Activity of Imipenem-Relebactam against a Large Collection of *Pseudomonas aeruginosa* Clinical Isolates and Isogenic β -Lactam-Resistant Mutants

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ABSTRACT Imipenem and imipenem-relebactam MICs were determined for 1,445 *Pseudomonas aeruginosa* clinical isolates and a large panel of isogenic mutants showing the most relevant mutation-driven β -lactam resistance mechanisms. Imipenem-relebactam showed the highest susceptibility rate (97.3%), followed by colistin and ceftolozane-tazobactam (both 94.6%). Imipenem-relebactam MICs remained $\leq 2 \mu\text{g/ml}$ in all 16 isogenic PAO1 mutants and in 8 pairs of extensively drug-resistant clinical strains that had developed resistance to ceftolozane-tazobactam and ceftazidime-avibactam due to mutations in OXA-10 or AmpC.

KEYWORDS *Pseudomonas aeruginosa*, imipenem-relebactam, whole-genome sequencing, multidrug resistance, extensively drug resistant, β -lactam resistance mechanisms, antibiotic resistance

The growing prevalence of nosocomial infections produced by multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas aeruginosa* strains is associated with significantly increased morbidity and mortality (1). This increasing threat results from the extraordinary capacity of *P. aeruginosa* to develop resistance to nearly all available antibiotics by the selection of mutations in chromosomal genes, the growing prevalence of transferable resistance determinants (particularly those encoding carbapenemases or extended-spectrum β -lactamases [ESBLs]), and the dissemination of MDR/XDR global strains, the high-risk clones (2, 3).

On the other hand, the recent introduction of novel β -lactam- β -lactamase inhibitor combinations, such as ceftolozane-tazobactam or ceftazidime-avibactam, which are stable against AmpC hydrolysis, have partially alleviated the urgent clinical need for new agents to combat infections by MDR/XDR *P. aeruginosa* strains (4, 5). However, emerging resistance to these antibiotics is concerning and calls for continuous development of new therapeutic options (6–8). One of these recently developed novel options for the treatment of MDR/XDR Gram-negative pathogens is the combination of the carbapenem imipenem with relebactam, a novel inhibitor of β -lactamases from classes A and C (9). Thus, the objective of this work was to analyze the added value, if any, of imipenem-relebactam to our antipseudomonal arsenal. For this purpose, we determined the activity of imipenem-relebactam against a recent well-characterized large collection of *P. aeruginosa* clinical isolates (1,445 isolates from 51 different Spanish hospitals), for which the MICs of ceftolozane-tazobactam, ceftazidime-avibactam, and other antipseudomonals had been determined (10). Moreover, resistance mechanisms and whole-genome sequences (WGSs) from 185 XDR isolates from that collection

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TABLE 1 Antimicrobial susceptibility data for 1,445 *P. aeruginosa* clinical isolates tested

Antibiotic ^a	MIC ($\mu\text{g/ml}$)		Susceptibility (%) ^b	
	MIC ₅₀	MIC ₉₀	S	R
TIC	32	256	18.8	81.2
PIP-TZ	8	128	73.5	26.5
CAZ	4	32	79.7	20.3
FEP	4	16	79.4	20.6
TOL-TZ	1	2	94.6	5.4
CAZ-AVI	2	8	94.2	5.8
ATM	4	32		14.8
IMI	2	16	78.3	13.5
IMI-REL	0.5	1	97.3	2.6
MER	1	16	70.1	14.1
CIP	0.25	>16	61.6	38.4
TOB	0.5	32	83.7	16.3
AMI	4	8	91.6	4.0
COL	1	2	94.6	5.4

^aTIC, ticarcillin; PIP-TZ, piperacillin-tazobactam; CAZ, ceftazidime; FEP, cefepime; TOL-TZ, ceftolozane-tazobactam; CAZ-AVI, ceftazidime-avibactam; ATM, aztreonam; IMI, imipenem; IMI-REL, imipenem-relebactam; MER, meropenem; CIP, ciprofloxacin; TOB, tobramycin; AMI, amikacin; COL, colistin. Except for IMI and IMI-REL, susceptibility data were obtained from a previous work (10).

^bS, susceptible; R, resistant; according to EUCAST 2018.

were available for analysis (10). Additionally, imipenem-relebactam was tested against a large collection of isogenic mutants showing the most relevant mutation-driven β -lactam resistance mechanisms, including those affecting ceftolozane-tazobactam and ceftazidime-avibactam.

Activity of imipenem-relebactam against *P. aeruginosa* clinical isolates. The collection of 1,445 isolates studied included up to 30 consecutive health care-associated nonduplicated (1/patient) *P. aeruginosa* clinical isolates collected during October 2017 from each of the 51 participating hospitals, covering all 17 Spanish regions (10). The distribution of sample types was as follows: respiratory, 32.8%; urine, 23.7%; soft tissue and osteoarticular, 23.1%; blood culture, 5.7%; and others, 14.9%. MICs of ticarcillin, piperacillin-tazobactam, ceftazidime, cefepime, ceftolozane plus tazobactam 4 $\mu\text{g/ml}$, ceftazidime plus avibactam 4 $\mu\text{g/ml}$, aztreonam, imipenem, meropenem, ciprofloxacin, tobramycin, amikacin, and colistin had been determined by broth microdilution according to EUCAST guidelines and v 8.1 clinical breakpoints (www.eucast.org). In this work, we used broth microdilution to determine the MICs of imipenem and imipenem plus relebactam 4 $\mu\text{g/ml}$ for the 1,445 isolates. For imipenem-relebactam, the imipenem breakpoints were used. The presence of carbapenemases and ESBLs were assessed in the previous study, and one representative XDR isolate from each clone and hospital ($n = 185$) were further analyzed through WGS, including multilocus sequence typing (MLST) and resistome analysis (10).

Antimicrobial susceptibility data for the 1,445 isolates tested are shown in Table 1. Imipenem-relebactam showed MIC_{50/90}s of 0.5/1 $\mu\text{g/ml}$, 4- and 16-fold lower, respectively, than those of imipenem alone. Moreover, imipenem-relebactam showed the highest susceptibility rate (97.3%), followed by colistin (94.6%), ceftolozane-tazobactam (94.6%), and ceftazidime-avibactam (94.2%). The susceptibility rate for imipenem-relebactam of imipenem-resistant strains was 80.5%, whereas that for XDR strains (17.4% of all isolates) was 86.4%. The distributions of imipenem and imipenem-relebactam MICs for the complete collection of clinical isolates and for those showing an XDR phenotype are shown in Fig. 1. Modal MIC values for imipenem and imipenem-relebactam were 2 and 0.25 $\mu\text{g/ml}$ for the overall collection of isolates and 16 and 1 $\mu\text{g/ml}$ for XDR isolates.

Up to 37 of the 1,445 isolates showed imipenem-relebactam resistance (MIC, >8 $\mu\text{g/ml}$), and all produced an acquired carbapenemase, including 26 VIM (3 VIM-1, 11 VIM-2, and 12 VIM-20), 4 IMP (1 IMP-1, 2 IMP-8, and 1 IMP-33), and 7 GES-5. Two additional isolates showed intermediate susceptibility (MIC, 8 $\mu\text{g/ml}$), with 1 of them

FIG 1 Distribution of imipenem (IMI) and imipenem-relebactam (IMI/REL) MICs for complete collection of clinical isolates and those showing an XDR phenotype.

producing a VIM-2; the second intermediate isolate was negative for acquired β -lactamases but showed, in addition to MexXY overexpression (due to *mexZ* inactivation) and AmpC overexpression (due to PBP4 mutation), unique mutations in the essential penicillin binding proteins PBP 2 (A269V) and PBP 3 (N242S). The potential involvement of these mutations in imipenem-relebactam resistance will be assessed in further studies.

On the other hand, none of the imipenem-relebactam-susceptible isolates showed acquired carbapenemases. However, imipenem-relebactam-susceptible isolates included ESBL-producing strains (4 PER-1, 2 GES-1, and 1 OXA-15) that were resistant to ceftolozane-tazobactam and ceftazidime-avibactam. Likewise, the 4 strains documented to show AmpC mutations (T96I, F147L, G183D, or E247G) involved in ceftolozane-tazobactam and ceftazidime-avibactam resistance were imipenem-relebactam susceptible. Moreover, imipenem-relebactam susceptibility was documented in 39 of 78 (50%) ceftolozane-tazobactam-resistant strains and 51 of 84 (60.7%) ceftazidime-avibactam-resistant strains that were noncarbapenemase producers.

Stability of imipenem-relebactam against *P. aeruginosa* β -lactam resistance mechanisms. Imipenem and imipenem-relebactam MICs were determined in duplicate in a well-characterized panel of 16 PAO1 isogenic mutants showing combinations of the most relevant resistance mutations, including AmpC hyperproduction, OprD inactivation, and efflux pump overexpression (11). MICs were also determined in 8 charac-

TABLE 2 MICs of imipenem and imipenem-relebactam against PAO1 isogenic mutants, showing combinations of most relevant β -lactam resistance mutations

Strain	Resistance mechanism	MIC (mg/liter)	
		IMI	IMI-REL
PAO1	Wild type	0.5	0.125
PAO1 $\Delta dacB$	AmpC $\uparrow$	1	0.125
PAO $\Delta dacC$	AmpC $\uparrow$	0.5	0.125
PAO $\Delta dacB \Delta dacC$	AmpC $\uparrow$	1	0.125
PAO $\Delta dacB \Delta dacC \Delta pbbG$	AmpC $\uparrow$	0.125	0.125
PAO $\Delta ampD$	AmpC $\uparrow$	0.5	0.125
PAO $\Delta ampD \Delta ampDh2 \Delta ampDh3$	AmpC $\uparrow$	0.5	0.0625
PAO $\Delta dacB \Delta ampD$	AmpC $\uparrow$	1	0.125
PAOD1 (<i>oprD</i> ⁻)	OprD ⁻	8	0.25
PAO $\Delta mexR$	MexAB-OprM $\uparrow$	0.5	0.125
PAO $\Delta nfxB$	MexCD-OprJ $\uparrow$	0.5	0.125
PAO $\Delta mexZ$	MexXY $\uparrow$	1	0.125
PAO $\Delta ampD \Delta mexR$	AmpC $\uparrow$ + MexAB-OprM $\uparrow$	0.5	0.125
PAOD1 $\Delta ampD$	OprD ⁻ + AmpC $\uparrow$	8	0.5
PAOD1 $\Delta dacB$	OprD ⁻ + AmpC $\uparrow$	8	0.5
PAOD $\Delta mexR$	OprD ⁻ + MexAB-OprM $\uparrow$	8	0.5
PAOD $\Delta mexZ$	OprD ⁻ + MeXY $\uparrow$	16	1

terized (including WGS) pairs of isogenic XDR clinical isolates that had developed resistance to ceftolozane-tazobactam during treatment (7, 12). Finally, imipenem-relebactam was tested in a panel of derivatives from a PAO1 OprD⁻ AmpC mutant expressing five different AmpC variants, conferring ceftolozane-tazobactam and ceftazidime-avibactam resistance, previously cloned from these clinical isolates.

Imipenem-relebactam MICs remained ≤ 1 μ g/ml (range, 0.125 to 1 μ g/ml), and were 4- to 16-fold lower than those of imipenem in the 16 isogenic PAO1 mutants tested, including those showing AmpC hyperproduction (such as AmpD and PBP4 mutants), OprD inactivation, and/or efflux pump (MexAB-OprM, MexXY, and MexCD-OprJ) overexpression (Table 2). Moreover, imipenem-relebactam was highly active (MIC, 0.5 to 2 μ g/ml) against the eight pairs of XDR clinical strains that had developed resistance to ceftolozane-tazobactam and ceftazidime-avibactam due to the selection of mutations in OXA-10 (OXA-14; $n = 2$) or AmpC (T96I, F147L, E247K, E247G, or Δ G299-E247; $n = 6$) (Table 3). Finally, the imipenem-relebactam MIC (0.5 μ g/ml) of the OprD⁻ AmpC PAO1 mutant was not increased by the expression of any of the AmpC variants, confirming the stability against these mutations (Table 4).

TABLE 3 Activity of imipenem-relebactam against isogenic XDR clinical isolates that had developed resistance to ceftolozane-tazobactam

Patient	Isolate ID	Isolation date (day/mo/yr)	Sample type	MLST	MIC (μ g/ml)				β -Lactam resistance genotype
					TOL-TZ (S $\leq$ 4)	CAZ-AVI (S $\leq$ 8)	IMP (S $\leq$ 4)	IMI-REL (S $\leq$ 4)	
1	96-C4	18/7/2016	Bronchial aspirate	ST179	4	2	1	0.5	OXA-10
	96-H6	31/7/2016	Bronchial aspirate	ST179	32	32	8	2	OXA-14, OprD W417X
2	101-E5	7/11/2016	Bronchial aspirate	ST175	2	4	8	1	OprD Q142X, AmpR G154R
	103-H8	14/12/2016	Bronchial aspirate	ST175	>32	>32	1	1	OprD Q142X, AmpR G154R, AmpC E247K
3	104-B7	16/12/2016	Urine	ST175	2	4	8	1	OprD Q142X, AmpR G154R
	104-I9	7/1/2016	Blood	ST175	>32	32	1	0.5	OprD Q142X, AmpR G154R, AmpC T96I
4	106-G2	3/2/2017	Bronchial aspirate	ST175	2	4	16	0.5	OprD Q142X, AmpR G154R
	107-H1	20/2/2017	Bronchial aspirate	ST175	>32	16	1	0.5	OprD Q142X, AmpR G154R, AmpC T96I
5	109-E9	27/3/2017	Sputum	ST175	2	4	16	1	OprD Q142X, AmpR G154R
	110-G8	7/4/2017	Bronchial aspirate	ST175	32	32	1	0.5	OprD Q142X, AmpR G154R, AmpC DelG229-E247
6	109-F7	28/3/2017	Bronchial aspirate	ST235	4	8	16	1	OXA-2, OprD 1bplns
	110-G6	19/4/2017	Bronchial aspirate	ST235	32	16	2	0.5	OXA-2, OprD 1bplns, AmpC F147L
7	114-G4	20/7/2017	Blood	ST179	8	8	8	1	OXA-10, OprD W6X
	117-C6	3/9/2017	Tracheal aspirate	ST179	32	>32	8	2	OXA-14, <i>mexR</i> 4bplns, <i>mexZ</i> 15bpDel, OprD W6X, <i>ftsI</i> (PBP3) F533L
8	116-A9	21/8/2017	Bronchial aspirate	ST175	2	4	8	1	OprD Q142X, AmpR G154R
	117-E3	4/9/2017	Tracheal aspirate	ST175	>32	8	2	0.5	OprD Q142X, AmpR G154R, AmpC E247G

TABLE 4 Activity of imipenem-relebactam against PAO1 OprD[−] ΔAmpC mutants expressing AmpC variants conferring ceftolozane-tazobactam and ceftazidime-avibactam resistance

PAO1 variant	MIC (μg/ml)			
	TOL-TZ (S ≤ 4)	CAZ-AVI (S ≤ 8)	IMP (S ≤ 4)	IMI-REL (S ≤ 4)
PAO1	≤0.5	1	0.5	0.125
PAOD1 (OprD [−])	≤0.5	1	8	0.25
PAOD1 ΔAmpC	≤0.5	1	0.5	0.5
PAOD1 ΔAmpC + pUCP AmpC _{WT} (PDC-1)	1	2	4	0.5
PAOD1 ΔAmpC + pUCP AmpC _{T96I} (PDC-222)	32/4	8	0.5	0.5
PAOD1 ΔAmpC + pUCP AmpC _{E247K} (PDC-221)	32	16	0.5	0.5
PAOD1 ΔAmpC + pUCP AmpC _{ΔG299-E247K} (PDC-223)	>32	32	0.5	0.5
PAOD1 ΔAmpC + pUCP AmpC _{F147L} (PDC-316)	16	8	0.5	0.5
PAOD1 ΔAmpC + pUCP AmpC _{E247G} (PDC-80)	32	4	1	0.5

Concluding remarks. According to WHO, MDR *P. aeruginosa*, along with *Acinetobacter baumannii* and *Enterobacteriales*, is in the list of top pathogens for which the development of novel antibiotic treatments is critical (13). One of the few novel agents on the horizon for the treatment of *P. aeruginosa* infections is the combination of the carbapenem imipenem with the novel β-lactamase inhibitor relebactam (9, 14). Up to 97% of *P. aeruginosa* strains isolated in 2017 from Spanish hospitals were found to be susceptible to imipenem-relebactam, slightly higher values than those recently documented in surveillance studies from Europe and the United States (93% to 94%) (15, 16). Note that there was a nearly perfect correlation between imipenem-relebactam resistance and carbapenemase production, which is both bad and good news. The bad news is that carbapenemase production, and particularly metallo-β-lactamase production, is still an unmet challenge for antimicrobial drug development. The good news is that imipenem-relebactam resistance appears to be extremely low among carbapenemase-nonproducing *P. aeruginosa* isolates. Only one carbapenemase-nonproducing *P. aeruginosa* isolate had an MIC of 8 μg/ml (intermediate). This isolate showed, in addition to *mexXY* and *ampC* overexpression, unique mutations in PBP 2 (A269V) and PBP 3 (N242S); the role of these mutations in reduced imipenem-relebactam susceptibility needs to be experimentally addressed.

Results from the analysis of the isogenic strains were complementary to those of the analysis of the clinical strains and indicated that imipenem-relebactam is highly stable against most relevant *P. aeruginosa* β-lactam mutation-driven resistance mechanisms, including combinations of OprD inactivation and AmpC and efflux overexpression. Possibly a more interesting finding of the study concerns the activity of imipenem-relebactam against *P. aeruginosa* isolates that had developed ceftolozane-tazobactam and ceftazidime-avibactam resistance during therapy. Indeed, resistance development mostly included specific mutations in the Ω-loop of AmpC, which conferred resistance to ceftolozane-tazobactam and ceftazidime-avibactam and increased susceptibility to imipenem. Interestingly, imipenem-relebactam remained highly active against both the parent XDR strains (imipenem resistant, ceftolozane-tazobactam susceptible) and the resistant mutants (ceftolozane-tazobactam and ceftazidime-avibactam resistant, imipenem susceptible). Thus, imipenem-relebactam is an attractive option for rescue therapy in cases of ceftolozane-tazobactam and ceftazidime-avibactam resistance development. Likewise, from the perspective of antimicrobial stewardship programs, the usefulness of imipenem-relebactam as a strategy to diversify the selective pressure exerted by ceftolozane-tazobactam and ceftazidime-avibactam should be explored.

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