



HAL
open science

In vitro evaluation of the inoculum effect of various β -lactamase-producing *Klebsiella pneumoniae* isolates on ceftazidime-avibactam

Alexia Chauzy, William Couet, Isabelle Martin, H el ene Mirfendereski,
Sandrine Marchand

► To cite this version:

Alexia Chauzy, William Couet, Isabelle Martin, H el ene Mirfendereski, Sandrine Marchand. In vitro evaluation of the inoculum effect of various β -lactamase-producing *Klebsiella pneumoniae* isolates on ceftazidime-avibactam. 34th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Apr 2024, Barcelona, Spain. hal-04571024

HAL Id: hal-04571024

<https://hal.science/hal-04571024>

Submitted on 7 May 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin ee au d ep ot et  a la diffusion de documents scientifiques de niveau recherche, publi es ou non,  emanant des  tablissements d'enseignement et de recherche fran ais ou  trangers, des laboratoires publics ou priv es.

In vitro evaluation of the inoculum effect of various β -lactamase-producing *Klebsiella pneumoniae* isolates on ceftazidime-avibactam

A. Chauzy¹, W. Couet^{1,2}, I. Martin^{1,2}, H. Mirfendereski^{1,2} and S. Marchand^{1,2}

Contact: alexia.chauzy@univ-poitiers.fr

¹ Université de Poitiers, Inserm U1070, France

² CHU de Poitiers, Département de Toxicologie et Pharmacocinétique, France

Introduction

Inoculum effect (IE) is a phenomenon widely recognized as having a potential effect on the efficacy of antibiotics, particularly β -lactams. One of the most frequently reported mechanism responsible for IE is an increased β -lactamases production with an increased bacterial burden¹.

The objective of this study was to characterize *in vitro* the IE produced by several *K. pneumoniae* strains with different β -lactamase profiles on ceftazidime-avibactam (CAZ-AVI), a β -lactam- β -lactamase inhibitor (BL-BLI) combination.

Materials and methods

- In vitro* IE was investigated on two clinical *K. pneumoniae* isolates producing KPC-2 or OXA-48, and on one non- β -lactamase-producing reference strain, used as a control (Table 1).
- Time-kill (TK) experiments were performed over 30h, using **low (10⁵-10⁶ CFU/mL)** and **high (10⁸ CFU/mL)** starting inocula in presence and absence of AVI. CAZ concentrations were adapted according to the inoculum.
- Bacterial counts were determined at various times.
- β -lactamase activity and AVI inhibitory effect were characterized by measuring CAZ concentrations by LC-MS/MS throughout TK experiments.

Table 1. Tested CAZ-AVI concentrations in the TK experiments for the three selected isolates.

Strain	β -lactamases	Concentration range of CAZ (mg/L)	Inoculum (CFU/mL)		
			10 ⁵	10 ⁶	10 ⁸
<i>K. pneumoniae</i> A28006	KPC-2	In absence of AVI	16-256		64-1024
		In presence of AVI at :			
		- 4 mg/L - 16 mg/L	0.25-8		2-256 16-256
<i>K. pneumoniae</i> CHA	OXA-48	In absence of AVI	0.03125-1		0.25-16
		In presence of AVI at :			
		- 4 mg/L - 16 mg/L	0.0625-1		0.25-16 0.25-16
<i>K. pneumoniae</i> ATCC 43816	-	In absence of AVI		0.125-4	1-32
		In presence of AVI at : - 4 mg/L		0.0625-2	0.5-16

Results

An IE on CAZ activity was observed for the three strains in absence of AVI (Fig.1-3, first column).

For the β -lactamase-producing strains, CAZ degradation was dependent on the bacterial density (Fig.1-2). However, in presence of AVI, CAZ degradation was reduced resulting in a greater bacterial decrease:

- At low inoculum, an AVI concentration of 4 mg/L was sufficient to prevent CAZ degradation, while at high inoculum a higher concentration of AVI was required to compensate for the overproduction of β -lactamases (Fig.1-2, bottom panels).
- For a starting inoculum of 10⁸ CFU/mL, an AVI concentration of 16 mg/L restored the sensitivity of *K. pneumoniae* A28006 to CAZ but was still not sufficient for *K. pneumoniae* CHA.

CAZ activity was also increased for the non- β -lactamase-producing strain in presence of AVI (Fig.3, top panels), although this was unrelated to its inhibitory activity (Fig.3, bottom panels).

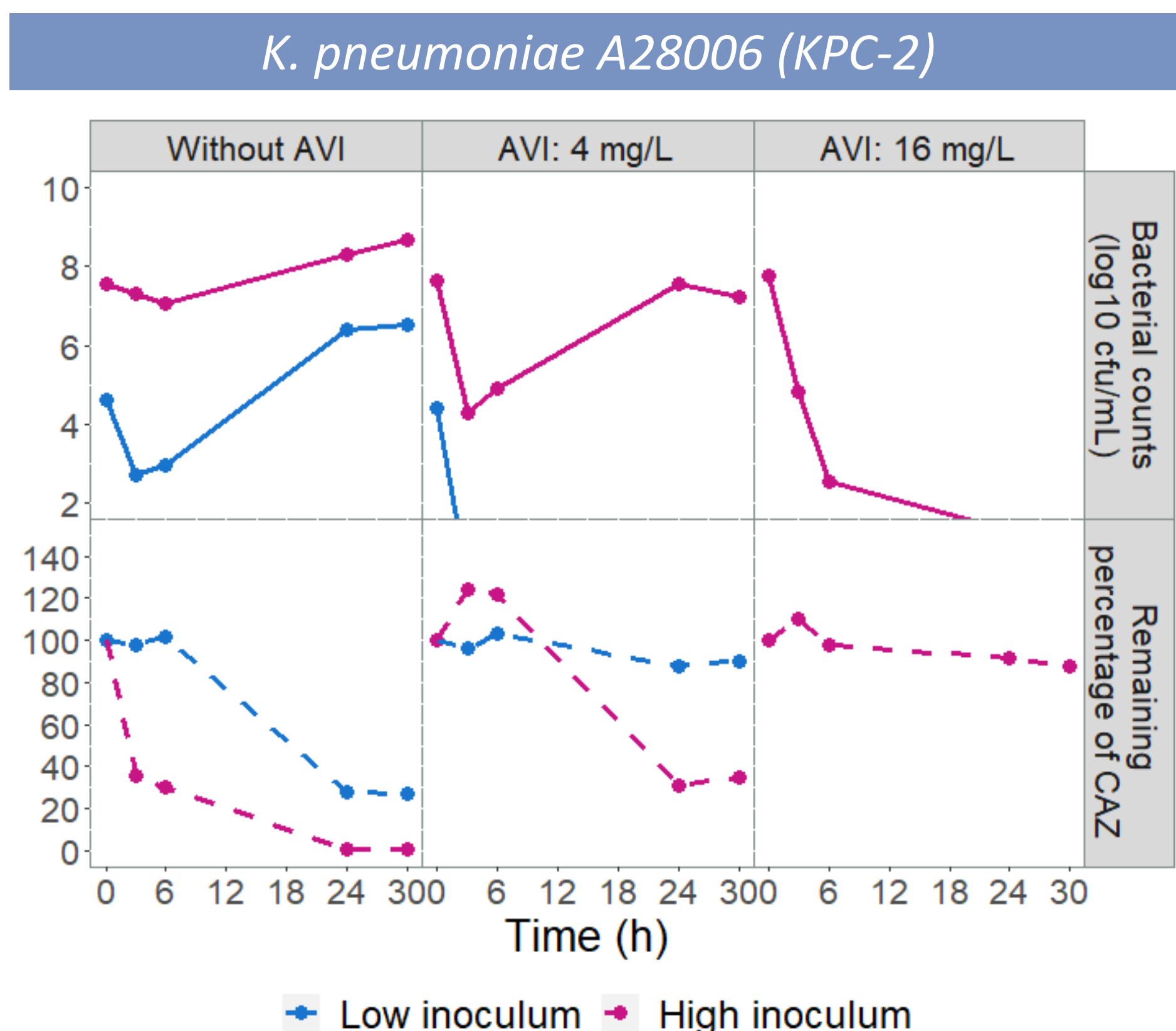


Figure 1. Comparison between low and high inocula of the efficacy of 64 mg/L CAZ against *K. pneumoniae* A28006 and its degradation in absence and presence of AVI

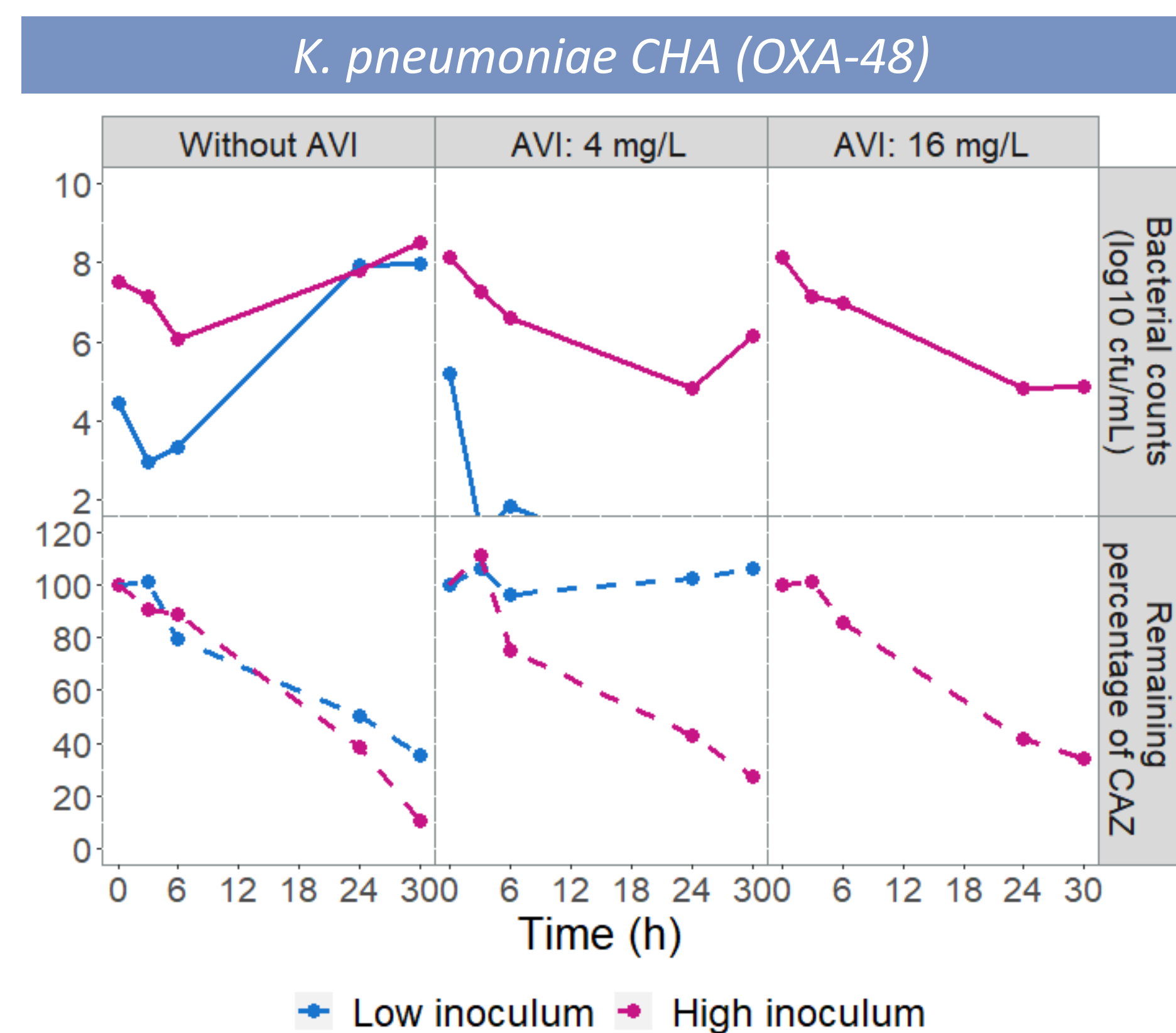


Figure 2. Comparison between low and high inocula of the efficacy of 0.25 mg/L CAZ against *K. pneumoniae* CHA and its degradation in absence and presence of AVI

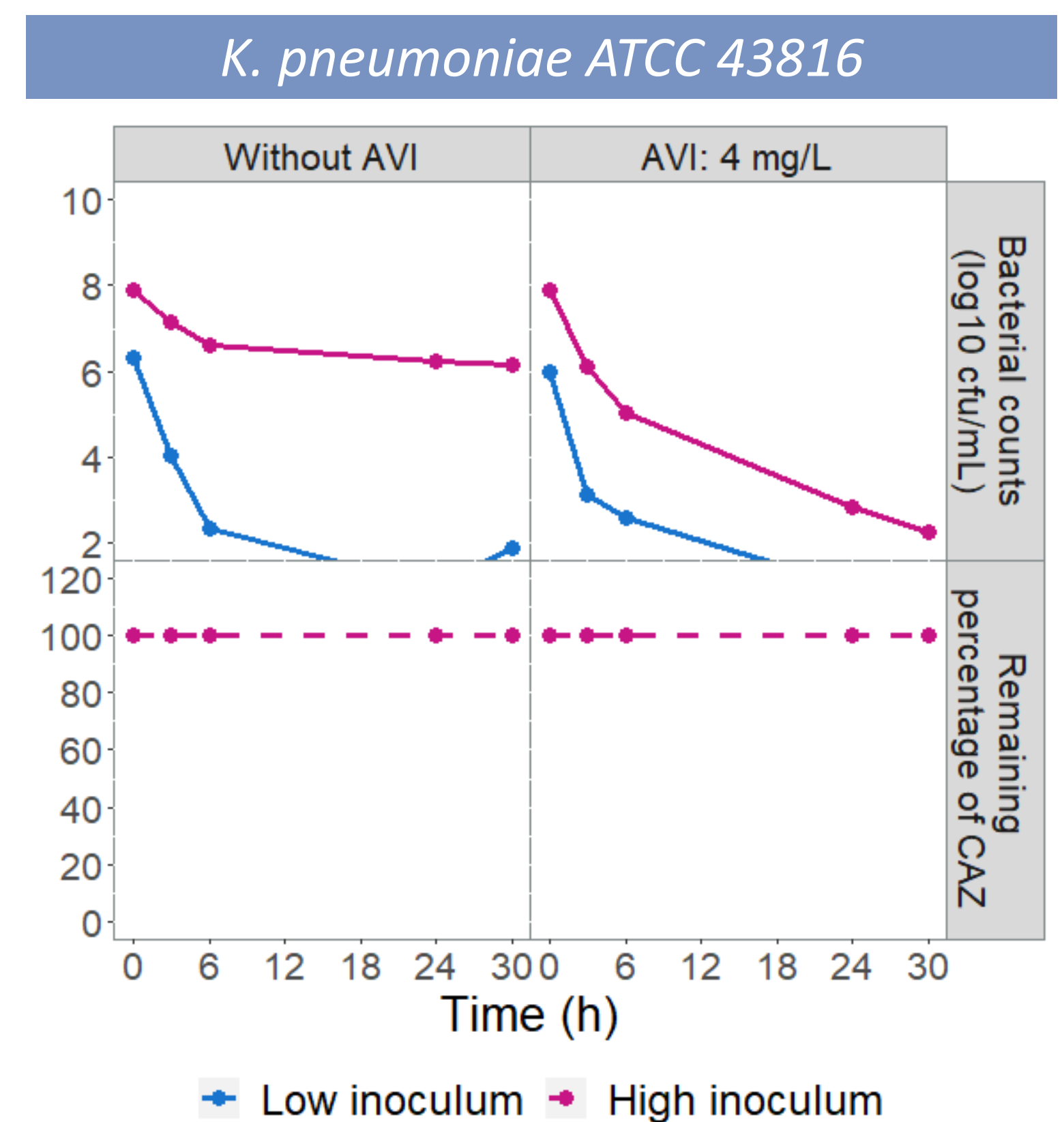


Figure 3. Comparison between low and high inocula of the efficacy of 1 mg/L CAZ against *K. pneumoniae* ATCC 43816 and its degradation in absence and presence of AVI

Conclusion

This study showed that different mechanisms might contribute to the IE of *K. pneumoniae* on CAZ-AVI: the first one corresponding to a reduction of CAZ activity and the second one, specific to BL-BLI, to an over-production of β -lactamases. A combination of these two types of IE cannot be ruled out.

AVI reduced the IE by enhancing CAZ activity² against *K. pneumoniae* ATCC 43816 or/and by protecting CAZ from degradation by β -lactamases in the other strains.

- References:**
- 1) Lenhard and Bulman. 10.1093/jac/dkz226
 - 2) Kristofferson et al. 10.1093/jac/dkz440



This project has been funded by a Research Grant 2022 from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) to AC.