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Activity of ceftazidime–avibactam against multidrug-resistance *Enterobacteriaceae* expressing combined mechanisms of resistance☆

Inmaculada López-Hernández[a,*], Noemí Alonso[b], Marta Fernández-Martínez[c], Laura Zamorano[d], Alba Rivera[b], Antonio Oliver[d], M. Carmen Conejo[e], Luis Martínez-Martínez[c,f], Ferrán Navarro[b], Alvaro Pascual[a,e]

[a] *Intercentre Unit of Infectious Diseases, Microbiology and Preventive Medicine, University Hospital Virgen Macarena-Virgen del Rocío, Spain*
[b] *Santa Creu i Sant Pau Hospital, Barcelona, Spain*
[c] *University Hospital Marqués de Valdecilla, Santander, Spain*
[d] *Son Espases Hospital, Palma de Mallorca, Spain*
[e] *School of Medicine, University of Sevilla, Spain*
[f] *Department of Molecular Biology, University of Cantabria, Santander, Spain*

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ABSTRACT

Introduction: Antimicrobial resistance in *Enterobacteriaceae* is increasing worldwide and is making treating infections caused by multidrug-resistant *Enterobacteriaceae* a challenge. The use of β-lactam agents is compromised by microorganisms harboring extended-spectrum β-lactamases (ESBLs) and other mechanisms of resistance. Avibactam is a non β-lactam agent that inhibits clinically relevant β-lactamases, such as ESBL and AmpC. The ceftazidime–avibactam combination (CAZ-AVI) was recently approved for use in certain complicated infections, and may provide a therapeutic alternative for infections caused by these microorganisms.

Methods: The *in vitro* activity of CAZ and CAZ-AVI (AVI at a fixed concentration of 4 mg/L) was tested against 250 clinical isolates of *Enterobacteriaceae* using broth microdilution. EUCAST breakpoint criteria were used for CAZ, and FDA criteria for CAZ-AVI. Clinical isolates included bacteria producing extended-spectrum β-lactamases (ESBLs) and acquired AmpC β-lactamases (AACBLs). Porin loss in *Klebsiella pneumoniae* was also evaluated.

Results: The combination of AVI with CAZ displayed excellent activity against clinical isolates of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*, rendering all the ceftazidime-resistant isolates susceptible to ceftazidime. CAZ-AVI retained activity against porin-deficient isolates of *K. pneumoniae* producing ESBLs, AACBLs, or both, although MIC values were higher compared to porin-expressing isolates. CAZ-AVI rendered all the ceftazidime-resistant AACBL-producing *Enterobacteriaceae* tested susceptible to ceftazidime.

Conclusion: CAZ-AVI showed potent *in vitro* activity against clinical isolates of *Enterobacteriaceae* producing ESBLs and/or AACBLs, including *K. pneumoniae* with loss of porins.

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* Corresponding author.
E-mail address: inlopezh@us.es (I. López-Hernández).

Actividad de ceftazidima/avibactam en enterobacterias multirresistentes con mecanismos de resistencia combinados

Palabras clave:
Ceftazidima
Avibactam
Enterobacterias
Porinas
AmpC
Resistencia

RESUMEN

Introducción: La resistencia antibiótica en enterobacterias está en aumento y el tratamiento de infecciones producidas por enterobacterias multirresistentes supone un reto terapéutico. El uso de betalactámicos se afecta con la producción de betalactamasas de espectro extendido (BLEE) y otros mecanismos de resistencia. Avibactam es un compuesto no betalactámico que inhibe betalactamasas como BLEE o AmpC. La combinación ceftazidima-avibactam (CAZ-AVI) ha sido aprobada recientemente para el tratamiento de infecciones complicadas y puede ser una alternativa terapéutica en estas infecciones.

Métodos: La actividad *in vitro* de CAZ y CAZ-AVI (AVI, concentración fija de 4 mg/mL) fue determinada en 250 aislamientos clínicos de enterobacterias mediante microdilución en caldo. Los puntos de corte de EUCAST fueron utilizados para CAZ, y los criterios de FDA se utilizaron para CAZ-AVI. Las enterobacterias estudiadas producían BLEE y/o AmpC adquiridas (BLAA). El papel de la pérdida de porinas en *Klebsiella pneumoniae* también fue evaluado.

Resultados: CAZ-AVI demostró una excelente actividad en *Escherichia coli* y *Klebsiella pneumoniae* productoras de BLEE, devolviendo la sensibilidad a CAZ en todos los aislamientos resistentes a CAZ. CAZ-AVI mantuvo su actividad en aislamientos de *K. pneumoniae* deficientes en porinas productoras de BLEE y/o BLAA, aunque los valores de CMI fueron más altos comparados con las cepas que expresaban porinas. En todas las enterobacterias resistentes a ceftazidima productoras de BLAA analizadas en este estudio CAZ-AVI devolvió la sensibilidad a ceftazidima.

Conclusión: CAZ-AVI demostró una potente actividad *in vitro* en aislamientos clínicos de enterobacterias productoras de BLEE y/o BLAA, incluyendo *K. pneumoniae* con pérdida de porinas.

Introduction

Infections caused by multidrug-resistant gram-negative bacteria are increasing worldwide and pose a therapeutic challenge in clinical practice because treatment choices are limited.[1]

Extended-spectrum β-lactamase (ESBLs) enzymes with the ability to hydrolyze and create resistance to oxyimino-cephalosporins and aztreonam appeared after the introduction of broad-spectrum cephalosporins.[2] The current incidence and prevalence of different ESBLs is a matter of great concern, limiting the therapeutic use of β-lactams.[3] In addition, other plasmid mediated β-lactamases such as acquired AmpC beta-lactamases (AACBLs), which prevent the action of cephalosporins, have also spread in recent years. Resistance due to AACBL enzymes is less common than ESBL production in most parts of the world, but may be broader in spectrum.[4]

With respect to porins, the periplasmic concentration of the β-lactam agent is a function of the permeability of the outer membrane; in particular, the porin channels through which the β-lactams penetrate may play an essential role and contribute to the level of susceptibility to certain β-lactams.[5]

The use of carbapenems as drugs of choice for the treatment of infections caused by the microorganisms mentioned above has facilitated the appearance and dissemination of carbapenemase-producing *Enterobacteriaceae*. Alternative agents to carbapenems are needed. In recent years, there has been an alarming decline in the research and development of new antibiotics to deal with the threat of antimicrobial resistance. In 2015, the FDA (Food and Drug Administration, USA) approved the use of ceftazidime–avibactam for the treatment of complicated intra-abdominal infections (in combination with metronidazole) and complicated urinary tract infections in adults including pyelonephritis.[6] Ceftazidime–avibactam is also under clinical development for treatment of nosocomial pneumonia, including ventilator-associated pneumonia (VAP) in a phase III clinical trial (NCT01808092).[7]

Ceftazidime–avibactam may improve the outcome of patients infected with multidrug-resistant gram-negative bacteria. Avibactam is a member of a new class of β-lactamase inhibitors, diazabicyclooctanes (non β-lactam compounds), that inhibit serine β-lactamases, including class A (KPC), class C (AmpC), as well as some class D enzymes (OXA-48). It binds covalently and reversibly to these β-lactamases, so preventing their action.[8,9]

The aim of this study was to evaluate the activity of avibactam in combination with ceftazidime against a well-defined collection of *Enterobacteriaceae* producing ESBLs or AACBLs. The role of porin loss was evaluated in *Klebsiella pneumoniae* isolates.

Material and methods

Bacterial strains

A total of 250 bacterial isolates were studied. Species identification was confirmed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany).

The strains were selected from four collections:

a) ESBL-producing *Escherichia coli*, isolated during a nationwide study performed in Spain, including the most prevalent ESBLs and *E. coli* ST131 ($n = 50$).[10] In this group, the isolates were not clonally related by REP-PCR (Repetitive Element Palindromic-PCR), with the exception of four isolates with identical REP-PCR profiles that belonged to the ST131 clone and produced CTX-M-15. Thirty-seven isolates were CTX-M producers, including eighteen from the M-1 group (CTX-M-1 $n = 2$, CTX-M-3 $n = 1$, CTX-M-15 $n = 9$, CTX-M-32 $n = 5$ and CTX-M-57 $n = 1$) and nineteen from the M-9 group (CTX-M-9 $n = 6$, CTX-M-14 $n = 12$ and CTX-M-27 $n = 1$) and ten isolates produced SHV-12, and three TEM-type ESBLs (TEM-4 $n = 1$ and TEM-52 $n = 2$).
b) ESBL-producing *K. pneumoniae* isolated in a nationwide study performed in Spain, including the most prevalent ESBLs and clones ($n = 50$).[11] They comprised twenty-two CTX-M producers: fourteen from the M-1 group (CTX-M-1 $n = 4$, CTX-M-15 $n = 9$ and CTX-M-32 $n = 1$), six from the M-9 group (CTX-M-9 n = 2, CTX-M-14 n = 3 and CTX-M-16 $n = 1$) and two CTX-M-type that

were not sequenced. Thirteen isolates produced SHV-type ESBLs (SHV-2 $n=4$, SHV-5 $n=2$, SHV-12 $n=6$ and SHV-54 $n=1$) and eight TEM-type (TEM-3 $n=1$, TEM-4 $n=3$, TEM-15 $n=1$, TEM-25 $n=1$, TEM-52 $n=1$ and TEM-133 $n=1$). Seven isolates produced two ESBLs, five presented one CTX-M-type (CTX-M-1 $n=2$, CTX-M-14 $n=1$, CTX-M-15 $n=1$ and CTX-M-type not sequenced $n=1$) and SHV-12, and two carried one CTX-M-type plus one TEM-type. (CTX-M-15 plus TEM-74 and CTX-M-type not sequenced plus TEM-3). The 50 isolates showed 45 different REP-PCR profiles.

c) *K. pneumoniae* strains were grouped according to their outer membrane protein profile (POR+/−) and their ability to produce AACBLs (AACBL+/−) and ESBLs (ESBL+/−) ($n=49$).[12] Isolates classified as POR− lacked both outer membrane proteins, OmpK35 and OmpK36. Most isolates POR+ lacked OmpK35.

d) AACBL-producing *Enterobacteriaceae* isolated in a nationwide study performed in Spain in 2009. These included the most prevalent species and enzymes ($n=100$).[13,14] As determined by PFGE (Pulsed field gel electrophoresis), the selected isolates were not clonally related.

Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) values of ceftazidime (0.015–32 mg/L), with and without avibactam (at a fixed concentration of 4 mg/L) were determined by microdilution assay in Mueller–Hinton II broth (Oxoid, Spain) following CLSI guidelines.[15] *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27583 and *K. pneumoniae* ATCC 700603 were used as reference strains for quality control of *in vitro* susceptibility testing. Ceftazidime was purchased from Sigma Aldrich (St. Louis, MO, USA) and avibactam was provided by AstraZeneca (Cheshire, England, UK). The interpretation of ceftazidime susceptibility was determined according to EUCAST criteria (susceptible, MIC ≤ 1 mg/L).[16] The FDA-approved breakpoint for *Enterobacteriaceae* was used for ceftazidime–avibactam (susceptible, MIC ≤ 8 mg/L), based on pharmacokinetic/pharmacodynamic analysis.[6]

Results

The results of the ESBL-producing *E. coli* group using EUCAST breakpoint criteria for ceftazidime (resistant if MIC > 4 mg/L) showed that 78% of isolates were resistant, 20% intermediate and 2% (one isolate producing CTX-M-9) susceptible. All isolates were susceptible to the ceftazidime/avibactam combination, displaying MIC values of ≤1 mg/L. The MIC range and MIC_{50} and MIC_{90} values revealed no differences in the ability of avibactam to protect ceftazidime from hydrolysis by different types of ESBL, as shown in Table 1. The four ST131 isolates showed MIC values for ceftazidime ranging from 4 mg/L to >32 mg/L, whereas in combination with avibactam they ranged from 0.25 mg/L to 1 mg/L.

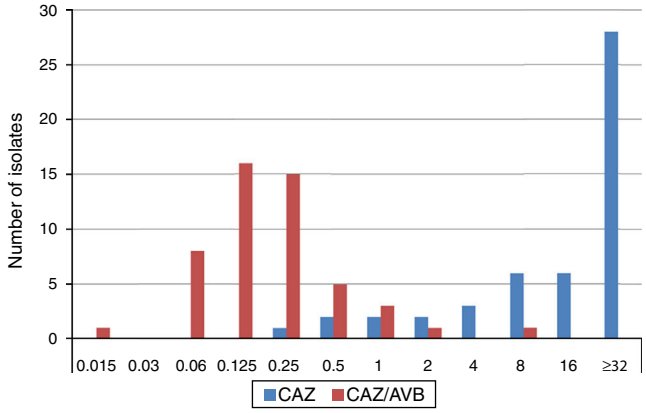

Fig. 1. Ceftazidime (CAZ) and ceftazidime/avibactam (CAZ/AVI) MIC distribution for 50 ESBL-producing *K. pneumoniae*.

The MIC distributions of ceftazidime, alone and in combination with avibactam, against ESBL-producing *K. pneumoniae* isolates are shown in Fig. 1. Applying EUCAST breakpoint criteria for ceftazidime, 80% of isolates were resistant, 10% intermediate and 10% susceptible, (five isolates producing CTX-M-type ESBLs, (CTX-M-9 $n=1$, CTX-M-14 $n=3$ y CTX-M-type not sequenced $n=1$) resulting in MIC_{50} and MIC_{90} values of ≥32 mg/L. The values of the ceftazidime/avibactam combination ranged between 0.125–0.25 mg/L and 0.5–1 mg/L, respectively. MIC range, and MIC_{50} and MIC_{90} values (when applicable) by type of ESBL produced are shown in Table 1. All but two isolates displayed ceftazidime/avibactam MIC values of ≤1 mg/L. The two isolates displaying the highest ceftazidime/avibactam MICs included one producing CTX-M-1, with ceftazidime and ceftazidime/avibactam MICs of >32 mg/L and 2 mg/L, respectively, and another producing SHV-12, with ceftazidime and ceftazidime/avibactam MICs of 32 mg/L and 8 mg/L, respectively.

When tested against two POR-, ESBL-, AACBL-*K. pneumoniae* isolates, the results showed, as expected, that avibactam did not improve the activity of ceftazidime (MIC ceftazidime, 1 mg/L; MIC ceftazidime/avibactam, 0.5–1 mg/L). The MIC range, MIC_{50} and MIC_{90} values (when applicable) of ceftazidime and ceftazidime/avibactam for the 47 isolates producing ESBLs, AACBLs or both, according to porin profile, are shown in Table 2. The MIC distributions of these antimicrobials are shown in Fig. 2. Applying the EUCAST breakpoint criteria for ceftazidime, 47 isolates were resistant to this cephalosporin, with MIC_{50} and MIC_{90} values of

Table 1
MIC range, MIC_{50} and MIC_{90} of ceftazidime and cetazidime/avibactam for 50 ESBL-producing *E. coli* and 50 ESBL-producing *K. pneumoniae*, grouped by type of ESBL produced. ESBL: Extended-Spectrum Beta-lactamase.

		MIC (mg/L)					
		Ceftazidime			Ceftazidime/avibactam		
ESBL	(no. of strains)	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
E. coli							
CTX-M-type	37	1 to 32	32	>32	0.06–1	0.25	1
SHV-12	10	8 to 32	>32	>32	0.06–1	0.25	0.5
TEM-type	3	32 to 32			0.125–0.5		
K. pneumonia							
CTX-M-type	22	0.25 to 32	32	>32	0.06–2	0.25	0.5
SHV-type	13	2 to 32	32	>32	≤0.015–8	0.125	1
TEM-type	8	4 to 32	16	>32	0.125–1	0.125	1
CTX-M-type plus SHV-12	5	4 to 32		>32	0.06–0.5	0.25	0.5
CTX-M-type plus TEM-type	2	32 to 32			0.125		

Table 2
MIC range, MIC_{50} and MIC_{90} of ceftazidime and ceftazidime/avibactam for 47 *K. pneumoniae* isolates producing ESBL, AACBL or both grouped on the basis of the beta-lactamase(s) produced and the porin profile.

		MIC (mg/L)					
		Ceftazidime			Ceftazidime/avibactam		
Group	(*n*)	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
POR+	**30**	**2** to **32**	**32**	**>32**	**0.125–4**	**1**	**2**
ESBL[a] +	10	2 to 32	32	>32	0.125–2	0.5	2
AACBL[b] +	16	>32	>32	>32	0.125–4	1	2
ESBL+AACBL[c] +	4	>32			0.5–2		
POR–	**17**	**16** to **32**	**>32**	**>32**	**1–4**	**2**	**4**
ESBL[d] +	13	16 to 32	>32	>32	1–4	2	4
AACBL[e] +	1	>32			1		
ESBL+AACBL[f] +	3	>32			2–4		

AACBL: Adquired AmpC type Beta-lactamase; ESBL: Extended-Spectrum Beta-lactamase; POR: Porin expression. *(Isolates classified as POR– lacked both outer membrane proteins, OmpK35 and OmpK36. Most isolates POR+ lacked OmpK35).*
Results for POR+/POR– in bold characters.
[a] SHV-2, SHV-3, SHV-5, SHV-27, TEM-12.
[b] ACT-1, DHA-1, FOX-5.
[c] FOX-5+SHV-2, ACT-1+TEM-10.
[d] SHV-2, SHV-5.
[e] ACT-1.
[f] ACT-1+TEM-10.

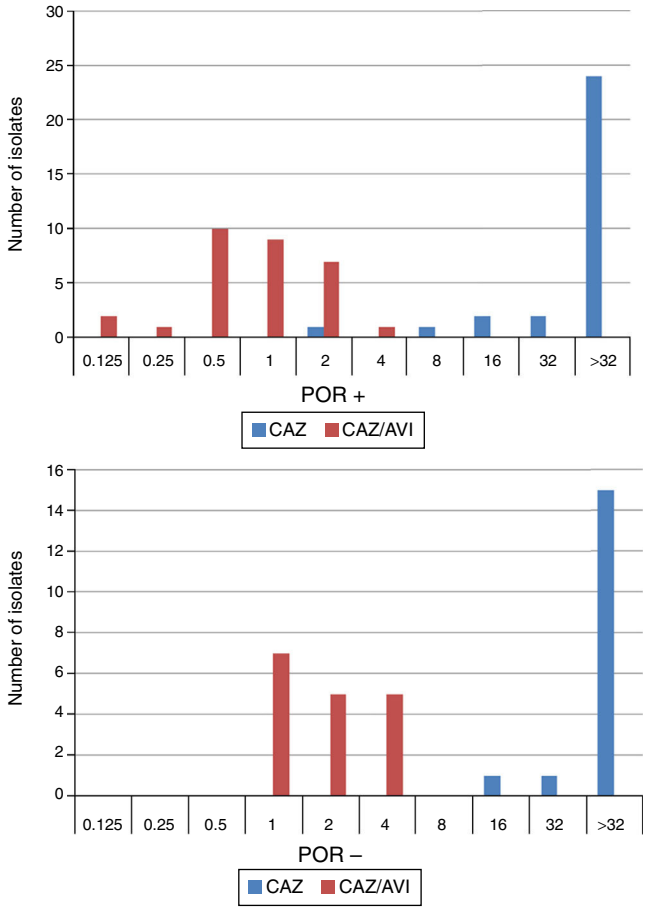

Fig. 2. Ceftazidime (CAZ) and ceftazidime/avibactam (CAZ/AVI) MIC distribution for 47 *K. pneumoniae* isolates producing ESBL, AACBL or both grouped on the basis their porin profile.

>32 mg/L. The addition of avibactam reduced the MIC_{50} and MIC_{90} values in the POR+ group to 1 mg/L and 2 mg/L, respectively. In the POR- group, the MIC_{50} and MIC_{90} values were 2 mg/L and 4 mg/L, respectively. In both POR+ and POR– groups, all isolates were susceptible to ceftazidime when avibactam was added. MIC values for ceftazidime/avibactam of ≤0.5 mg/L were observed only for POR+ isolates. All but one of the isolates that displayed the highest MIC value of 4 mg/L for this combination were POR–.

The results for AACBL-producing *Enterobacteriaceae*, demonstrated that all isolates were resistant to ceftazidime, with MIC_{50} and MIC_{90} values of 32 mg/L and ≥32 mg/L, respectively. The addition of avibactam significantly improved the activity of ceftazidime in *E. coli*, lowering the MIC_{50} and MIC_{90} values to 0.25 mg/L and 0.5 mg/L, respectively. MIC ranges, MIC_{50} and MIC_{90} values (when applicable) of ceftazidime and ceftazidime/avibactam for the different species of *Enterobacteriaceae* studied are shown in Table 3. These values according to AACBL type are shown in Table 4. No significant differences were observed for the excellent ability of avibactam to protect ceftazidime from hydrolysis by different types of AACBL or species.

Discussion

The emergence and spread of antimicrobial resistance (ESBLs, AACBLs and carbapenemases) have become challenges for the treatment of gram-negative infections.[1] This study set out to determine the activity of the novel combination agent, ceftazidime–avibactam, in well-characterized isolates of *Enterobacteriaceae*, including those with combined mechanisms of resistance.

Ceftazidime–avibactam is active against a wide variety of clinical ESBL-harboring *Enterobacteriaceae* as reported in previous studies.[17] The results of the present study showed that ceftazidime–avibactam combination inhibited all ESBLs producing isolates at ≤1 mg/L. The activity of ceftazidime–avibactam was not affected by the presence of more than one ESBL or any specific ESBL. Only two *K. pneumoniae* isolates were inhibited by higher concentrations of ceftazidime–avibactam, one a CTX-M-1 producer (MIC, 2 mg/L), the other a SHV-12 producer (MIC, 8 mg/L). There are no approved ceftazidime/avibactam EUCAST breakpoints,[16] but after applying the FDA breakpoint, these isolates should be considered as susceptible to this combination. It is possible that other non-hydrolytic mechanisms contributing to ceftazidime resistance, such as decreased permeability or efflux pumps, were present in these isolates and also affected the activity of ceftazidime–avibactam, although further studies would be needed to elucidate the mechanism. In this respect, preliminary studies have produced no evidence of any impact of efflux on ceftazidime–avibactam activity in selected isolates

Table 3
MIC ranges, MIC_{50} and MIC_{90} values of ceftazidime and cetazidime/avibactam displayed by the different species of AACBL-producing *Enterobacteriaceae*.

		MIC (mg/L)					
		Ceftazidime			Ceftazidime/avibactam		
Species	(*n*)	Range	MIC_{50}	CMI_{90}	Range	MIC_{50}	CMI_{90}
E. coli[a]	40	4 to 32	32	>32	0.06–2	0.25	0.5
K. pneumoniae[b]	29	8 to 32	>32	>32	0.125–1	0.25	0.5
P. mirabilis[c]	22	4 to 32	16	32	0.06–0.5	0.06	0.25
K. oxytoca[d]	4	8 to 32			0.125–0.25		
C. koseri[e]	3	4–32			0.125		
E. cloacae[f]	1	>32			0.5		
S. enterica[g]	1	>32			0.5		

[a] *E. coli*: CMY-2 (21), DHA-1 (11), CMY-4 (1), CMY-7 (1), CMY-27 (1), CMY-54 (1), CMY-55 (1), CMY-57 (1), CMY-59 (1), FOX-8 (1).
[b] *K. pneumoniae*: DHA-1 (16), CMY-2 (10), ACC-1 (2), CMY-2 plus DHA-1 (1).
[c] *P. mirabilis* CMY-2 (18), DHA-1 (2), ACC-1 (2).
[d] *K. oxytoca* DHA-1 (3), CMY-2 (1).
[e] *C. koseri* CMY-2 (1), DHA-1 (1), ACC-1 plus ACT-2 (1).
[f] *E. cloacae* DHA-1.
[g] *S. enterica* DHA-1.

Table 4
MIC ranges, MIC_{50} and MIC_{90} values of ceftazidime and cetazidime/avibactam for 100 AACBL-producing *Enterobacteriaceae*, grouped on the basis of the AACBL(s) produced. AACBL: Adquired AmpC type Beta-lactamase.

		Ceftazidime			Ceftazidime/avibactam		
AACBL	(*n*)	Range	MIC_{50}	CMI_{90}	Range	MIC_{50}	CMI_{90}
CMY-type	58	4 to 32	32	>32	0.06–2	0.25	0.5
DHA-1	35	8 to 32	32	>32	0.06–1	0.125	0.5
ACC-1	4	8 to 32			0.125–0.5		
FOX-8	1	16			1		
ACT-2 plus ACC-1	1	4			0.125		
CMY-2 plus DHA-1	1	>32			0.5		

of *E. coli*, *K. pneumoniae* and *Enterobacter aerogenes* using PAβN (phenylalanine-arginine β-naphthylamide) as efflux inhibitor during assays.[18]

β-Lactams penetrate the outer membrane through the porin channels and it is possible that altered expression of these porins may affect the activity of the ceftazidime–avibactam combination if it leads to reduced concentrations of the antibiotic in the periplasm. The major outer membrane porins in *K. pneumoniae* are OmpK35 and OmpK36.[19] In order to investigate the role of these porins in susceptibility to ceftazidime–avibactam, 49 isolates with different porin profiles were selected (porin-active and porin-defective). Some of the strains presented other associated mechanisms of resistance, such as ESBLs and/or AACBLs, which made it possible to study their combined effect. Recent studies have demonstrated that OmpK35 and OmpK36 are not the major channels through which avibactam penetrates into the periplasm in *K. pneumoniae*[18,20]; these studies showed that diffusion also occurs through other pathways since the ceftazidime–avibactam MIC remained low against an OmpK35/OmpK36 mutation. The results of the present study with *K. pneumoniae* confirmed those results, since, against clinical isolates of ceftazidime-resistant *K. pneumoniae* lacking both porins, the MIC values of ceftazidime–avibactam remained lower than or equal to the FDA approved breakpoint (MIC ≤ 8 mg/L). It appears that porin deficiency does not limit the penetration of avibactam into the periplasmic space. However, the ceftazidime–avibactam MIC in these strains was higher (MIC_{90}/MIC_{50} 4/2) when compared to isolates expressing both porins (MIC_{90}/MIC_{50} 2/1). Only one isolate in the porin-active group expressing the AACBL ACT-1 showed an MIC value within the range of the porin-defective group (MIC 4 mg/L). Further characterization studies would be needed for this strain. Pagés et al.[18] found similar results for the *K. pneumoniae* KP74 strain (lacking only OmpK35 and expressing the TEM-3 ESBL), which displayed a higher ceftazidime–avibactam MIC than strains lacking both porins.

Avibactam is the first β-lactamase inhibitor with activity against class C enzymes. Previous inhibitors such as clavulanic acid inhibit only class A β-lactamase enzymes. For this reason, in the present study, we tested 100 clinical isolates of ceftazidime-resistant AACBL-producing *Enterobacteriaceae*, including *E. coli, K. pneumoniae, P. mirabilis, K. oxytoca, C. koseri, E. cloacae* and *S. enterica.* The results showed that ceftazidime–avibactam inhibited 100% of isolates tested at ≤8 mg/L, confirming the excellent ability of avibactam to protect ceftazidime from hydrolysis by different types of AACBL in different species, as Karlowsky et al. also showed.[17] Moreover, no significant differences were found for ceftazidime–avibactam MIC values associated with any specific AACBL.

The results of the present study demonstrate the potent activity of avibactam to hydrolyze ESBLs and AmpC in *Enterobacteriaceae*. These data combined with the results reported by Lahiri et al.[21] demonstrate that the development of high-level resistance to ceftazidime/avibactam appears to occur at a low frequency and provides evidence that ceftazidime–avibactam is an excellent alternative for the treatment of infections caused by multidrug-resistant isolates.

Nevertheless, the ceftazidime–avibactam MIC of 8 mg/L against a *K. pneumoniae* isolate producing SHV-12 obtained in the present study, which is just within the limit of the currently accepted FDA breakpoint, gives cause for concern.

The results of this study will have to be evaluated in the future when EUCAST validates a susceptibility breakpoint for ceftazidime–avibactam. In the case that the breakpoint equaled the current EUCAST breakpoint for ceftazidime alone (susceptible, ≤1 mg/L),[16] the susceptibility category of some isolates examined in this study would n to be modified.

In conclusion, the results of this study, using a wide range of isolates and well characterized resistance mechanisms, support previous data and confirm the potential utility of

ceftazidime–avibactam as a therapeutic agent for treating serious infections caused by multidrug-resistant *Enterobacteriaceae*.

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Conflicts of interest

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References

1. Center for Disease Dynamics, Economics & Policy. State of the World's antibiotics, 2015. Washington, DC: CDDEP; 2015.
2. Kliebe C, Nies BA, Meyer JF, Tolxdorff-Neutzling RM, Wiedemann B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob Agents Chemother. 1985;28:302–7.
3. Adler A, Katz DE, Marchaim D. The continuing plague of extended-spectrum β-lactamase-producing *Enterobacteriaceae* Infections. Infect Dis Clin North Am. 2016;30:347–75.
4. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev. 2009;22:161–82.
5. Fernández L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev. 2012;25:661–81.
6. US Food and Drug Administration. 2015. Avycaz (ceftazidime–avibactam) package insert. NDA 206494. Product labeling: http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206494s000lbl.pdf.
7. Astra Zeneca. A phase III, randomized, multicentre, double-blind, double-dummy, parallel-group comparative study to determine the efficacy, safety and tolerability of ceftazidime–avibactam versus meropenem in the treatment of nosocomial pneumonia including ventilator-associated pneumonia in hospitalized adults. In: ClinicalTrials.gov. Bethesda (MD): National Library of Medicine (US). 2013–2016. Available from: https://clinicaltrials.gov/ct2/show/study/NCT01808092 NLM Identifier: NCT01808092.
8. van Duin D, Bonomo RA. Ceftazidime/avibactam and ceftolozane/tazobactam: second generation β-lactam/β-lactamase combinations. Clin Infect Dis. 2016. Advance Access published May 8, 2016.
9. Syue LS, Chen YH, Ko WC, Hsueh PR. New drugs for the treatment of complicated intra-abdominal infections in the era of increasing antimicrobial resistance. Int J Antimicrob Agents. 2016;47:250–8.
10. Díaz MA, Hernández-Bello JR, Rodríguez-Baño J, Martínez-Martínez L, Calvo J, Blanco J, et al., Spanish Group for Nosocomial Infections (GEIH). Diversity of *Escherichia coli* strains producing extended-spectrum beta-lactamases in Spain: second nationwide study. J Clin Microbiol. 2010;48:2840–5.
11. Ruiz de Alegría C, Rodríguez-Baño J, Cano ME, Hernández-Bello JR, Calvo J, Román E, et al., Spanish Group for Nosocomial Infections (GEIH). *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases in Spain: microbiological and clinical features. J Clin Microbiol. 2011;49:1134–6.
12. Conejo MC, Hernández JR, Pascual A. Effect of porin loss on the activity of tigecycline against *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases or plasmid-mediated AmpC-type beta-lactamases. Diagn Microbiol Infect Dis. 2008;61:343–5.
13. Miró E, Agüero J, Larrosa MN, Fernández A, Conejo MC, Bou G, et al. Prevalence and molecular epidemiology of acquired AmpC β-lactamases and carbapenemases in *Enterobacteriaceae* isolates from 35 hospitals in Spain. Eur J Clin Microbiol Infect Dis. 2013;32:253–9.
14. Pérez-Llarena FJ, Kerff F, Zamorano L, Fernández MC, Nuñez ML, Miró E, et al. Characterization of the new AmpC β-lactamase FOX-8 reveals a single mutation, Phe313Leu, located in the R2 loop that affects ceftazidime hydrolysis. Antimicrob Agents Chemother. 2013;57:5158–61.
15. Clinical and Laboratory Standards Institute. 2015. M100-S25. Performance standards for antimicrobial susceptibility testing, 25th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
16. European Committee on Antimicrobial Susceptibility Testing. 2016. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, valid from 2016-01-01. European Committee on antimicrobial susceptibility Testing, Vaxjo, Sweden.
17. Karlowsky JA, Biedenbach DJ, Kazmierczak KM, Stone GG, Sahm DF. Activity of ceftazidime–avibactam against extended-spectrum- and AmpC β-lactamase-producing Enterobacteriaceae collected in the INFORM Global Surveillance Study from 2012 to 2014. Antimicrob Agents Chemother. 2016;60:2849–57.
18. Pages JM, Peslier S, Keating TA, Lavigne JP, Nichols WW. Role of the outer membrane and porins in susceptibility of β-Lactamase-producing *Enterobacteriaceae* to ceftazidime–avibactam. Antimicrob Agents Chemother. 2016;60:1349–59.
19. Tsai YK, Fung CP, Lin JC, Chen JH, Chang FY, Chen TL, et al. *Klebsiella pneumoniae* outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2011;55:1485–93.
20. Dupont H, Gaillot O, Goetgheluck AS, Passart C, Emond JP, Lecuru M, et al. Molecular characterization of carbapenem-non susceptible enterobacterial isolates collected during a prospective interregional survey in France and susceptibility to the novel ceftazidime–avibactam and aztreonam–avibactam combinations. Antimicrob Agents Chemother. 2016;60:215–21.
21. Lahiri SD, Johnstone MR, Ross PL, McLaughlin RE, Olivier NB, Alm RA. Avibactam and class C β-lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. Antimicrob Agents Chemother. 2014;58:5704–13.