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Antimicrobial Susceptibility of KPC-Producing Enterobacteriaceae Stratified by Infection Site (USA, 2012-2015)

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Abstract

Background: KPC-producing strains have been increasingly reported worldwide and these isolates, predominantly *K. pneumoniae* (KPN), are often resistant to most or all available antimicrobials. We evaluated the antimicrobial susceptibility of KPC-producing Enterobacteriaceae isolates from various infection types.

Methods: 465 KPC-producing Enterobacteriaceae clinical isolates were collected from 49 USA medical centers in 2012–2015 as part of the the INFORM Surveillance Program, and tested for susceptibility by reference broth microdilution methods using ceftazidime-avibactam (CAZ-AVI; AVI at fixed 4 µg/mL), gentamicin (GEN), amikacin (AMK), tigecycline (TIG), colistin (COL) and others. CLSI, US-FDA (CAZ-AVI and TIG) and EUCAST (COL) interpretative criteria were applied. KPC and other β-lactamase genes were detected by PCR/sequencing.

Results: KPN represented 83.9% of the collection, and *Enterobacter* spp. was the second most common organism (n=31; 6.7%). The most active agents overall were CAZ-AVI (99.4% susceptible [S]) and TIG (98.9% S; Table). Only 81.5% and 59.4% of isolates were S to COL and AMK, respectively. Only three isolates were CAZ-AVI-non-S, and two of them co-produced a metallo-β-lactamase (NDM-1 or VIM-4). KPC-producing isolates were more commonly isolated from pneumonia (n=153), urinary tract (UTI; n=99) and skin/soft tissue infections (SSSI; n=74). CAZ-AVI (98.0-100.0% S) and TIG (94.1-100.0% S) were consistently active against isolates from all sites of infections, whereas S rates varied from 77.8 to 92.8% for COL, 51.5 to 64.7% for AMK and 43.2 to 60.4% for GEN. Levofloxacin was active against only 15.7% of isolates. Among KPN, S rates were 99.2% for CAZ-AVI and only 80.5% for COL. S rates for CAZ-AVI among isolates from intensive care unit (ICU) patients did not vary substantially compared to those from non-ICU patients.

Conclusions: The novel β-lactam/β-lactamase inhibitor combination CAZ-AVI was very active against this large collection of KPC-producing isolates (99.4% S) and represents a very valuable addition to the limited armamentarium currently available for the treatment of infections caused by KPC-producing Enterobacteriaceae.

Infection site/ organism	MIC_{50}/MIC_{90} (% Susceptible)				
	CAZ-AVI	TIG	COL	AMK	GEN
All (465)	0.5 /2 (99.4)	0.5 / 1 (98.9)	≤0.5 / >8 (81.5)	16 / 32 (59.4)	8 / >8 (49.0)
BSI (53)	0.5 / 2 (98.1)	0.5 / 2 (100.0)	≤0.5 / >8 (80.4)	16 / 32 (60.4)	2 / >8 (60.4)
Pneumonia (153)	0.5 / 1 (100.0)	0.5 / 1 (100.0)	≤0.5 / >8 (78.1)	16 / 32 (64.1)	4 / >8 (53.6)
SSSI (74)	0.5 / 2 (100.0)	0.5 / 1 (100.0)	≤0.5 / >8 (82.2)	16 / 32 (63.5)	8 / >8 (43.2)
UTI (99)	0.5 / 2 (98.0)	0.5 / 1 (98.0)	≤0.5 / >8 (77.8)	16 / >32 (51.5)	8 / >8 (43.4)
IAI (17)	0.5 /1 (100.0)	0.5 / 2 (94.1)	≤0.5 / >8 (88.2)	16 / 32 (64.7)	8 / >8 (47.1)
Other infections (69)	1 / 2 (100.0)	0.5 / 2 (97.1)	≤0.5 / 2 (92.8)	16 / 32 (53.6)	8 / >8 (44.9)
K. pneumoniae (390)	0.5 / 2 (99.2)	0.5 / 1 (99.0)	≤0.5 / >8 (80.5)	16 / 32 (52.6)	4 / >8 (52.8)

BSI=bloodstream infections; SSSI=skin/soft tissue infections; UTI=urinary tract infections; IAI=intra-abdominal infections

Introduction

KPC-producing isolates are a matter of concern worldwide. In the United States (USA), these organisms became very prevalent in the New York City area and recent data demonstrate that these enzymes have been detected in all but two USA states according to the Center for disease Control (CDC).

KPC-encoding genes are carried by transposons and plasmids that are mobile genetic structures usually harboring resistance genes to additional antimicrobial classes and conferring a multidrug resistance profile to the isolates carrying them. Furthermore, the genes encoding KPC are usually associated with internationally successful *Klebsiella pneumoniae* clones that facilitate its dissemination.

KPC enzymes hydrolyze and encode resistance to virtually all β-lactams and these enzymes are poorly inhibited by older β-lactamase inhibitors such as clavulanic acid and tazobactam. In this study, we evaluated the activity of ceftazidime-avibactam and comparator agents tested against 465 KPC-producing Enterobacteriaceae isolates stratified by infection types. Additionally, the antimicrobial susceptibility of isolates from intensive care unit (ICU) was compared to those from non-ICU.

Methods

Bacterial isolates. A total of 465 KPC-producing Enterobacteriaceae clinical isolates were collected from 49 USA hospitals during 2012–2015 as part of the the INFORM Surveillance Program. 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 MALDI Biotyper (Billerica, Massachusetts, USA). by following manufacturer instructions. Isolates belonged to the following bacterial species/groups: *Citrobacter freundii* species complex (7 isolates), *Enterobacter aerogenes* (2), *Enterobacter cloacae* species complex (29), *Escherichia coli* (16), *Klebsiella oxytoca* (14), *Klebsiella pneumoniae* (390), *Proteus mirabilis* (1), and *Serratia marcescens* (6).

Antimicrobial susceptibility testing. All isolates were susceptibility tested using reference broth microdilution methods against ceftazidime-avibactam (avibactam at fixed 4 µg/mL) and comparator antimicrobial agents as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A10). Categorical interpretations were those found in CLSI document M100-S26, the EUCAST website for colistin or United States Food and Drug Administration (US-FDA) package inserts for tigecycline and ceftazidime-avibactam. 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 M100-S26 document.

KPC screening. KPC encoding genes were screened using a microarray based assay (Check-MDR CT101 kit; Check-points, Wageningen, Netherlands).

Characterization of ceftazidime-avibactam non-susceptible isolates. Whole genome sequencing analysis was performed employing total genomic DNA prepared using the Nextera XT™ library construction protocol and index kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions and sequenced on a MiSeq Sequencer (Illumina). Data analysis of *de novo* assembled sequences were analyzed using a proprietary software applying a criteria of >94% identity and 40% minimum length.

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

Results

- Among 465 KPC-producing isolates collected from USA hospitals during 2012-2015, 153 (32.9%) were collected from pneumonia in hospitalized patients, 99 (21.3%) from urinary tract infections (UTI), 74 (15.9%) from skin/soft tissue infection (SSSI), 53 (11.4%) from bloodstream infections (BSI), 17 (3.7%) from intra-abdominal infections (IAI) and 69 (14.8%) from other infection sites (Figure 1).
- Overall, ceftazidime-avibactam ($MIC_{50/90}$, 0.5/2 µg/mL; Table 1) was very active against KPC-producing isolates and this combination inhibited 99.4% of the isolates at the current US-FDA breakpoints.
- Only three isolates were non-susceptible to ceftazidime-avibactam. These isolates were all *K. pneumoniae* displaying ceftazidime MIC values at 16 or >32 µg/mL (Table 2).
- Whole genome sequencing analysis demonstrated that two of these isolates (ceftazidime-avibactam MIC, >32 µg/mL) co-harbored metallo-β-lactamase genes (bla_{NDM-1} or bla_{VIM-4}) among other β-lactamases (Table 2). The isolate co-harboring bla_{VIM-4} also had deletions and decreased expression of the outer membrane protein *ompK37* and elevated expression of the efflux system AcrAB-TolC.
- The remaining isolate displaying a ceftazidime-avibactam MIC value of 16 µg/mL had decreased expression of *ompK36* and insertions in *ompK36* and *ompK37* (Table 2).
- The activities of ceftazidime-avibactam (98.0 to 100.0% susceptible) and tigecycline (94.1 to 100.0%) were consistent among infection types. However, susceptibility rates varied from 77.8 to 92.8% for colistin, 51.5 to 64.7% for amikacin and 43.2 to 60.4% for gentamicin (Figure 2). Levofloxacin was active against only 15.7% of isolates (data not shown).
- A total of 111 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.
- The most common organism carrying KPC-encoding genes was *K. pneumoniae* (n=390; 83.9% of the collection) and ceftazidime-avibactam inhibited 99.2% of these isolates at US-FDA breakpoint (Table 1).

Figure 1. Distribution of the infection types among 465 KPC-producing isolates collected from 2012 to 2015 in USA hospitals.

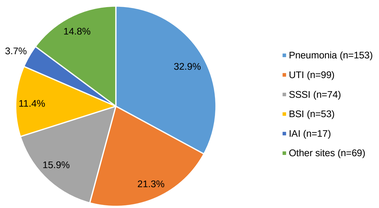


Table 1. MIC distributions for ceftazidime-avibactam when tested against 465 KPC-producing Enterobacteriaceae isolates collected from 2012 to 2015 in USA hospitals.

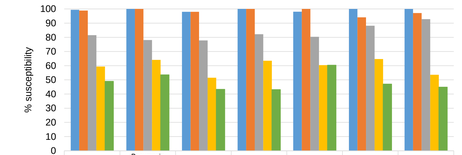
Organism/ Infection site	No. of isolates tested	No. of isolates inhibited at ceftazidime-avibactam MIC (µg/mL; cumulative %)[a]:													MIC_{50}	MIC_{90}
		≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32		
All isolates	465	14 (3.0)	5 (4.1)	16 (7.5)	35 (15.1)	69 (29.9)	133 (58.5)	131 (86.7)	48 (97.0)	11 (99.4)	0 (99.4)	1 (99.6)	0 (99.6)	2 (100.0)	0.5	2
Pneumonia	153	4 (2.6)	2 (3.9)	6 (7.8)	11 (15.0)	22 (29.4)	42 (56.9)	52 (90.8)	13 (99.3)	1 (100.0)					0.5	1
UTI	99	6 (6.1)	2 (8.1)	6 (14.1)	7 (21.2)	15 (36.4)	29 (65.7)	21 (86.9)	11 (98.0)	0 (98.0)	0 (98.0)	1 (99.0)	0 (99.0)	1 (100.0)	0.5	2
SSSI	74	1 (1.4)	1 (2.7)	2 (5.4)	7 (14.9)	10 (28.4)	19 (54.1)	23 (85.1)	6 (93.2)	5 (100.0)					0.5	2
BSI	53	2 (3.8)	0 (3.8)	1 (5.7)	5 (15.1)	11 (35.8)	16 (66.0)	6 (77.4)	9 (94.3)	2 (98.1)	0 (98.1)	0 (98.1)	0 (98.1)	1 (100.0)	0.5	2
IAI	17	1 (5.9)	0 (5.9)	0 (5.9)	1 (11.8)	3 (29.4)	6 (64.7)	5 (94.1)	1 (100.0)						0.5	1
Other sites	69	0 (0.0)	1 (1.4)	4 (7.2)	8 (18.8)	21 (49.3)	24 (84.1)	8 (95.7)	3 (100.0)						0.5	1
ICU	111	3 (2.7)	2 (4.5)	7 (10.8)	9 (18.9)	14 (31.5)	30 (58.6)	33 (88.3)	11 (98.2)	2 (100.0)					0.5	2
Non-ICU	162	5 (3.1)	2 (4.3)	3 (6.2)	13 (14.2)	28 (31.5)	44 (58.6)	47 (87.7)	13 (95.7)	6 (99.4)	0 (99.4)	1 (100.0)			0.5	2
K. pneumoniae	390	14 (3.6)	4 (4.6)	8 (6.7)	29 (14.1)	59 (29.2)	122 (60.5)	99 (85.9)	44 (97.2)	8 (99.2)	0 (99.2)	1 (99.5)	0 (99.5)	2 (100.0)	0.5	2

Table 2. Sequencing analysis and gene expression results for three KPC-producing isolates displaying non-susceptible ceftazidime-avibactam MIC results collected during 2012 to 2015 in USA hospitals.

Bacterial species City, State	CAZ-AVI MIC in µg/mL	Intrinsic β-lactam resistance genes: β-lactamases detected	Efflux or OMP	Sequencing analysis[a]	Relative quantification (interpretation)[a]
K. pneumoniae Houston, TX	>32 µg/mL	NDM-1 KPC-17 CTX-M-55 TEM-1 DHA-1 SHV-122	OmpK35	Internal stop codon	0.5 (similar to baseline)
			OmpK36	134DG135 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)
K. pneumoniae Philadelphia, PA	16 µg/mL	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)
K. pneumoniae New York, NY	>32 µg/mL	VIM-4 KPC-2 SHV-1 TEM-1	OmpK35	G211S, V241I	0.1 (decreased expression)
			OmpK36	R345H	6.6 (similar to baseline)
			OmpK37	R239K, 237 TERY 238 insertion, E244D, N274S, D275T, 275 SSTNGG 276 insertion, V277I, V295G, D350G	0.0 (decreased expression)
			AcrAB-TolC	NA	15.3 (elevated expression)

a. Relevant deletions/insertion, stop codons that might be incompatible with protein function or altered gene expression are underlined. NA= not applicable.

Figure 2. Activity of ceftazidime-avibactam and comparator agents tested against 465 KPC-producing isolates collected from 2012 to 2015 in USA hospitals and stratified by infection type. Percent susceptibility applying CLSI, US-FDA (ceftazidime-avibactam and tigecycline) or EUCAST (colistin) breakpoints.



	All (465)	Pneumonia (153)	UTI (99)	SSSI (74)	BSI (53)	IAI (17)	Other (69)
Ceftazidime-avibactam	99.4	100	98	100	98.1	100	100
Tigecycline	98.9	100	98	100	100	94.1	97.1
Colistin	81.5	78.1	77.8	82.2	80.4	88.2	92.8
Amikacin	59.4	64.1	51.5	63.5	60.4	64.7	53.6
Gentamicin	49	53.6	43.4	43.2	60.4	47.1	44.9

Conclusions

- Ceftazidime-avibactam was very active against this contemporary (2012-2015) collection of KPC-producing isolates collected in USA hospitals, regardless of the type of infection, ICU status or organism species. With exception of tigecycline, other comparator agents displayed varied activity against isolates collected from different infection types.
- Only three (0.6%) out of 465 isolates displayed non-susceptible ceftazidime-avibactam MIC results. Two of these isolates co-produced metallo-β-lactamases that still challenge the treatment with clinically available antimicrobial agents and alternative options to treat infections caused by these organisms are needed.

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