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Ceftazidime-Avibactam Activity Tested against Eleven Enterobacteriaceae Species Producing KPC Enzymes

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Abstract

Background: KPC enzymes are usually detected in *K. pneumoniae* (KPN); however other Enterobacteriaceae (ENT) species often carry these carbapenemases. We evaluated the activity of ceftazidime-avibactam (CAZ-AVI) and comparators tested against KPC-producing ENT belonging to 11 bacterial species.

Methods: 662 KPC-producing ENT clinical isolates collected worldwide from 2009–2014 were susceptibility (S) tested by reference broth microdilution methods using CAZ-AVI (AVI at fixed 4 µg/ml) and comparators. CLSI, US-FDA (tigecycline [TIG] and CAZ-AVI) and EUCAST interpretative criteria were applied. KPC and metallo-β-lactamase (MBL) genes were detected by PCR/sequencing.

Results: Overall, CAZ-AVI inhibited 98.8% of the isolates at the breakpoint recently established by the US-FDA. Among other antimicrobial agents, TIG (97.4 and 90.8% S by US-FDA and EUCAST criteria, respectively) and colistin (COL; 82.1% S [EUCAST]) were the only agents with S rates >80.0%. Amikacin inhibited 56.2 and 40.2% of the isolates according to CLSI and EUCAST S criteria, respectively. Isolates displaying elevated CAZ-AVI (>8 µg/ml) were one *E. cloacae* (ECL; Poland) and seven KPN isolates from China, Greece, USA and Italy (n=4). These 8 isolates co-produced MBL: five VIM-1-producers and one each of IMP-4, VIM-4 and VIM-26. KPN isolates displayed higher resistance rates for comparators; CAZ-AVI, TIG and COL were the agents with higher S rates (98.7, 97.5/91.1 and 81.9% S, respectively). KPC-producing ECL isolates had higher S rates to amikacin (95.2/85.7% S) compared to KPN and CAZ-AVI displayed the highest S rates (97.6% S). CAZ-AVI was active against all isolates belonging to other ENT species (100.0% S; Table).

Conclusions: CAZ-AVI was very active against this large collection of KPC-producing isolates, inhibiting >97.0% of the isolates at the US-FDA breakpoint criteria. Eight isolates in this collection displaying CAZ-AVI MIC values >8 µg/ml and co-produced MBLs that are not inhibited by AVI.

KPC-producing species (no. tested)	% susceptible CLSI (US-FDA for CAZ-AVI and tigecycline)/EUCAST				
	CAZ-AVI	Meropenem	Amikacin	Tigecycline	Colistin
K. pneumoniae (554)	98.7/-	1.8/6.0	49.5/32.5	97.5/91.1	-/81.9
E. cloacae (42)	97.6/-	4.8/11.9	95.2/85.7	95.2/81.0	-/83.3
E. coli (24)	100.0/-	12.5/37.5	83.3/62.5	100.0/100.0	-/95.8
K. oxytoca (12)	100.0/-	0.0/33.3	91.7/83.3	100.0/100.0	-/91.7
C. freundii (10)	100.0/-	10.0/30.0	90.0/90.0	100.0/90.0	-/100.0

Introduction

KPC was first described from a *Klebsiella pneumoniae* isolate collected in 1991 of a patient hospitalized in New York City. Isolates producing KPC enzymes quickly became prevalent in the New York City area and recent data demonstrate that these enzymes have been detected in all but two states in the United States (USA) according to the Centers for disease Control (CDC). Outside of the USA, KPC-producing isolates have been reported in Germany, Poland, Belgium, Hungary, Croatia and the United Kingdom. Furthermore, these genes are considered endemic in Greece and Italy and these enzymes are also very prevalent in other countries such as Israel, China and Brazil.

KPC enzymes hydrolyze and encode resistance to virtually all β-lactams and isolates carrying genes encoding these β-lactamases are often resistant to other antimicrobial classes and although these enzymes are mainly detected in *K. pneumoniae*, several other Enterobacteriaceae species and non-fermentative Gram-negative bacilli have been reported carrying KPC genes.

In this study, we evaluated the activity of ceftazidime-avibactam and comparator agents tested against 662 KPC-producing Enterobacteriaceae isolates, including 11 bacterial species.

Methods

Bacterial isolates. A total of 662 KPC-producing Enterobacteriaceae clinical isolates collected during 2009 to 2014 were tested. These isolates were collected in Argentina (17 isolates), Brazil (46), China (15), Colombia (10), Ecuador (3), Germany (2), Greece (13), Israel (23), Italy (26), Panama (4), Poland (13), Turkey (3), USA (478) and Venezuela (9). Only one isolate per patient infection episode was included in the study. Isolates were collected from bloodstream infection (205 isolates; 31.0%), intra-abdominal infections (17; 2.6%), pneumonia in hospitalized patients (180; 27.2%), skin/soft tissue infection (126; 19.0%), urinary tract infection (101; 15.3%) or other or unknown sites (33; 5.0%).

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. Isolates belonged to the following bacterial species/groups: *Citrobacter freundii* species complex (10 isolates), *Enterobacter aerogenes* (7), *Enterobacter cloacae* species complex (42), *Escherichia coli* (24), *Klebsiella oxytoca* (12), *Klebsiella pneumoniae* (554), *Morganella morganii* (1), *Proteus mirabilis* (1), *Proteus penneri* (1), *Serratia marcescens* (5), and *Citrobacter* spp. (5).

Antimicrobial susceptibility testing. All isolates were susceptibility tested using reference broth microdilution method against ceftazidime-avibactam (avibactam at fixed 4 µg/ml) and comparator antimicrobial agents as described by the Clinical and Laboratory Standards Institute (CLSI). Categorical interpretations were those found in CLSI document M100-S26, the EUCAST website or United States Food and Drug Administration (US-FDA) package inserts for tigecycline and ceftazidime-avibactam. Quality control (QC) was performed using *Escherichia coli* ATCC 25922 and 35218, *K. pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within published ranges as published in CLSI documents.

KPC Screening. KPC encoding genes were screened using PCR methods or a microarray based assay (Check-MDR CT101 kit; Check-points, Wageningen, Netherlands). Sequencing of selected amplicons was performed and protein alignments were compared with available sequences.

Results

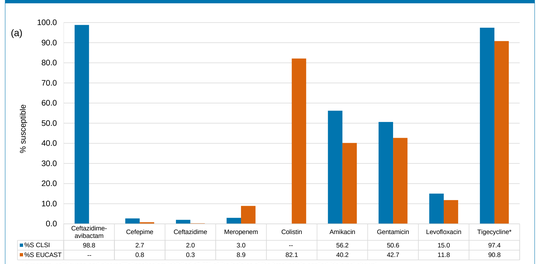
- Ceftazidime-avibactam inhibited 98.8% of the KPC-producing Enterobacteriaceae isolates at ≤8 µg/ml, the breakpoint recently established by the US-FDA (Table 1).
- Ceftazidime-avibactam inhibited 98.7% (547/554) of the KPC-producing *K. pneumoniae* isolates at ≤8 µg/ml (Table 1). Seven isolates displayed ceftazidime-avibactam MIC values >8 µg/ml: four VIM-1-producers and one each of IMP-4, VIM-4 and VIM-26 and were collected in China, Greece, USA and Italy (n=4).
- All but one (97.6%) of the *E. cloacae* isolates were inhibited by ceftazidime-avibactam at ≤8 µg/ml (Table 1). The isolate displaying a ceftazidime-avibactam MIC value >8 µg/ml was a KPC-producing *E. cloacae* that co-produced VIM-1 collected during 2011 in a Polish hospital.
- Ceftazidime-avibactam inhibited all (100.0%) of the KPC-producing *E. coli*, *C. freundii* and *K. oxytoca* at the current US-FDA breakpoint (Table 1).
- When KPC-2- and KPC-3-types are analysed separately, 99.3 and 98.1% of the isolates are inhibited by ceftazidime-avibactam at the US-FDA breakpoint. Noteworthy, the majority of KPC-2-producing isolates seem to be inhibited by ceftazidime-avibactam at lower concentrations when compared to KPC-3 isolates (90.3 versus 79.3% at ≤2 µg/ml for KPC-2 and KPC-3, respectively; Table 1).
- Other β-lactams displayed limited activity against KPC-producing Enterobacteriaceae and susceptibility rates were 0.3 to 8.9% being lowest for ceftazidime (CLSI/EUCAST) and highest for meropenem (EUCAST; Figure 1a).
- Among comparators from other antimicrobial classes, tigecycline (97.4/90.8% susceptible by US-FDA/EUCAST criteria, respectively) and colistin (82.1% by EUCAST) were the most active non-β-lactam agents. Amikacin inhibited 56.2 and 40.2% of the isolates according to CLSI and EUCAST susceptibility criteria, respectively (Figure 1a).
- KPC-producing *K. pneumoniae* isolates displayed higher resistance rates for amikacin, gentamicin and colistin (49.5, 52.9 and 81.9% susceptible CLSI for aminoglycosides and EUCAST for colistin, respectively) when compared to other bacterial species producing KPC (Figure 1b).
- KPC-2-producing isolates displayed slightly higher susceptibility rates for amikacin (57.5%), gentamicin (58.6%) and levofloxacin (17.5%) when compared to KPC-3 (51.4, 43.8, 12.5% susceptible, respectively). Susceptibility rates to β-lactams, although very low for both groups, were also slightly greater for KPC-2 when compared to KPC-3 (Figure 1c).

Table 1. MIC distributions for ceftazidime-avibactam when tested against 662 KPC-producing Enterobacteriaceae isolates collected from 2009 to 2014 worldwide.

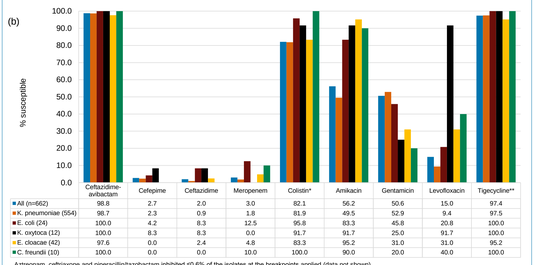
Organism/Organism group/KPC type[a]	No. of isolates tested	No. of isolates inhibited at ceftazidime/avibactam MIC (µg/mL; cumulative %)[b]: ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32	MIC_{50}	MIC_{90}
All Enterobacteriaceae	662		25 (3.8)	19 (6.6)	51 (14.4)	102 (29.8)	194 (59.1)	169 (84.6)	76 (96.1)	15 (98.3)	3 (98.8)	3 (99.2)	0 (99.2)	5 (100.0)	0.5	2
Klebsiella pneumoniae	554		25 (4.5)	11 (6.5)	41 (13.9)	82 (28.7)	179 (61.0)	132 (84.8)	65 (96.6)	11 (98.6)	1 (98.7)	3 (99.3)	0 (99.3)	4 (100.0)	0.5	2
Escherichia coli	24			5 (20.8)	7 (50.0)	10 (91.7)	2 (100.0)								0.12	0.25
Klebsiella oxytoca	12					2 (16.7)	1 (25.0)	8 (91.7)	0 (91.7)	1 (100.0)					1	1
Enterobacter cloacae species complex	42					3 (7.1)	5 (19.0)	21 (69.0)	8 (88.1)	2 (92.9)	2 (97.6)	0 (97.6)	0 (97.6)	1 (100.0)	1	4
Citrobacter freundii species complex	10					1 (10.0)	1 (20.0)	6 (80.0)	2 (100.0)						1	2
KPC-2	268		3 (1.1)	8 (4.1)	29 (14.9)	57 (36.2)	91 (70.1)	54 (90.3)	22 (98.5)	0 (98.5)	2 (99.3)	0 (99.3)	0 (99.3)	2 (100.0)	0.5	1
KPC-3	208		7 (3.4)	7 (6.7)	10 (11.5)	19 (20.7)	50 (44.7)	72 (79.3)	29 (93.3)	9 (97.6)	1 (98.1)	3 (99.5)	0 (99.5)	1 (100.0)	1	2

a. KPC sequencing results were available for 478 isolates, including 268 KPC-2, 208 KPC-3 and one each of KPC-4 and KPC-20 (not analyzed).

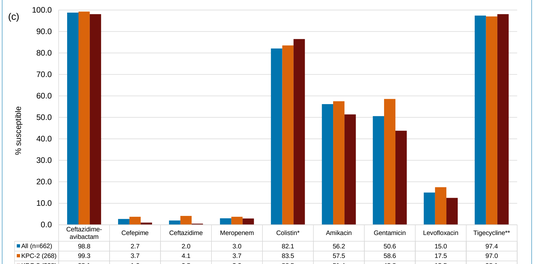
Figure 1. Activity of ceftazidime-avibactam and comparator antimicrobial agents (a) using CLSI and ECAST breakpoints when tested against all 662 KPC-producing Enterobacteriaceae isolates, (b) using CLSI breakpoints when tested against the most common bacterial species and (c) using CLSI breakpoints when tested against the most common KPC types.



Aztreonam, ceftriaxone and piperacillin/tazobactam inhibited ≤0.6% of the isolates at the breakpoints applied (data not shown).
*Tigecycline interpretation on %S CLSI was performed according to US-FDA breakpoint criteria.



Aztreonam, ceftriaxone and piperacillin/tazobactam inhibited ≤0.6% of the isolates at the breakpoints applied (data not shown).
*Colistin interpretation was performed according to EUCAST breakpoint criteria.
**Tigecycline interpretation was performed according to US-FDA breakpoint criteria.



*Colistin interpretation was performed according to EUCAST breakpoint criteria.
**Tigecycline interpretation was performed according to US-FDA breakpoint criteria.

Conclusions

- Ceftazidime-avibactam was very active against a large global collection of KPC-producing Enterobacteriaceae isolates that included 11 bacterial species. The only KPC-producing isolates displaying ceftazidime-avibactam >8 µg/ml co-produced metallo-β-lactamases that are not inhibited by β-lactamase inhibitors clinically available or in late development stages.
- KPC-producing *K. pneumoniae* (the most prevalent species) displayed higher resistance rates to comparator agents when compared to other bacterial species; however, ceftazidime-avibactam inhibited 98.7% of these isolates at current susceptibility breakpoint.
- Although minor differences in the ceftazidime-avibactam MIC distributions were noted when comparing isolates producing KPC-2 and KPC-3, this combination inhibited 99.3 and 98.1% of the isolates at current susceptibility breakpoint.
- KPC-producing isolates become very prevalent in the USA and other countries and ceftazidime-avibactam is very active against a large collection of these isolates.

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