Published in "Journal of Antimicrobial Chemotherapy doi: 10.1093/jac/dkx393, "
which should be cited to refer to this work.

Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing *Klebsiella pneumoniae*

Aurélie Jayol[1–4], Patrice Nordmann[1–3,5], Laurent Poirel[1–3]* and Véronique Dubois[4,6]

[1]Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland; [2]INSERM European Unit (LEA-IAME Paris, France), University of Fribourg, Fribourg, Switzerland; [3]National Reference Center for Emerging Antibiotic Resistance, University of Fribourg, Fribourg, Switzerland; [4]Laboratory of Bacteriology, University Hospital of Bordeaux, Bordeaux, France; [5]University of Lausanne and University Hospital Center, Lausanne, Switzerland; [6]CNRS UMR5234, University of Bordeaux, Bordeaux, France

*Corresponding author. Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, rue Albert Gockel 3, CH-1700 Fribourg, Switzerland. Tel: +41-26-300-9582; E-mail: laurent.poirel@unifr.ch

Sir,

The spread of carbapenemase-producing *Klebsiella pneumoniae* is a major public health concern since such isolates are basically resistant to most available antibiotics, including β-lactams, fluoroquinolones and aminoglycosides.[1] Infections due to carbapenemase-producing *K. pneumoniae* are therefore commonly treated with a regimen containing colistin.[1] However, acquired resistance to colistin now occurs frequently and has few therapeutic options.[2] Outbreaks with colistin-resistant and carbapenemase-producing *K. pneumoniae* isolates have been reported worldwide[2] and mortality rates are high owing to limited treatment options.[3]

Recently, a new therapeutic option, namely ceftazidime/avibactam, combining a broad-spectrum cephalosporin and a novel β-lactamase inhibitor, has been marketed. The addition of avibactam expands the spectrum of activity of ceftazidime to many MDR Enterobacteriaceae including producers of ESBLs and carbapenemases.[4] Indeed, avibactam is active against all types of ESBLs and against carbapenemases of class A (KPC) and of some class D (OXA-48 and its derivatives), but is not active against class B β-lactamases (MBLs).[4]

In two reports, the combination of ceftazidime/avibactam with aztreonam demonstrated a synergistic effect against MBL-producing Gram-negative pathogens, but only a small number of isolates were tested.[5,6]

The objective of this study was to determine the *in vitro* activity of ceftazidime/avibactam, alone (for class A and D carbapenemase producers) or in combination with aztreonam (for class B carbapenemase producers), against a collection of colistin-resistant and carbapenemase-producing *K. pneumoniae* isolates.

A collection of 63 *K. pneumoniae* isolates recovered from clinical samples in France, Colombia and Turkey were tested in this study. All the isolates were resistant to colistin (MICs of colistin ranging from 8 to >128 mg/L) and produced a carbapenemase. The nature of the carbapenemase and the mechanisms responsible for colistin resistance have been previously characterized. Our collection included 11 KPC-like producers, 32 OXA-48 producers, 5 OXA-181 producers, 8 NDM-like producers and 7 isolates that co-produced two carbapenemases (NDM and OXA-48 or NDM and OXA-181) (Table S1, available as Supplementary data at *JAC* Online). The mechanisms responsible for colistin resistance were various and are indicated in Table S1.

MICs of ceftazidime/avibactam were determined using MIC test strips (Liofilchem, I2A, Montpellier, France) according to the manufacturer's guidelines. Following the EUCAST breakpoints (http://www.eucast.org/), isolates with an MIC of ceftazidime/avibactam of ≤8 mg/L were categorized as susceptible, whereas those with an MIC >8 mg/L were categorized as resistant. All the *K. pneumoniae* isolates producing a class A (KPC) or class D (OXA-48 and OXA-181) carbapenemase alone were susceptible to ceftazidime/avibactam with MICs ranging from 0.12 to 6 mg/L. As expected, the isolates producing a class B carbapenemase (NDM alone or associated with another carbapenemase) presented a high level of resistance to ceftazidime/avibactam (MIC ≥256 mg/L) (Table S1).

For the 15 isolates producing an MBL, MICs of aztreonam were also determined using MIC test strips (Liofilchem). According to EUCAST breakpoints, isolates with an MIC of ≤1 mg/L were categorized as susceptible, whereas those with an MIC >4 mg/L were categorized as resistant. Out of the 15 isolates, only a single isolate was actually susceptible to aztreonam. Resistance to aztreonam was mainly due to production of ESBLs (data not shown).

The *in vitro* synergy of ceftazidime/avibactam and aztreonam against MBL-positive isolates was studied using MIC test-strip-based synergy methods as previously described.[6] The combination was tested by first applying a ceftazidime/avibactam strip to the Mueller–Hinton agar, removing it after 5 min, then applying an aztreonam strip to the exact same location and replacing the ceftazidime/avibactam strip on top of the aztreonam strip.[6] Despite a high level of resistance to each antibiotic, the combination was synergistic for all the isolates with MICs of the combination <2 mg/L (Figure 1).

This study further suggests that ceftazidime/avibactam is an effective therapeutic option for treating infections caused by colistin-resistant and KPC- or OXA-48-producing *K. pneumoniae*. Moreover, the association of ceftazidime/avibactam and aztreonam is effective against NDM-producing *K. pneumoniae*. This combination was efficient in particular against *K. pneumoniae* isolates producing two carbapenemases. The synergy of the combination of

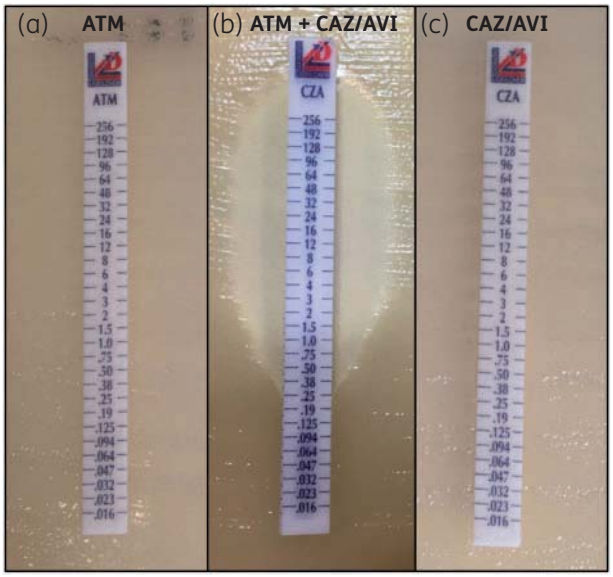

Figure 1. Example of synergistic combination of ceftazidime/avibactam (CAZ/AVI) and aztreonam (ATM) for an NDM + ESBL-producing *K. pneumoniae*. Susceptibility testing of ATM alone (a), combination of CAZ/AVI with ATM (b) and CAZ/AVI alone (c). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

ceftazidime/avibactam with aztreonam against NDM producers could be explained by the neutralization of the ESBL activity by avibactam allowing a restoration of the susceptibility to aztreonam. This study suggests that further commercialization of aztreonam/avibactam as a pharmaceutical preparation could be an interesting option to treat infections caused by MBL producers.

Notably, synergy of ceftazidime/avibactam and aztreonam can be easily evaluated using MIC test-strip synergy assays in clinical microbiology laboratories. Further investigations using experimental models and clinical trials are required to further confirm that this might be a relevant and effective therapeutic option in clinical practice.

Acknowledgements

We thank Drs A. Brink, Z. Gülay, M. Yilmaz and M. V. Villegas who provided colistin-resistant and carbapenemase-producing *K. pneumoniae* isolates.

Funding

This work was financed by the University of Bordeaux (France) and the University of Fribourg (Switzerland).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data

References

1 Meletis G. Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis* 2016; **3**: 15–21.

2 Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev* 2017; **30**: 557–96.

3 Monaco M, Giani T, Raffone M *et al*. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill* 2014; **19**: pii=20939.

4 Lagacé-Wiens P, Walkty A, Karlowsky JA. Ceftazidime-avibactam: an evidence-based review of its pharmacology and potential use in the treatment of Gram-negative bacterial infections. *Core Evid* 2014; **9**: 13–25.

5 Wenzler E, Deraedt MF, Harrington AT *et al*. Synergistic activity of ceftazidime-avibactam and aztreonam against serine and metallo-β-lactamase-producing Gram-negative pathogens. *Diagn Microbiol Infect Dis* 2017; **88**: 352–4.

6 Davido B, Fellous L, Lawrence C *et al*. Ceftazidime-avibactam and aztreonam, an interesting strategy to overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2017; **61**: e01008–17.