceptibility tested using the reference broth microdilution method as described by the CLSI. Categorical interpretations for all antimicrobials were those found in M100-S23 (2013) and quality control (QC) was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within acceptable ranges as published in CLSI (M100-S23, 2013) documents.

Screening for β-lactamases. Isolates displaying the CLSI screening criteria for an ESBL-phenotype (MIC, >1 μg/mL for aztreonam and/or ceftazidime and/or ceftriaxone) 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 (which includes CTX-M-15-like), 2, 8+25 and 9 (which includes CTX-M-14-like), TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM-1.

Results

- Overall, 99.7% of the ESBL-phenotype isolates were inhibited by ceftazidime-avibactam at ≤4 μg/mL (current breakpoint for ceftazidime; Table 1), whereas only 26.2% of the isolates were inhibited by ceftazidime alone at the same concentration
- Only two isolates had ceftazidime-avibactam MIC results at ≥4 μg/mL (Table 1): two *E. cloacae* isolates producing NDM-1 from a unique medical center in Colorado (data not shown)
- All isolates producing CTX-M-14-like and CMY-2-like enzymes were inhibited by ceftazidime-avibactam at ≤1 μg/mL; and strains producing CTX-M-15-like and SHV ESBL variants were inhibited by ceftazidime-avibactam at ≤2 μg/mL (Table 1)
- KPC-producers were highly resistant to most antimicrobial agents tested (Table 2); however, ceftazidime-avibactam was very active against these isolates (MIC$_{50/90}$, 0.5/2 μg/mL). Tigecycline and tetracycline were the agents displaying greatest coverage but inhibited only 98.3% and 63.6% of the isolates at current CLSI/FDA breakpoints, respectively
- Ceftazidime-avibactam was very potent against isolates producing CTX-M-15-like (MIC$_{50/90}$, 0.12/0.5 μg/mL) and CTX-M-14-like (MIC$_{50/90}$, 0.12/0.25 μg/mL; Table 2) β-lactamases that did not carry carbapenemases. Meropenem (MIC$_{50/90}$, ≤0.06/≤0.06 μg/mL for both CTX-M groups), and tigecycline (MIC$_{50/90}$, 0.12/1 μg/mL for both groups) were the only comparators displaying high activity against CTX-M-producing isolates (inhibited >94.0% of the isolates using the current CLSI or FDA breakpoint criteria)
- Against isolates that produced a SHV ESBL enzyme and no concurrent carbapenemases, meropenem (MIC$_{50/90}$, ≤0.06/0.12 μg/mL), ceftazidime-avibactam (MIC$_{50/90}$, 0.25/1 μg/mL) and tigecycline (MIC$_{50/90}$, 0.5/1 μg/mL) were the most active compounds tested. Meropenem and tigecycline inhibited 98.8% and 100.0%, respectively, of these isolates at the current breakpoints and the highest ceftazidime-avibactam MIC was only 2 μg/mL (Table 2)
- Isolates producing CMY-2-like transferable cephalosporinase that did not carry carbapenemase genes were more susceptible to several of the comparator agents tested (Table 2). The activity of ceftazidime-avibactam (MIC$_{50/90}$, 0.12/0.5 μg/mL) was notably elevated when compared to other agents tested (MIC$_{90}$ range, 2->64 μg/mL, except meropenem at 0.12 μg/mL).

Table 1. MIC Frequency distribution of the β-lactamase producing Enterobacteriaceae tested against ceftazidime with and without avibactam

Organism group (no. tested)/ Antimicrobial agent	No. of isolates at MIC in μg/mL (cumulative %):												
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
All isolates (701)													
Ceftazidime-avibactam	10 (1.4)	17 (3.9)	88 (16.4)	249 (51.9)	132 (70.8)	118 (87.6)	65 (96.9)	17 (99.3)	3 (99.7)	0 (99.7)	0 (99.7)	0 (99.7)	2 (100.0)
Ceftazidime	--	--	1 (0.1)	12 (1.9)	9 (3.1)	13 (5.0)	36 (10.1)	70 (20.1)	43 (26.2)	74 (36.8)	91 (49.8)	111 (65.6)	241 (100.0)
KPC-producers (118)													
Ceftazidime-avibactam	--	--	2 (1.7)	11 (11.0)	15 (23.7)	43 (60.2)	32 (87.3)	12 (97.5)	3 (100.0)	--	--	--	--
Ceftazidime	--	--	--	--	--	--	--	--	--	--	6 (5.1)	9 (12.7)	103 (100.0)
CTX-M-15-like-producers (288)													
Ceftazidime-avibactam	4 (1.4)	10 (4.9)	39 (18.4)	119 (59.7)	67 (83.0)	32 (94.1)	15 (99.3)	2 (100.0)	--	--	--	--	--
Ceftazidime	--	--	--	--	--	4 (1.4)	4 (2.8)	22 (10.4)	12 (14.6)	41 (28.8)	62 (50.3)	69 (74.3)	74 (100.0)
CTX-M-14-like-producers (70)													
Ceftazidime-avibactam	--	5 (7.1)	14 (27.1)	33 (74.3)	11 (90.0)	4 (95.7)	3 (100.0)	--	--	--	--	--	--
Ceftazidime	--	--	--	3 (4.3)	2 (7.1)	5 (14.3)	15 (35.7)	14 (55.7)	13 (74.3)	8 (85.7)	4 (91.4)	4 (97.1)	2 (100.0)
SHV ESBL-producers (83)													
Ceftazidime-avibactam	3 (3.6)	0 (3.6)	9 (14.5)	22 (41.0)	14 (57.8)	24 (86.7)	9 (97.6)	2 (100.0)	--	--	--	--	--
Ceftazidime	--	--	--	--	--	--	2 (2.4)	4 (7.2)	4 (12.0)	6 (19.3)	8 (28.9)	15 (47.0)	44 (100.0)
CMY-2-like-producers (54)													
Ceftazidime-avibactam	--	2 (3.7)	12 (25.9)	23 (68.5)	10 (87.0)	5 (96.3)	2 (100.0)	--	--	--	--	--	--
Ceftazidime	--	--	--	--	--	--	--	1 (1.9)	6 (13.0)	13 (37.0)	9 (53.7)	14 (79.6)	11 (100.0)

Table 2. Activity of ceftazidime-avibactam and comparator antimicrobial agents when tested against 701 ESBL-phenotype-positive Enterobacteriaceae isolates collected from 72 USA hospitals located in the nine USA Census regions

Isolate group (no. tested)/ Antimicrobial agent	MIC (μg/mL)			CLSI[a]	EUCAST[a]
	MIC$_{50}$	MIC$_{90}$	Range	%S / %I / %R	%S / %I / %R
KPC-producers (118)					
Ceftazidime-avibactam	0.5	2	0.06 – 4	- / - / -	- / - / -
Ceftazidime	>32	>32	16 – >32	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Ceftriaxone	>8	>8	8 – >8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	>8	>8	2 – >8	0.0 / 4.2 / 95.8	4.2 / 24.6 / 71.2
Piperacillin/tazobactam	>64	>64	>64	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Levofloxacin	>4	>4	≤0.12 – >4	10.2 / 1.7 / 88.1	5.9 / 4.3 / 89.8
Gentamicin	8	>8	≤1 – >8	47.5 / 17.8 / 34.7	32.2 / 15.3 / 52.5
Tetracycline	4	>32	1 – >32	63.6 / 15.2 / 21.2	- / - / -
Tigecycline[b]	0.5	1	0.06 – 8	98.3 / 0.8 / 0.9	93.2 / 5.1 / 1.7
CTX-M-15-like-producers (288)[c]					
Ceftazidime-avibactam	0.12	0.5	≤0.015 – 2	- / - / -	- / - / -
Ceftazidime	16	>32	0.5 – >32	14.6 / 14.2 / 71.2	2.8 / 11.8 / 85.4
Ceftriaxone	>8	>8	8 – >8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	≤0.06	≤0.06	≤0.06 – 2	99.7 / 0.3 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	16	>64	≤0.5 – >64	67.2 / 17.3 / 15.5	48.3 / 18.9 / 32.8
Levofloxacin	>4	>4	≤0.12 – >4	12.5 / 4.2 / 83.3	11.5 / 1.0 / 87.5
Gentamicin	2	>8	≤1 – >8	53.3 / 2.1 / 44.6	51.6 / 1.7 / 46.7
Tetracycline	>32	>32	0.5 – >32	30.2 / 0.7 / 69.1	- / - / -
Tigecycline[b]	0.12	1	0.06 – 4	98.3 / 1.7 / 0.0	94.8 / 3.5 / 1.7
CTX-M-14-like-producers (70)[d]					
Ceftazidime-avibactam	0.12	0.25	0.03 – 1	- / - / -	- / - / -
Ceftazidime	2	16	0.12 – >32	74.3 / 11.4 / 14.3	35.7 / 38.5 / 25.7
Ceftriaxone	>8	>8	>8	0.0 / 0.0 / 100.0	0.0 / 0.0 / 100.0
Meropenem	≤0.06	≤0.06	≤0.06 – 0.25	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	2	8	≤0.5 – >64	92.9 / 2.8 / 4.3	91.4 / 1.5 / 7.1
Levofloxacin	>4	>4	≤0.12 – >4	17.1 / 2.9 / 80.0	17.1 / 0.0 / 82.9
Gentamicin	2	>8	≤1 – >8	55.7 / 0.0 / 44.3	54.3 / 1.4 / 44.3
Tetracycline	>32	>32	0.5 – >32	22.9 / 0.0 / 77.1	- / - / -
Tigecycline[b]	0.12	1	0.06 – 4	94.3 / 5.7 / 0.0	94.3 / 0.0 / 5.7
SHV ESBL-producers (83)[e]					
Ceftazidime-avibactam	0.25	1	≤0.015 – 2	- / - / -	- / - / -
Ceftazidime	>32	>32	1 – >32	12.0 / 7.3 / 80.7	2.4 / 9.6 / 88.0
Ceftriaxone	>8	>8	0.12 – >8	9.6 / 7.3 / 83.1	9.6 / 7.3 / 83.1
Meropenem	≤0.06	0.12	≤0.06 – 2	98.8 / 1.2 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	>64	>64	1 – >64	45.8 / 3.6 / 50.6	36.1 / 9.7 / 54.2
Levofloxacin	>4	>4	≤0.12 – >4	39.8 / 7.2 / 53.0	37.3 / 2.5 / 60.2
Gentamicin	2	>8	≤1 – >8	61.4 / 10.9 / 27.7	51.8 / 9.6 / 38.6
Tetracycline	4	>32	0.5 – >32	55.4 / 9.7 / 34.9	- / - / -
Tigecycline[b]	0.5	1	0.06 – 2	100.0 / 0.0 / 0.0	94.0 / 6.0 / 0.0
CMY-2-like-producers (54)[f]					
Ceftazidime-avibactam	0.12	0.5	0.03 – 1	- / - / -	- / - / -
Ceftazidime	16	>32	2 – >32	13.0 / 24.0 / 63.0	0.0 / 13.0 / 87.0
Ceftriaxone	>8	>8	0.5 – >8	1.9 / 14.8 / 83.3	1.9 / 14.8 / 83.3
Meropenem	≤0.06	0.12	≤0.06 – 0.25	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
Piperacillin/tazobactam	4	>64	≤0.5 – >64	81.5 / 7.4 / 11.1	72.2 / 9.3 / 18.5
Levofloxacin	≤0.12	>4	≤0.12 – >4	70.4 / 1.8 / 27.8	64.8 / 5.6 / 29.6
Gentamicin	≤1	>8	≤1 – >8	79.6 / 1.9 / 18.5	77.8 / 1.8 / 20.4
Tetracycline	>32	>32	0.5 – >32	33.3 / 1.9 / 64.8	- / - / -
Tigecycline[b]	0.12	2	0.06 – 4	96.3 / 3.7 / 0.0	85.2 / 11.1 / 3.7

a. Criteria as published by the CLSI and EUCAST.
b. USA-FDA breakpoints were applied.
c. Listed CTX-M-15-like do not include KPC- or NDM-1-producing isolates.
d. Listed CTX-M-14-like do not include CTX-M-15-like-producing isolates.
e. Listed SHV ESBL do not include CTX-M-15-like-, CTX-M-14-like-, KPC- or NDM-1-producing isolates.
f. Listed CMY-2-like do not include CTX-M-15-like-, CTX-M-14-like-, KPC- or NDM-1-producing isolates.

Conclusions

- Despite the β-lactamase enzymes or combination of enzymes produced, ceftazidime-avibactam displayed potent activity against these contemporary isolates producing β-lactamases that are prevalent in USA hospitals during 2012 (except those harboring NDM-1)
- MDR rates among Enterobacteriaceae isolates are a matter of great concern and organisms carrying carbapenemases and other prevalent β-lactamases also display resistance to most clinically available antimicrobial agents. Ceftazidime-avibactam will be a potentially useful addition to the armamentarium of agents to treat infections cause by MDR Enterobacteriaceae.

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