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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).

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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

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