Activity of ceftazidime-avibactam against clinical and isogenic laboratory *Pseudomonas aeruginosa* isolates expressing combinations of most relevant β-lactam resistance mechanisms.

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Abstract

The activity of ceftazidime-avibactam was compared with that of ceftazidime alone and meropenem against a collection of 190 *P. aeruginosa* clinical isolates recovered from a multicenter study of bloodstream infections. The addition of avibactam increased ceftazidime susceptibility in the complete collection of strains (64.7% to 91.1%) and particularly among subsets of isolates showing AmpC hyperproduction (10.9% to 76.1%) or MDR profiles (27% to 77.8%). MICs of ceftazidime-avibactam, in contrast with those of ceftazidime or meropenem, remained ≤4 µg/mL for a panel of 16 PAO1 isogenic mutants, expressing multiple combinations of the most relevant β-lactam resistance mechanisms.

Pseudomonas aeruginosa causes a wide range of severe infections, and represents a therapeutic challenge due to its low intrinsic susceptibility to most antimicrobials and its extraordinary ability to develop resistance to nearly all available antibiotics through chromosomal mutations (1). Although the prevalence of acquired β-lactamases, particularly class B carbapenemases (metallo-β-lactamases, MBLs), is increasing in certain areas, the overexpression of AmpC is still the most frequent and relevant resistance mechanism to penicillins and cephalosporins in *P. aeruginosa*, frequently leading to pan-β-lactam-resistance profiles when combined with the inactivation of carbapenem porin OprD and/or the overexpression of diverse efflux pumps (2, 3).

Avibactam is a new broad spectrum inhibitor of β-lactamases from classes A and C as well as some from class D, recently commercialized in combination with ceftazidime in the USA and Europe, with treatment indications for complicated urinary tract infections, complicated intra-abdominal infections and hospital-acquired pneumonia (Europe) (4).

The objective of this study was to evaluate the activity of ceftazidime-avibactam, compared with that of ceftazidime alone and meropenem, against a collection of 190 *P. aeruginosa* clinical isolates recovered from a bloodstream infections multicenter study performed in Spain (5). Resistance mechanisms produced by this collection have been deeply characterized previously (5, 6). Additionally, a panel of 16 PAO1 isogenic mutants, expressing multiple combinations of the most relevant β-lactam resistance mechanisms, such as AmpC hyperproduction, OprD inactivation and efflux pumps overexpression were tested. MICs were determined for ceftazidime alone or combined with avibactam (at a fixed concentration of 4 µg/mL) and for meropenem by broth microdilution using CLSI breakpoints (7).

59 Recommendations by Magiorakos *et al*. were followed for the definition of multidrug
60 resistance (MDR) profiles (8).

61 Consistently with previous reports (9, 10), the addition of avibactam
62 significantly increased overall ceftazidime susceptibility in the collection of clinical
63 strains, from 64.7% to 91.1% (Table 1). Up to 74.6% of the isolates non-susceptible to
64 ceftazidime remained susceptible to ceftazidime-avibactam. Moreover, ceftazidime-
65 avibactam overall susceptibility percentages were well above those of meropenem
66 (77.4%). The effect of the addition of avibactam was even higher for the subset of
67 isolates showing MDR profiles (27% to 77.8%) and AmpC hyperproduction (10.9% to
68 76.1%). The MIC distributions (Figure 1) corroborated the significant increase of
69 activity, with modal MIC values of ceftazidime for MDR and AmpC hyperproducing
70 strains decreasing from 32 to 4 µg/mL with the addition of avibactam; MIC_{50}s and
71 MIC_{90}s (Table 1) also revealed an at least 4-fold higher potency of ceftazidime-
72 avibactam compared to ceftazidime alone in these subsets of strains. Moreover, up to
73 74.1% of pan-β-lactam resistant isolates were susceptible to ceftazidime-avibactam
74 (Table 1).

75 Ceftazidime MIC_{50}s were not much different in the presence of avibactam for
76 the isolates overexpressing major efflux pumps (MexAB or MexXY), likely indicating
77 that, as expected, avibactam does not provide protection against these resistance
78 mechanisms. However, MIC_{90S} and non-susceptibility percentages were lower for
79 ceftazidime-avibactam than for ceftazidime alone in these subsets of isolates, likely due
80 to the co-expression of additional resistance mechanisms, particularly AmpC
81 hyperproduction. On the other hand, the activity of comparator meropenem was much
82 lower than that of ceftazidime-avibactam among all subgroups of isolates (MDR, AmpC

hyperproduction and efflux pump overexpression), with susceptibility rates below 50% in all cases (Table 1).

Of the 17 (8.9%) isolates non-susceptible to ceftazidime-avibactam (MICs >8 µg/mL), two of them produced the MBL VIM-2. These two isolates showed the highest ceftazidime-avibactam MIC values, 64 and 128 µg/mL, and were the only isolates found to produce an acquired β-lactamase in the complete collection of 190 isolates (5). MICs for all other non-susceptible strains ranged from 16 (14 isolates) to 32 (1 isolate) µg/mL. Thus, most non-susceptible isolates remained within the CLSI ceftazidime intermediate category (16 µg/ml). The analysis of resistance mechanisms (AmpC and efflux pumps) in this subset of isolates failed to detect specific differences with ceftazidime-avibactam susceptible isolates, arguing in favour of the existence of yet unidentified mechanisms modulating ceftazidime-avibactam susceptibility (11).

The activity of ceftazidime-avibactam, compared with that of ceftazidime alone and meropenem was also evaluated in a collection of PAO1 isogenic mutants expressing multiple combinations of the most relevant β-lactam resistance mechanisms, including multiple levels of AmpC hyperproduction, mutation of nonessential penicillin-binding proteins (PBPs), inactivation of the porin OprD, and/or efflux pumps overexpression. As shown in Table 2, MICs of ceftazidime-avibactam, in contrast with those of ceftazidime or meropenem remained ≤4 µg/mL in all cases. The potentiation of the activity of ceftazidime by avibactam was highest among isolates showing multiple combinations of mutations leading to very high-level of AmpC production, such as the triple *ampD* mutant, the mutant defective in all 3 nonessential PBPs or the AmpD-PBP4 double mutant, for which the MIC of ceftazidime was reduced from 64 to 4 µg/mL. Thus, results are similar to those documented for the novel combination ceftolozane-tazobactam (3, 12). It should be noted, however, that resistance to both novel

combinations may emerge through the (infrequent) selection of different mutations leading to the modification of AmpC structure (13). MexAB-OprM overexpression determined a reduction (4-fold MIC increase) of ceftazidime susceptibility which was not restored, as expected (14), by the addition of avibactam. However, the positive effect of avibactam on AmpC hyperproducing strains was still seen even when they simultaneously overexpressed MexAB-OprM (See MexR and AmpD-MexR mutants in Table 2). On the other hand, consistently with previous data (2), susceptibility of meropenem was highly compromised by combinations of OprD inactivation and AmpC or efflux pumps (MexAB-OprM) hyperproduction.

Thus, ceftazidime-avibactam, could be a new useful therapeutic option for the treatment of nosocomial infections by *P. aeruginosa*, including non-MBL-producing MDR strains.

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212 **Legends to figures**

213

214 **Figure 1. A**. Ceftazidime (CAZ) and ceftazidime-avibactam (CAZ-AVI) MIC distributions for a collection of 190 *P. aeruginosa* bloodstream
215 isolates recovered from a 10-hospital multicenter study performed in Spain. **B.** CAZ and CAZ-AVI MIC distribution for the 46 isolates from the
216 collection showing AmpC hyperproduction. **C**. CAZ and CAZ-AVI MIC distribution for the 63 isolates from the collection showing a MDR
217 profile.

Table 1. $MIC_{50/90}$ and susceptibility percentages for the entire collection of blood stream isolates, and subsets of isolates showing AmpC or efflux pumps hyperproduction or MDR profiles.

	All Isolates (n = 190)			**AmpC hyperproducing isolates**[b] (n = 46)			**MexAB hyperproducing isolates**[b] (n = 24)			**MexXY Hyperproducing isolates**[b] (n = 25)			**MDR isolates** (n = 63)			**Pan-β-lactam resistant isolates**[c] (n = 27)
	CAZ	*CAZ-AVI*	*MER*	*CAZ*	*CAZ-AVI*	*MER*	*CAZ*	*CAZ-AVI*	*MER*	*CAZ*	*CAZ-AVI*	*MER*	*CAZ*	*CAZ-AVI*	*MER*	*CAZ-AVI*
***MIC*$_{50}$**	4	4	1	32	4	8	8	8	8	8	4	8	32	4	8	8
***MIC*$_{90}$**	32	8	16	128	16	32	64	16	32	32	8	16	128	16	32	16
% Susceptible[a]	64.7	91.1	77.4	10.9	76.1	41.3	50.0	87.5	41.7	60.0	96.0	44.0	27.0	77.8	41.3	74.1

[a] Breakpoints for ceftazidime (CAZ), S≤ 8 µg/mL; ceftazidime-avibactam (CAZ-AVI) S≤ 8/4 µg/mL; meropenem (MER) S≤4 µg/mL.

[b] Previously definitions were used (5). Strains were considered positive for *ampC* or *mexY* overexpression when the corresponding mRNA level was at least 10-fold higher than that of PAO1. Strains were considered positive for *mexB* overexpression when the corresponding mRNA level was at least 3-fold higher than that of PAO1.

[c] Pan-β-lactam resistant isolates are defined as non-susceptible to ceftazidime, cefepime, aztreonam, piperacillin-tazobactam, imipenem and meropenem.

226 **Table 2**. MIC for ceftazidime (CAZ), ceftazidime-avibactam (CAZ-AVI) and meropenem (MER) for PAO1 isogenic mutants expressing
227 multiple combinations of most relevant β-lactam resistance mechanisms.

Strain	Phenotype[a]	Reference	MIC (µg/mL)		
			CAZ	CAZ-AVI	MER
PAO1	Wild type strain		1	1	0.5
PAOΔ*dacB*	PAO1 PBP4 mutant [↑*ampC* (Ca. 50-fold)]	15	32	2	0.5
PAOΔ*dacC*	PAO1 PBP5 mutant	16	1	1	0.5
PAOΔ*dacB*Δ*dacC*	PAO1 PBP4-PBP5 mutant [↑*ampC* (Ca. 500-fold)]	16	64	2	0.5
PAOΔ*dacB*Δ*pbpG*Δ*dacC*	PAO1 PBP4-PBP5-PBP7 mutant [↑*ampC* (Ca. 1200-fold)]	16	64	2	0.5
PAOΔ*ampD*	PAO1 AmpD mutant [↑*ampC* (Ca. 50-fold)]	17	16	2	1
PAOΔDΔDh2ΔDh3	PAO1 AmpD-AmpDh2-AmpDh3 mutant [↑*ampC* (Ca. 1000-fold)]	17	64	4	1
PAOD1	OprD- spontaneous PAO1 mutant (W65X)	12	1	1	2
PAOD1Δ*ampD*	PAOD1 (OprD-) AmpD mutant [↑*ampC* (Ca. 50-fold)]	12	16	2	8
PAOΔdBΔ*ampD*	PAO1 PBP4 AmpD mutant [↑*ampC* (Ca. 1800-fold)]	15	64	4	1
PAOD1Δ*dacB*	PAOD1 (OprD-) PBP4 mutant [↑*ampC* (Ca. 50-fold)]	12	32	2	2
PAOΔMxR	PAO1 MexR mutant [↑*mexB* (Ca. 10-fold)]	18	4	4	2
PAODΔMxR	PAOD1 (OprD-) MexR mutant [↑*mexB* (Ca. 10-fold)]	2	4	4	8
PAOΔDΔMxR	PAO1 AmpD-MexR mutant [↑*ampC* (Ca. 50-fold) + ↑*mexB* (Ca. 10-fold)]	2	32	4	4
PAOΔNB	PAO1 NfxB mutant [↑*mexD* (Ca. 150-fold)]	19	1	1	0.25
PAOΔMxZ	PAO1 MexZ mutant [↑*mexY* (Ca. 15-fold)]	20	1	1	0.5
PAODΔMxZ	PAOD1 (OprD-) MexZ mutant [↑*mexY* (Ca. 15-fold)]	This work	1	1	2

228 [a]Expression levels and *oprD* aminoacid changes, are referred to PAO1.

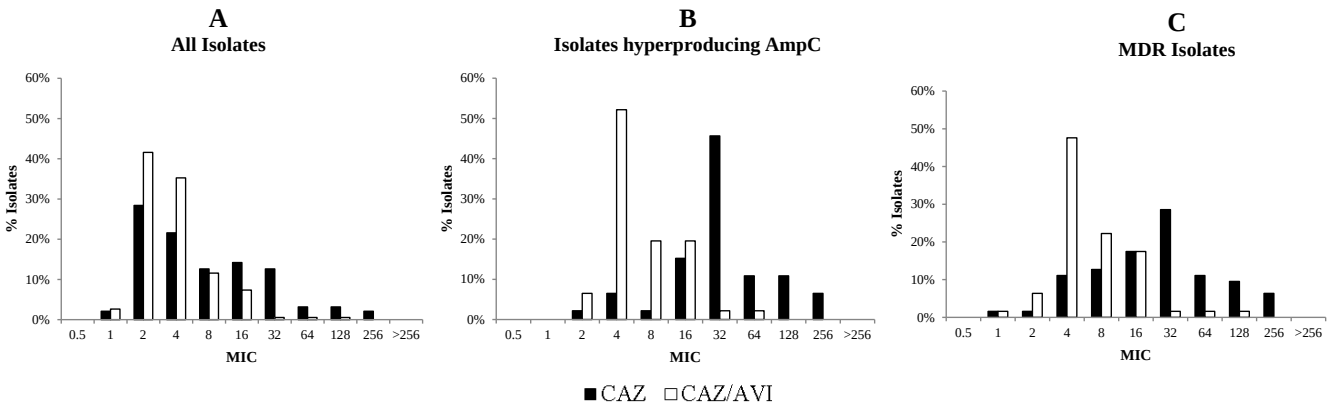

Figure 1. A. Ceftazidime (CAZ) and ceftazidime-avibactam (CAZ-AVI) MIC distributions for a collection of 190 *P. aeruginosa* bloodstream isolates recovered from a 10-hospital multicenter study performed in Spain. **B.** CAZ and CAZ-AVI MIC distribution for the 46 isolates from the collection showing AmpC hyperproduction . **C**. CAZ and CAZ-AVI MIC distribution for the 63 isolates from the collection showing a MDR profile.