P0334

Activity of ceftazidime-avibactam against carbapenem-non-susceptible *Enterobacteriaceae* with and without additional ESBL and/or class C β-lactamases isolated from a global surveillance program, 2012–2014

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Revised Abstract

Background: Carbapenem resistance in *Enterobacteriaceae* can be mediated through expression of serine carbapenemases (KPC, OXA-48-like), metallo β-lactamases (MBLs), or extended-spectrum-β-lactamases (ESBLs) and AmpC cephalosporinases combined with changes in membrane permeability. Treatment options for carbapenem-resistant *Enterobacteriaceae* (CRE) are limited. We evaluated the *in vitro* activity of ceftazidime-avibactam against CRE collected globally in 2012–2014 through the INFORM surveillance program. Methods: 804 CRE isolates were collected from 109 sites in 35 countries. Susceptibility testing was performed by broth microdilution and interpreted using FDA breakpoints (ceftazidime-avibactam; ≤8 mg/L [susceptible]; ≥16 mg/L [resistant]). Ceftazidime-avibactam was tested with doubling dilutions of ceftazidime at a fixed concentration of 4 mg/L avibactam. Isolates were screened for the presence of β-lactamase genes using PCR and microarray, followed by sequencing. Results: 688 CRE isolates contained carbapenemases; the majority were KPC (459), followed by MBL (131) and OXA-48-like (98) enzymes. Most of these contained additional β-lactamases (ESBL and AmpC). Ceftazidime-avibactam showed potent activity against all subgroups, with the exception of isolates that contained an MBL. No regional differences were observed with the exception of diminished activity against carbapenemase-negative isolates from the Asia/Pacific region.

Percentage of isolates susceptible to ceftazidime-avibactam according to enzyme type					
Phenotype/ Enzyme content[a] (n)	**Ceftazidime-avibactam %S (n)[c]**				
	Global (804)[b]	**EUR (448)**	**AP (87)**	**MEA (61)**	**LA (208)**
Carbapenemase- (116)					
ESBL+	94 (72)	100 (27)	37 (8)	100 (4)	97 (33)
AmpC+	91 (23)	100 (10)	71 (7)	--	100 (6)
ESBL + AmpC	92 (12)	100 (4)	75 (4)	--	100 (4)
ESBL-, AmpC-	56 (9)	60 (5)	0 (2)	100 (1)	100 (1)
KPC+, MBL- (459)					
ESBL+	98 (199)	100 (110)	92 (13)	100 (1)	99 (75)
AmpC+	100 (16)	100 (3)	100 (6)	100 (2)	100 (5)
ESBL + AmpC	100 (9)	100 (2)	100 (3)	100 (1)	100 (3)
ESBL-, AmpC-	98 (235)	97 (140)	100 (4)	100 (15)	100 (76)
OXA-48-like, MBL- (98)					
ESBL+	100 (81)	100 (76)	--	100 (5)	--
AmpC+	100 (2)	100 (1)	--	100 (1)	--
ESBL + AmpC	50 (2)	0 (1)	--	100 (1)	--
ESBL-, AmpC-	92 (13)	91 (11)	--	--	100 (2)
MBL+ (131)	4 (131)	2 (58)	0 (40)	13 (30)	0 (3)

[a] Includes isolates that co-carry original spectrum β-lactamases
[b] Global, all; EUR, Europe; AP, Asia/Pacific; MEA, Middle East/Africa; LA, Latin America
[c] %S, percent susceptible (MIC ≤8 mg/L); n, number of isolates

Conclusions: CRE with different complex genotypes remained susceptible to ceftazidime-avibactam, except for isolates where carbapenem resistance was mediated by MBLs.

Introduction

Carbapenem resistance in *Enterobacteriaceae* can be mediated through expression of serine carbapenemases (KPC, OXA-48-like), metallo β-lactamases (MBLs), or extended-spectrum-β-lactamases (ESBLs) and AmpC cephalosporinases combined with changes in membrane permeability. Treatment options for carbapenem-resistant *Enterobacteriaceae* (CRE) are limited. We evaluated the *in vitro* activity of ceftazidime-avibactam against CRE collected globally in 2012–2014 through the INFORM (International Network For Optimal Resistance Monitoring) surveillance program.

Materials & Methods

- 804 CRE isolates were collected from 109 sites in 35 countries.
- Susceptibility testing was performed by broth microdilution following CLSI guidelines [1]. Ceftazidime-avibactam was tested with doubling dilutions of ceftazidime at a fixed concentration of 4 mg/L avibactam.
- MICs for ceftazidime-avibactam were interpreted using U.S. FDA breakpoints (susceptible, ≤8 mg/L; resistant, ≥16 mg/L) [2]. MICs for all other agents were interpreted according to EUCAST 2015 breakpoints [3].
- Isolates that were non-susceptible to meropenem (MIC ≥4 mg/L) were screened for the presence of β-lactamase genes encoding ESBLs (SHV, TEM, CTX-M, VEB, PER, GES), original spectrum β-lactamases (e.g. TEM-1, TEM-2, SHV-1, SHV-11), AmpC cephalosporinases (ACC, ACT, CMY, DHA, FOX, MOX, MIR), and carbapenemases (KPC, OXA-48-like, NDM, IMP, VIM, SPM, GIM) using PCR and microarray, followed by sequencing [4].

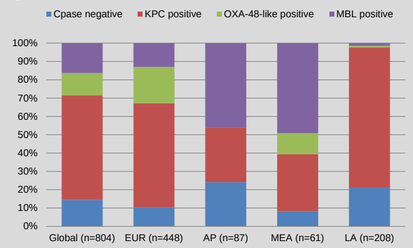
Results

Table 1. *In vitro* activity of ceftazidime-avibactam and comparator agents against CRE collected globally

Region	Phenotype/ enzyme content (n)	MIC (mg/L)			% Susceptible[a]
		Range	MIC_{50}	MIC_{90}	
Global	CRE All (804)				
	Ceftazidime-avibactam	≤0.015 - >128	1	>128	82.0
	Ceftazidime	0.12 - >128	>128	>128	2.5
	Cefepime	≤0.12 - >16	>16	>16	2.0
	Meropenem	4 - >8	>8	>8	0.0
	Colistin[b]	≤0.12 - >4	≤0.12	>4	81.0
	Amikacin	≤0.25 - >32	16	>32	40.8
	Tigecycline	0.06 - >8	1	2	66.9
	Levofloxacin	≤0.03 - >4	>4	>4	14.3
	CRE, MBL-negative (673)				
	Ceftazidime-avibactam	≤0.015 - >128	1	4	**97.2**
	Ceftazidime	0.12 - >128	128	>128	2.5
	Cefepime	≤0.12 - >16	>16	>16	1.5
	Meropenem	4 - >8	>8	>8	0.0
	Colistin[b]	≤0.12 - >4	≤0.12	>4	80.7
	Amikacin	≤0.25 - >32	16	>32	39.5
	Tigecycline	0.06 - >8	1	2	67.3
	Levofloxacin	≤0.03 - >4	>4	>4	12.8
Europe	CRE All (448)				
	Ceftazidime-avibactam	≤0.015 - >128	1	>128	85.5
	Ceftazidime	0.25 - >128	>128	>128	2.5
	Cefepime	≤0.12 - >16	>16	>16	1.6
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	>4	77.0
	Amikacin	≤0.25 - >32	32	>32	32.1
	Tigecycline	0.12 - >8	1	4	66.3
	Levofloxacin	≤0.03 - >4	>4	>4	10.9
	CRE, MBL-negative (390)				
	Ceftazidime-avibactam	≤0.015 - 128	1	4	**97.9**
	Ceftazidime	0.25 - >128	>128	>128	2.8
	Cefepime	0.25 - >16	>16	>16	1.3
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	>4	76.9
	Amikacin	≤0.25 - >32	32	>32	31.8
	Tigecycline	0.12 - 8	1	4	66.7
	Levofloxacin	≤0.03 - >4	>4	>4	10.8
Asia/Pacific	CRE All (87)				
	Ceftazidime-avibactam	0.06 - >128	64	>128	43.7
	Ceftazidime	0.5 - >128	>128	>128	2.3
	Cefepime	1 - >16	>16	>16	1.1
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	1	**92.0**
	Amikacin	≤0.25 - >32	4	>32	55.2
	Tigecycline	0.06 - 8	1	4	73.6
	Levofloxacin	0.06 - >4	>4	>4	24.1
	CRE, MBL-negative (47)				
	Ceftazidime-avibactam	0.06 - >128	2	128	80.9
	Ceftazidime	0.5 - >128	128	>128	4.3
	Cefepime	2 - >16	>16	>16	0.0
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	0.5	**93.6**
	Amikacin	≤0.25 - >32	4	>32	57.4
	Tigecycline	0.06 - 8	1	4	70.2
	Levofloxacin	0.25 - >4	>4	>4	19.1
Middle East/ Africa	CRE All (61)				
	Ceftazidime-avibactam	0.12 - >128	2	>128	57.4
	Ceftazidime	0.5 - >128	>128	>128	4.9
	Cefepime	≤0.12 - >16	>16	>16	6.6
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	>4	86.9
	Amikacin	1 - >32	16	>32	47.5
	Tigecycline	0.25 - 8	1	2	63.9
	Levofloxacin	0.06 - >4	>4	>4	21.3
	CRE, MBL-negative (31)				
	Ceftazidime-avibactam	0.12 – 4	1	2	**100**
	Ceftazidime	4 - >128	128	>128	0.0
	Cefepime	0.5 - >16	>16	>16	3.2
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	2	**93.5**
	Amikacin	1 - >32	32	>32	32.3
	Tigecycline	0.25 - 4	1	2	74.2
	Levofloxacin	0.12 - >4	>4	>4	12.9
Latin America	CRE All (208)				
	Ceftazidime-avibactam	0.03 – >128	1	2	**97.6**
	Ceftazidime	0.12 - >128	64	>128	1.9
	Cefepime	≤0.12 - >16	>16	>16	1.9
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	>4	83.2
	Amikacin	0.5 - >32	8	>32	51.4
	Tigecycline	0.25 - 8	1	2	66.3
	Levofloxacin	0.06 - >4	>4	>4	15.4
	CRE, MBL-negative (205)				
	Ceftazidime-avibactam	0.03 – >128	1	2	**99.0**
	Ceftazidime	0.12 - >128	64	>128	2.0
	Cefepime	≤0.12 - >16	>16	>16	2.0
	Meropenem	4 - >8	>8	>8	0.0
	Colistin	≤0.12 - >4	≤0.12	>4	82.9
	Amikacin	0.5 - >32	8	>32	51.2
	Tigecycline	0.25 - 8	1	2	66.8
	Levofloxacin	0.06 - >4	>4	>4	15.1

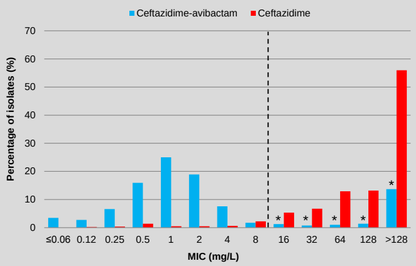
[a]MICs were interpreted according to EUCAST 2015 breakpoints with the exception of ceftazidime-avibactam, where MIC interpretive criteria according to U.S FDA were applied [2,3].
[b]Colistin was tested in the presence of 0.002% polysorbate-80; percent susceptible based on EUCAST breakpoint for colistin [3].

Figure 1. Distribution of CRE isolates with different resistance mechanisms by region.



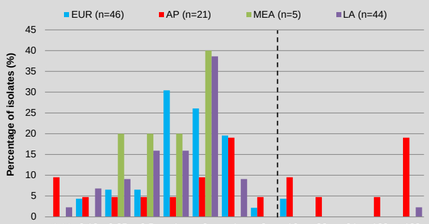
Cpase, carbapenemase; MBL, metallo-β-lactamase; Global, all; EUR, Europe; AP, Asia/Pacific; MEA, Middle East/Africa; LA, Latin America.
Isolates carrying KPC and OXA-48-like carbapenemases were collected in Europe (n=1), MBL and KPC in Europe (n=4) and the Asia/ Pacific region (n=2), and MBL and OXA-48-like in Europe (n=5) and the Middle East/Africa (n=2).

Figure 2A. Ceftazidime and ceftazidime-avibactam MIC distributions against all CRE collected globally (n=804).



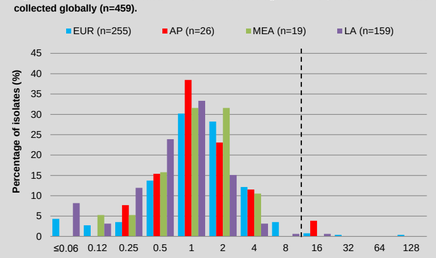
Dashed line represents the FDA susceptibility breakpoint of ≤8 mg/L for ceftazidime-avibactam.
* 126 of 145 isolates (86.9%) with ceftazidime-avibactam MIC values >8 mg/L carried MBLs.

Figure 2B. Ceftazidime-avibactam MIC distributions against carbapenemase-negative CRE isolates collected globally (n=116).



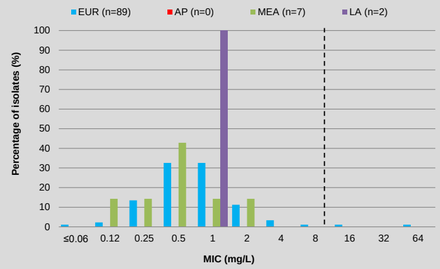
Dashed line represents the FDA susceptibility breakpoint of ≤8 mg/L for ceftazidime-avibactam.
Eleven isolates with ceftazidime-avibactam MIC values >8 mg/L (5 *K. pneumoniae*, 2 *E. coli*, 1 each *C. brakii*, *E. aerogenes*, and *P. vulgaris*) carried ESBL (CTX-M-15 (n=2), CTX-M-15 and SHV-12 (n=1), CTX-M-2 (n=1), CTX-M-3 (n=1)); AmpC (CMY-2 and DHA-1 (n=1)); only original-spectrum β-lactamases (SHV-type (n=2)); or no detected plasmid-mediated β-lactamase (n=3).

Figure 2C. Ceftazidime-avibactam MIC distributions against KPC-positive CRE isolates collected globally (n=459).



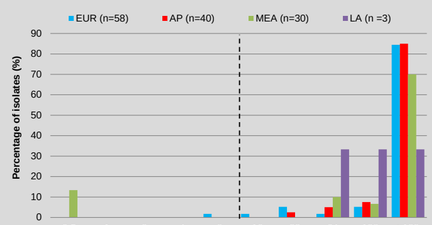
Dashed line represents the FDA susceptibility breakpoint of ≤8 mg/L for ceftazidime-avibactam.
Six isolates with ceftazidime-avibactam MIC values >8 mg/L (4 *K. pneumoniae*, 2 *K. oxytoca*) carried KPC-3 (n=3), KPC-2 (n=2), or KPC-2 and SHV-12 (n=1).

Figure 2D. Ceftazidime-avibactam MIC distributions against OXA-48-like-positive CRE isolates collected globally (n=98).



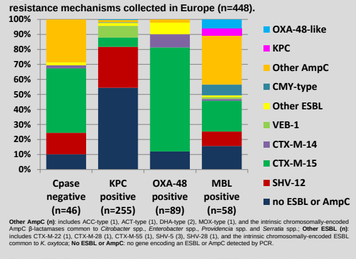
Dashed line represents the FDA susceptibility breakpoint of ≤8 mg/L for ceftazidime-avibactam.
Two isolates with ceftazidime-avibactam MIC values >8 mg/L (1 each *K. pneumoniae* and *S. marcescens*) carried OXA-48, and OXA-48 and CTX-M-22, respectively

Figure 2E. Ceftazidime-avibactam MIC distributions against MBL-positive CRE isolates collected globally (n=131).



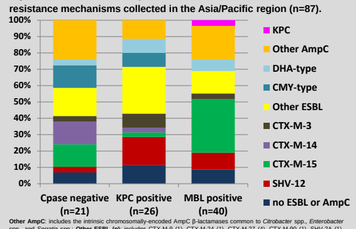
Dashed line represents the FDA susceptibility breakpoint of ≤8 mg/L for ceftazidime-avibactam.
Four *P. mirabilis* carrying VIM-5 that were collected in Nigeria tested with ceftazidime-avibactam MIC values <8 mg/L.

Figure 3A. Distribution of β-lactamases in CRE isolates with different resistance mechanisms collected in Europe (n=448).



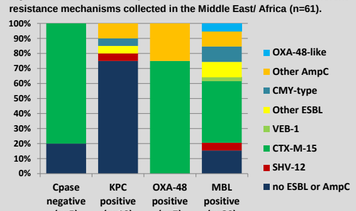
Other AmpC (n): includes ACC-type (1), ACT-type (1), DHA-type (2), MOX-type (1), and the intrinsic chromosomally-encoded AmpC β-lactamases common to *Citrobacter* spp., *Enterobacter* spp., *Providencia* spp. and *Serratia* spp.; **Other ESBL (n)**: includes CTX-M-22 (1), CTX-M-28 (1), CTX-M-55 (1), SHV-5 (3), SHV-28 (1), and the intrinsic chromosomally-encoded ESBL common to *K. oxytoca*; **No ESBL or AmpC**: no gene encoding an ESBL or AmpC detected by PCR.

Figure 3B. Distribution of β-lactamases in CRE isolates with different resistance mechanisms collected in the Asia/Pacific region (n=87).



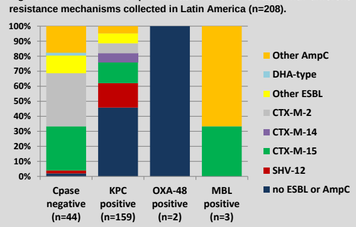
Other AmpC: includes the intrinsic chromosomally-encoded AmpC β-lactamases common to *Citrobacter* spp., *Enterobacter* spp., and *Serratia* spp.; **Other ESBL (n)**: includes CTX-M-9 (1), CTX-M-24 (1), CTX-M-27 (4), CTX-M-90 (1), SHV-2A (1), SHV-5 (2), SHV-28 (1), SHV-31 (1), and the intrinsic chromosomally-encoded ESBL common to *K. oxytoca*; **No ESBL or AmpC**: no gene encoding an ESBL or AmpC enzyme detected by PCR.

Figure 3C. Distribution of β-lactamases in CRE isolates with different resistance mechanisms collected in the Middle East/ Africa (n=61).



Other AmpC (n): includes the intrinsic chromosomally-encoded AmpC β-lactamases common to *Citrobacter* spp., *Enterobacter* spp., and *Providencia* spp.; **Other ESBL (n)**: includes CTX-M-2 (1), SHV-55 (1), SHV-134 (1), and the intrinsic chromosomally-encoded ESBL common to *K. oxytoca*; **No ESBL or AmpC**: no gene encoding an ESBL or AmpC detected by PCR.

Figure 3D. Distribution of β-lactamases in CRE isolates with different resistance mechanisms collected in Latin America (n=208).



Other AmpC (n): includes the intrinsic chromosomally-encoded AmpC β-lactamases common to *Citrobacter* spp., *Enterobacter* spp., and *Serratia* spp.; **Other ESBL (n)**: includes CTX-M-8 (4), CTX-M-12 (4), CTX-M-28 (1), CTX-M-67 (1), SHV-5 (4), and the intrinsic chromosomally-encoded ESBL common to *K. oxytoca*; **No ESBL or AmpC**: no gene encoding an ESBL or AmpC detected by PCR.

Results Summary

- The *in vitro* activity of ceftazidime-avibactam was greater than that of other tested agents (ceftazidime, cefepime, meropenem, amikacin, tigecycline, levofloxacin) against MBL-negative CRE collected in all regions. The activity of ceftazidime-avibactam was comparable to or greater than that of colistin in all regions but AP. (Table 1)
- Ceftazidime-avibactam was active *in vitro* against >97% of CRE carrying KPC, OXA-48, and isolates in which resistance was not mediated by known carbapenemases collected in all regions but AP, where 81% of CRE with these resistance mechanisms were susceptible to ceftazidime-avibactam. (Figure 2A-D and Table 1)
- Ceftazidime-avibactam was not active *in vitro* against isolates carrying MBLs. (Figure 2E)
- The proportion of CRE with different resistance mechanisms varied among regions. KPC-positive isolates were identified in all regions but were most common in LA and EUR. MBL-positive isolates were also found globally but were proportionately more common in AP and MEA, whereas isolates in which carbapenemases were not detected were more common in AP and LA. CRE carrying OXA-48-like enzymes were rare in LA and not found in AP. (Figure 1)
- 65% of CRE, including 62% of MBL-negative isolates, carried ESBLs and/or AmpC β-lactamases. CTX-M-15 and SHV-12 were detected in CRE collected from all regions. Additionally, CTX-M-14 was found in isolates from all regions but MEA, CTX-M-2 was common in isolates from LA, and plasmid-mediated AmpC (CMY- and DHA-type) were detected more often among CRE from AP. (Figure 3)

Conclusions

- CRE with different complex genotypes remained susceptible to ceftazidime-avibactam, except for isolates where carbapenem resistance was mediated by MBLs.
- Regional differences in resistance mechanisms are important to consider when assessing the value of ceftazidime-avibactam.

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