# Efficacy of aztreonam with ß-lactamase inhibitors against metallo-carbapenemase-producing Enterobacteria

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**Title:** Brief Report: Efficacy of aztreonam with ß-lactamase inhibitors against metallo-carbapenemase-producing Enterobacteria

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**Declarations**

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**Abstract**

**Purpose**: The combination of aztreonam (ATM), poorly hydrolyzed by metallo-ß-lactamase (MBL) and ß-lactamase inhibitors inhibiting extended spectrum ß-lactamases represents a theoretical therapeutic option against carbapenemase-producing Enterobacteriaceae (CPE). We evaluated the *in vitro* activity of aztreonam combined with ceftazidime-avibactam (CZA) or ceftolozane-tazobactam (C/T) against MBL CPE.

**Methods**: The effects of the combinations were tested against 42 clinical MBL CPE resistant to ATM by using E-test strips.

**Results**: CZA and ATM were synergistic and restored ATM susceptibility in 26/42 isolates (average MIC gain: 11-fold), while the second combination was poorly active.

**Conclusion**: CZA+ATM needs further evaluation for treating patients with MBL CPE.

**Sir,**

Metallo-β-lactamases (MBL) produced by Enterobacteriaceae inhibit the clinical activity of most β-lactams but aztreonam (ATM). However, carbapenemase-producing Enterobacteriaceae (CPE) are frequently carrying additional resistance mechanisms such as extended-spectrum-β-lactamase (ESBL) or over-production of chromosomally-determined cephalosporinase conferring additional resistance to ATM. Hence, such isolates represent a therapeutic challenge. Some hope comes from the combination of ATM with ceftazidime-avibactam (CZA), which has recently demonstrated *in vitro* and *in vivo* activities on MBL-producing Enterobacteriaceae [1–4]. Therefore, we assessed the *in vitro* synergistic activity of two antibiotic combinations with ATM, *i.e.* CZA and ceftolozane-tazobactam (C/T). Unselected MBL CPE isolates were consecutively collected from clinical samples from 2016 to 2019 in a University hospital in Paris, France. The phenotypic test Resist-4 OKNV (Coris Bioconcept®, Gembloux, Belgium) and the genotypic test Xpert Carba-R (Cepheid®, Sunnyvale USA) were used to characterize carbapenemase enzymes. MICs of ATM, CZA, and C/T were determined by the E-test strip method (BioMérieux®, Durham, USA – Liofilchem®, Waltham, USA). The synergistic activities of the ATM-CZA and ATM-C/T combinations were determined as previously described [1], by first applying on a Mueller-Hinton agar the CZA or C/T strip, that was replaced after 10 minutes, by an ATM strip at the very same place. Plates were incubated at 37°C for 16 – 18 hours under aerobiosis conditions. The MICs of the combinations were interpreted according to the 2018 EUCAST (http://www.eucast.org) breakpoints for ATM (1 and 4 mg/L). In order to quantify the decrease in MICs values of the synergistic combinations as compared to ATM alone, values of MICs ≥ 256 mg/L were converted to 512 mg/L. ESBL-production was assessed by the double-disk diffusion method [4]. The median decreases in MIC dilutions were compared by using the Mann Whitney test (R project, version 4.0.0).

A total of 47 MBL-producing Enterobacteriaceae (37 NDM and 10 VIM) were collected. *Escherichia coli* was the most frequent species (43%), followed by *Klebsiella pneumoniae* (26%) and *Enterobacter cloacae complex* (17%). Other species were *Citrobacter freundii* (n=4), *C. koseri* (n=1), *K. oxytoca* (n=1) and *Proteus mirabilis* (n=1). A total of 73% (27/37) of NDM- and half (5/10) of VIM-producing isolates displayed an ESBL phenotype. According to EUCAST breakpoints, all isolates were resistant (R) to CZA and C/T, and five were ATM-susceptible (S). ATM-CZA and ATM-C/T combinations MICs distribution were firstly plotted (see Figure 1) as a global visualization purpose and to seek the impact of the additive ESBL-producing phenotype. As expected, the five ATM-S isolates remained ATM-CZA-S and ATM-C/T-S. Among the 42 ATM non-S isolates,

only four (9%) were ATM-C/T-S, whereas 26 (62%) were ATM-CZA-S and 12 (29%) of intermediate susceptibility to ATM-CZA (as shown in Figure 2). There was no significant difference between ESBL and non-ESBL isolates. Among the four ATM-CZA-R (MICs > 256 and 128 mg/L) isolates, two (one NDM and one VIM producer) were ATM-C/T-S (MICs: 0.38 and 0.19 mg/L), both displaying an ESBL phenotype. The confidence interval of MICs distribution among all MBL-producing Enterobacteriaceae for ATM-CZA and ATM-C/T were respectively [0 ; 34.47] and [48.04 ; 137.08] (p-value < 0.05).

Amongst the two ATM-CZA-R remaining isolates, one was of intermediate susceptibility and one was resistant to ATM-C/T. Among the 30 ATM-C/T-R isolates, 17 (57%) were ATM-CZA-S. The median reduction in MICs dilution for the combinations with ATM were 14 (range: 0 to 24) fold for ATM-CZA compared to 5 (range: 0 to 22) fold for ATM-C/T and the difference was statistically significant ($p < 0.05$). However, for only three isolates (VIM-positive *E. coli*; NDM-positive *K. pneumoniae*; NDM and ESBL-positive *E. cloacae*), the decrease in MICs was higher with the ATM-C/T than with the ATM-CZA combination.

In summary, by using a set of unselected clinical isolates, we confirmed the interest of the ATM-CZA combination, while the ATM-C/T combination was seldom synergistic. Of interest, ESBL production had no impact on the MIC of the ATM-CZA combination. Of note, even though the ATM-C/T combination appeared of minimal interest, the latter may warrant testing when the ATM-CZA combination is ineffective. Indeed, two of four of the ATM-CZA-R strains appeared to be susceptible to the ATM-C/T combination. In the latter cases, there was no obvious link between the synergistic effect of ATM-C/T and the resistance phenotypic pattern of the isolates. Our report confirms previous studies on the interest of the ATM-AVI (avibactam) combination on MBL [1,2,4–7]. Nevertheless, besides one study [5] we tested a larger number of species and included VIM MBL. Because AVI has no inhibitory activity against MBL [5], the efficacy of the combination is likely due to the inhibition of class A or class C ß-lactamases by AVI, including carbapenemases [3] thus protecting ATM from hydrolysis by these enzymes. We report also herein on the new combination C/T and demonstrated its limited interest in this purpose.

Because new ß-lactamase inhibitors combined to carbapenems have little *in vitro* efficacy against MBL CPE, our results advocate for discussing the clinical use of the ATM-CZA combination as salvage therapy when no alternatives are available for the treatment of infections due to MBL-producing isolates.

**Figure 1 – Distribution of MIC values**

Minimum inhibitory concentration (MIC) distributions of ceftazidime-avibactam (CZA), and ceftolozane-tazobactam (C/T) in combination with aztreonam (ATM) against metallo-ß-lactamase producing isolates. White and grey bars represent ATM-CZA and ATM-C/T MICs, respectively. Dotted and solid bars represent extended spectrum β-lactamase- (ESBL) and non-ESBL-producing isolates, respectively. The two vertical solid lines represent the lower (1 mg/L) and higher (4 mg/L) ATM clinical breakpoints.

**Figure 2 – Scatter plot of Minimum Inhibitory Concentrations (MIC) of combinations with aztreonam**

Minimum inhibitory concentration (MIC) values of aztreonam + ceftazidime-avibactam (ATM-CZA) plotted against aztreonam + ceftolozane-tazobactam (ATM-C/T) on a double-logarithmic scale. The lower and higher clinical breakpoints (www.eucast.org) of ATM are represented by the two solid black lines on each axes (1 and 4 mg/L, respectively).

Grey and black dots represent one and two isolates, respectively

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Figure 1



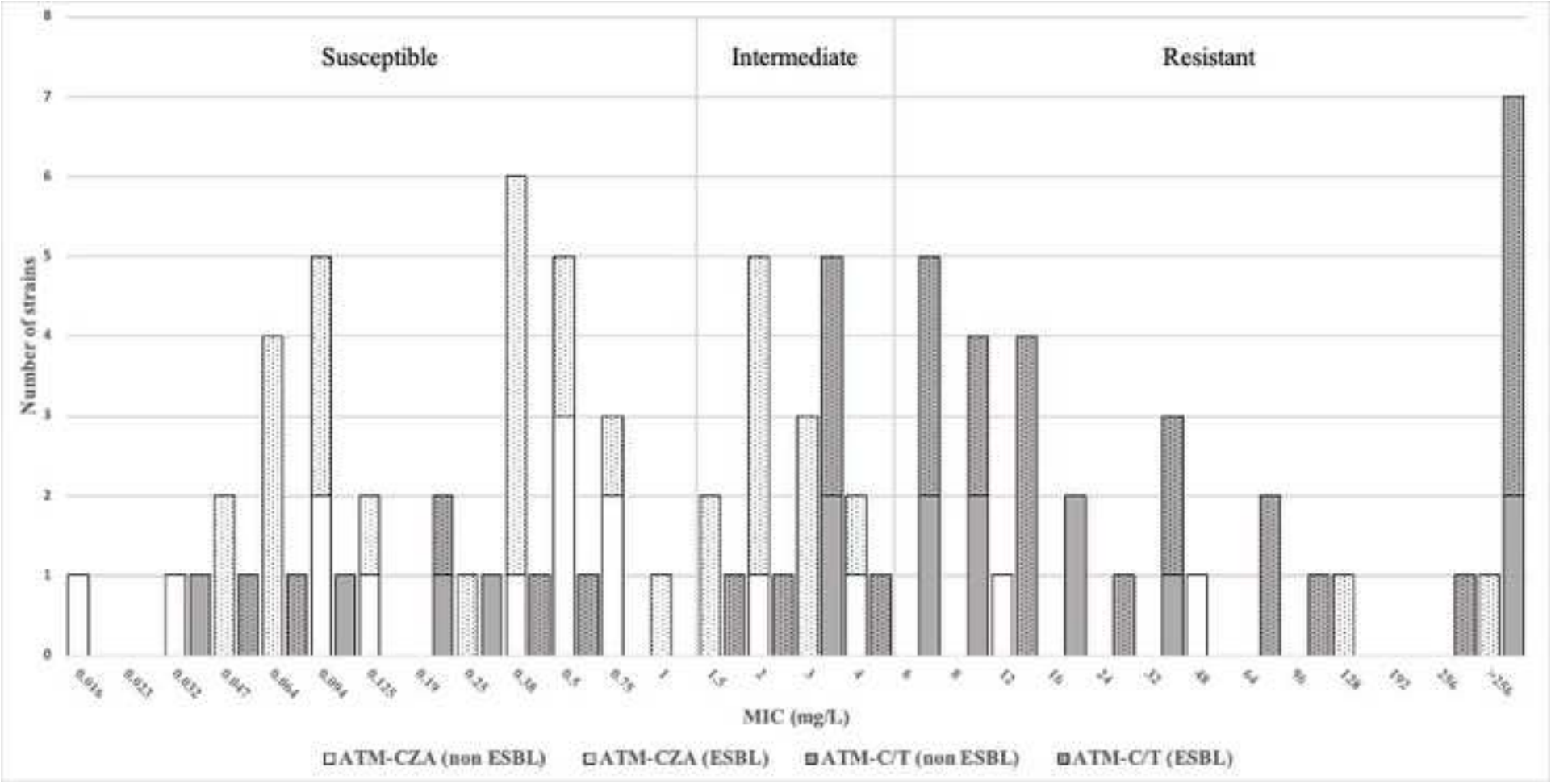

Figure 2

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