Activity of imipenem/relebactam against *Pseudomonas aeruginosa* with ESBLs and carbapenemases

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Running head: Imipenem/relebactam versus ESBL *P. aeruginosa*

Introduction. ESBL- and carbapenemase- producing *Pseudomonas aeruginosa* are prevalent in e.g. the Middle East, Eastern Europe and Latin America, though rarer elsewhere. Because *P. aeruginosa* readily mutates to carbapenem resistance via loss of OprD, those with ESBLs often are as broadly resistant as those with carbapenemases. We hypothesised (a) that relebactam might overcome class A carbapenemases directly in *P. aeruginosa* and (b) that relebactam's inhibition of AmpC, which gives a generalised potentiation of imipenem against the species, might restore imipenem susceptibility in OprD-deficient ESBL producers. **Methods.** MICs were determined by CLSI agar dilution for *P. aeruginosa* isolates with ESBLs, principally VEB types, and for those with GES-5, KPC and other carbapenemases. **Results.** Relebactam potentiated imipenem by around 4- to 8- fold for most *P. aeruginosa* isolates with VEB and other ESBLs; however, MICs typically were reduced only to 4-16 mg/L, thus remaining largely above EUCAST's susceptible range and only partly overlapping CLSI's intermediate range. Strong (*c.* 64-fold) potentiation was seen for isolates with KPC carbapenemases, but only 2-fold synergy for those with GES-5. Predictably, potentiation was not seen for isolates with class B or D carbapenemase activity. **Conclusions.** Relebactam did potentiate imipenem against ESBL-producing *P. aeruginosa*, which mostly are imipenem resistant via OprD loss, but this potentiation generally was insufficient to reduce imipenem MICs to the clinical range. Imipenem resistance owing to KPC carbapenemases was reversed by relebactam in *P. aeruginosa*, just as for Enterobacterales.

Introduction

Most resistance to β-lactams in *Pseudomonas aeruginosa* in Western Europe and North America arises via mutations that up-regulate efflux mediated by MexAB-OprM and other pumps, derepress AmpC-β-lactamase expression, or cause inactivation of the 'carbapenem-specific' porin, OprD.[1] Only small minorities of isolates owe resistance to acquired ESBLs or carbapenemases, though isolates with these enzymes are prevalent in Eastern Europe,[2] Russia,[3] the Middle East,[4,5] and Latin America.[6,7] Unlike for Enterobacterales, where CTX-M, SHV and TEM types predominate, most ESBLs in *P. aeruginosa* are VEB and PER types.[5,8] Among carbapenemases, MBLs (class B), dominate in most countries,[9] but KPC enzymes (class A) are prevalent in Colombia and Caribbean,[6,10] with reports of OXA-48-like (class D) enzymes from India and Turkey.[11,12] GES enzymes (Class A) occur too: some are ESBLs but others are carbapenemases.[13] Because *P. aeruginosa* readily mutates resistant to carbapenems (via inactivation of its OprD porin)[1] ESBL producers frequently are as broadly resistant as carbapenemase producers. This is in contrast to ESBL-producing Enterobacterales, which mostly remain susceptible to carbapenems.

New antibiotics offer potential to overcome some of these challenges. Ceftolozane/tazobactam remains active against most *P. aeruginosa* with mutational resistance to β-lactams regardless of mechanism, and ceftazidime/avibactam is active against those with derepressed AmpC or inactivated OprD. However, both these cephalosporin/inhibitor combinations lack reliable activity against strains with ESBLs or carbapenemases.[14,15] We hypothesised that imipenem/relebactam might have greater potential in these cases. First, and most simply, we posited that relebactam should inhibit class A carbapenemases, potentially restoring the activity of imipenem. Secondly, we reasoned that there was a potential for activity against carbapenem-resistant ESBL producers, as these owe their carbapenem resistance to a combination of loss of OprD loss and AmpC activity meaning that, if relebactam inhibited the AmpC enzyme, the imipenem MIC might be lowered into the clinical range. In context it is vital to understand that the chromosomal AmpC

β-lactamase of *P. aeruginosa,* ubiquitous in the species, ordinarily provides a small degree of protection against imipenem, such that imipenem/relebactam MICs for wild-type *P. aeruginosa* are *c.* 4- fold lower than those of imipenem alone. This differential extends to 8-fold for isolates lacking OprD.[16,17] Potentiation of imipenem likewise was seen with other AmpC inhibitors that were not developed,[18,19] and mutational loss of AmpC restores imipenem susceptibility in OprD-deficient strains.[16] Thus, somewhat counterintuitively, an AmpC inhibitor may overcome what is ordinarily considered to be an 'impermeability-mediated' resistance.

Panels of *P. aeruginosa* isolates with ESBLs and carbapenemases were used to test these hypotheses; these were selected from submissions to PHE and reflected the distribution of ESBLs and carbapenemases seen over the past decade, except that we under-represented MBL producers since there was no reasonable expectation that imipenem/relebactam would be active against them.

Materials and methods

Isolates

Isolates were non-replicate submissions of *P. aeruginosa* with ESBLs or non-metallo carbapenemases referred to the PHE Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit between 2012 and 2019. β-Lactamase genes were identified by PCR [for primers used, which were refined over time, see Supplementary Table S1] or by WGS using Illumina methodology, which was undertaken when an isolate had a phenotype suggesting an ESBL or carbapenemase, but routine PCR failed to identify a corresponding gene. When WGS was performed, reads from each genome were assembled de novo and screened for antimicrobial resistance genes using Blast software and PHE's in-house Genefinder bioinformatics pipeline.[14] ESBL producers variously expressed VEB (n=97), PER (n=9), GES ESBLs (n=7, comprising to one each with GES-1 and GES-7, three GES-9 and two with GES-26), SHV (n=2, one each with SHV-5 and -12) and CTX-M-15 (n=1) enzymes. Carbapenemase producers variously expressed GES-5 (n=37), OXA-48-like (n=4,

one with known OXA-181), MBLs (5 with NDM enzymes, 5 with VIM types and one with both) and KPC (n=2, unsequenced) carbapenemases. Variable Number Tandem Repeat (VNTR) typing or WGS data were available for most isolates with VEB and GES enzymes, with STs thereby deduced.[20] Among the 97 isolates with VEB ESBLs 75 (from at least 27 different hospitals) belonged to ST357 or its single locus variants (SLVs) and eight to ST654 or its SLVs; the remainder were sporadic types (n=6) or not typed (n=8). Among 37 isolates with GES-5 enzymes, 25 (from 7 hospitals) belonged to ST235, two to the 'Nottingham strain'[21] whilst 10 were not typed.

MIC determinations

MICs were determined by CLSI agar dilution,[22] with all β-lactamase inhibitors used at 4 mg/L. Imipenem, relebactam and ceftolozane were from Merck, Sharp and Dohme (Hoddesdon, UK) Imipenem, meropenem, ceftazidime, tobramycin, amikacin, gentamicin, colistin and tazobactam were from Merck KGaA (Gillingham, UK) and avibactam from Pfizer.

Results

MIC distributions of imipenem/relebactam and its comparators are shown in Table 1; fold reductions in imipenem MIC achieved with relebactam are shown in Table 2. Susceptibility data for β-lactams are reviewed against current EUCAST and CLSI breakpoints in Table 3.

The great majority of ESBL producers were resistant to imipenem and meropenem on all criteria, including >90% of those expressing VEB enzymes and >66% of those with other ESBLs. Since ESBLs do not attack carbapenems, such resistance must reflect other factors, putatively inactivation of OprD, as the near universal mechanism of non-carbapenemase-mediated carbapenem resistance in *P. aeruginosa*[1] All the carbapenemase-producing *P. aeruginosa* isolates also were resistant to both carbapenems on all criteria.

Relebactam at 4 mg/L achieved 4- to 8- fold reductions in imipenem MICs for most isolates expressing VEB ESBLs, and 2- to 4- fold reductions for those with other ESBLs (Table

2). Nevertheless, MICs of the combination mostly remained around 4-16 mg/L, thus falling above EUCAST's susceptible range (S ≤2 mg/L; R >2 mg/L) and with only a small overlap into the FDA's intermediate range (S ≤2 mg/L;R >4 mg/L values that it is understood will be adopted by CLSI). MICs of the combination for *P. aeruginosa* with VEB enzymes belonging to the widespread ST357 lineage tended to exceed those for non-ST357 isolates (fig 1), with 5 of the latter inhibited at <0.5+4 mg/L. Potentiation of imipenem against carbapenemase producers was generally two-fold or less, including for isolates with GES-5 enzyme. Striking exceptions were the two isolates with KPC enzymes, where imipenem MICs were reduced from 128 mg/L to 1-2 mg/L. There was no potentiation of imipenem for isolates with class B (VIM or NDM) and class D (OXA-48-like) enzymes; these β-lactamases are not inhibited by relebactam.[23]

Ceftazidime was tested as a comparator, alone and combined with clavulanate and avibactam. Almost all ESBL producers were highly resistant to the unprotected cephalosporin, with MICs ≥128 mg/L, as were those MBLs or KPC enzymes. Isolates with GES-5 enzymes were less resistant, with an MIC mode straddling 16-32 mg/L; three of four isolates expressing OXA-48-like enzyme were fully susceptible, with MICs of 2 mg/L. The remaining isolate with OXA-48-like activity was ceftazidime-resistant and likely had a further mechanism. Clavulanate and avibactam reduced the MICs of ceftazidime for most ESBL-producing *P. aeruginosa*, though rarely sufficiently to bring MICs into clinical ranges. Thus, the modal MIC of ceftazidime for isolates with VEB ESBLs fell from >128 mg/L with no inhibitor to 16 mg/L with clavulanate and to 64 mg/L with avibactam. Avibactam did reduce the modal MIC for isolates with GES-5 enzymes from 16-32 mg/L to 4 mg/L, with MICs for 35/37 isolates reduced to the EUCAST and CLSI breakpoint of ≤8 mg/L. Avibactam also reduced MICs of ceftazidime for the two isolates with KPC carbapenemase activity from >128 mg/L to 8-16 mg/L.

The two commercial tazobactam combinations were also tested. In keeping with previous experience, ceftolozane/tazobactam was found to lack activity at accepted breakpoints against most ESBL and carbapenemase producers,[14] exceptions being: (i) the

three isolates with OXA-48 carbapenemase also susceptible to ceftazidime, and (ii) 20/37 isolates with GES-5 carbapenemase, which scored as susceptible or (mostly) intermediate on CLSI criteria, though only 3/37 were susceptible on EUCAST criteria. Piperacillin/tazobactam lacked activity against almost all the ESBL- and carbapenemase-producing *P. aeruginosa* isolates at 16 mg/L, corresponding to EUCAST's I/R breakpoint and CLSI's S/I breakpoint; it was active against 58.7% of VEB isolates at CLSI's I/R breakpoint of 64 mg/L, though MICs of this level are associated with poor outcomes.[24]

The final comparators were aminoglycosides and colistin. Resistance to tobramycin and gentamicin was seen for the great majority of isolates in all groups whereas susceptibility to amikacin was frequent (64.9% on both CLSI and EUCAST criteria) only among those with GES-5 carbapenemases. Colistin susceptibility appeared general in all groups, with only a few isolates found resistant; a caveat is that agar dilution was used for MIC testing and this may occasionally miss resistance found by broth microdilution.

Discussion

P. aeruginosa isolates producing ESBLs - principally VEB enzymes - and carbapenemases present major resistance challenges. Although uncommon in *P. aeruginosa* in Western Europe and North America MBLs were present in 32% of carbapenem-resistant *P. aeruginosa* from Dubai[25] and 60% in Russia,[3] where a successful ST235 strain with VIM-2 enzyme activity has disseminated nationally. VEB ESBLs and various GES enzymes have repeatedly been shown to be widespread in *P. aeruginosa* in the Middle East,[5] also Mexico,[26] with dissemination of VEB types also reported in Bulgaria [2] and Thailand.[27]

AMRHAI receives a steady flow of carbapenemase- and ESBL-producing *P. aeruginosa*, substantially from London private hospitals with international clienteles. Referrals with VEB enzymes were stable at 4-10 p.a. up to 2016, then 54 in 2017, 24 in 2018 and 66 in 2019, with the 2017 numbers augmented by an outbreak that saw eight representatives referred from one UK NHS site. Most belong to the ST357 lineage,[20] indicating an international clonal type, and this was strongly represented (75/97 isolates) in the present panel.

As illustrated here, and with previous collections,[14,20] *P. aeruginosa* strains with ESBLs typically are as broadly resistant as those with carbapenemases, almost certainly owing to the ease with which carbapenem resistance develops via loss of OprD. Many ESBL- and carbapenemase-encoding plasmids simultaneously determine aminoglycoside-modifying enzymes or 16S rRNA methyltransferases, expanding the spectrum of resistance. We hypothesised (i) that the general potentiation of imipenem by relebactam for *P. aeruginosa*, contingent on inhibition of AmpC, might overcome OprD-loss-mediated imipenem resistance in ESBL-producing isolates, and (ii) that relebactam might directly overcome imipenem resistance mediated by class A carbapenemases.

Both hypotheses proved partially correct. For isolates with VEB enzymes, relebactam achieved 4- or 8- fold MIC reductions for imipenem (Table 2), with 77/97 producers inhibited at 8+4 mg/L, corresponding to EUCAST's high imipenem breakpoint from 2013-18. This positive finding is, however, negated by two developments since 2018, when this project was initiated. First, EUCAST's imipenem breakpoint for *P. aeruginosa* was lowered from ≤4/>8 mg/L (pre-2018) to ≤2/>4 mg/L (2019) and then to ≤0.001/>4 mg/L (2020).[28] This latter change aimed to move the wild-type population of *P. aeruginosa* to 'I', underscoring EUCAST's view that the imipenem should ordinarily be used at high dose (1g q6h) for infections caused by *P. aeruginosa*. Secondly, imipenem/relebactam was licensed, by the EMA as well as the FDA, at a regimen of 0.5 + 0.25g q6h, (i.e. half the licensed maximum dose for imipenem) and EUCAST assigned a ≤2/>2 mg/L breakpoint. The FDA has proposed a ≤2/>4 mg/L breakpoint for imipenem/relebactam, and this now been adopted also by CLSI, which has an identical value for imipenem itself despite the dosage maximum difference.

Thus, although relebactam potentiated imipenem against isolates with VEB and other ESBLs, MICs largely remained beyond the clinical range: only 10/97 (10.3%) isolates with VEB enzymes were susceptible on EUCAST's criteria whilst 26/97 (26.8%) were susceptible or intermediate on the FDA/CLSI criteria. Few isolates with other ESBLs were included, these being extremely rare among AMRHAI submissions, but there was no suggestion of better performance than against those with VEB types. Nor would differences based on ESBL type

be expected, given that any general potentiation of imipenem against *P. aeruginosa* is contingent on inhibition of AmpC, not upon relebactam's interactions with particular ESBLs.

Among carbapenemase producers, we predominantly tested isolates with GES-5 enzymes, as the most prevalent class A carbapenemase in UK *P. aeruginosa*. MICs of imipenem alone were 64-128 mg/L and were reduced only by one doubling dilution by relebactam. Since GES-5 is a class A enzyme, this lack of potentiation is surprising, and contrasts with the behaviour of avibactam, a structurally related diazabicyclooctane, which potentiated ceftazidime against these isolates. On the other hand, relebactam strongly potentiated imipenem against the two isolates with KPC enzymes, with MICs reduced to the clinical range. Whilst these carbapenemases are extremely rare among *P. aeruginosa* isolates in Europe and the UK – these two were the sole examples available to AMRHAI– they are prevalent in Colombia and on several Caribbean islands.[6,7,10]

Comparator results were in keeping with published data, except that we found ceftolozane/tazobactam widely inactive against isolates with GES-5 activity, with MICs mostly 8-16 mg/L, whereas previously we found values of 2-4 mg/L, falling within EUCAST's susceptible range.[14] This difference may reflect use of CLSI methodology with Mueller-Hinton agar whereas BSAC methodology with IsoSensitest agar was used previously.

Given that imipenem/relebactam only narrowly failed to achieve activity against many isolates with VEB and other ESBLs, with MICs that would have counted as intermediate under EUCAST's 2018 imipenem breakpoints, it may be worth exploring whether pharmacodynamic exposure could usefully be increased with altered regimens. Although imipenem's seizurogenic potential[29] is some constraint, the drug is licensed at regimens up to 1g q6h when used alone – i.e. double the exposure of imipenem/ relebactam, implying that some 'headroom 'may exist. Likewise, although imipenem's chemical instability complicates the use of extended infusions this should not be a barrier to increasing dosage frequency. In short, there may be routes to increase exposure and, given the paucity of alternatives against ESBL-producing *P. aeruginosa*, these deserve further exploration.

Funding. This work was funded by MSD as an Investigator-Initiated Project

Acknowledgements. We are grateful to Dr Katie Hopkins of PHE for helpful comments and discussion.

Transparency declarations:

DML: Advisory Boards or ad-hoc consultancy Accelerate, Allecra, Antabio, Centauri, Entasis, GlaxoSmithKline, Meiji, Melinta, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, T.A.Z., Tetraphase, VenatoRx, Wockhardt, Zambon, Paid lectures – Astellas, bioMérieux, Beckman Coulter, Cardiome, Cepheid, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer, Pfizer, T.A.Z, amounting to <10% of portfolio value. **All others:** nothing to declare but PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Accelerate, Achaogen, Allecra, Amplex, AstraZeneca, AusDiagnostics, Basilea, Becton Dickinson, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist, Department of Health, Enigma Diagnostics, ECDC, Food Standards Agency, GenePOC™, GlaxoSmithKline, Helperby Therapeutics, Henry Stewart Talks, IHMA, Innovate UK, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme, Meiji Seika, Mobidiag, Momentum Biosciences, Neem Biotech, NIHR, Nordic Pharma, Norgine Pharmaceuticals, Rempex Pharmaceuticals, Roche, Rokitan, Smith & Nephew, Shionogi, VenatoRx Pharmaceuticals, Wockhardt Ltd and the WHO.

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345 **Table 1**. MIC distributions of *P. aeruginosa* isolates with ESBLs and carbapenemases
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	No isolates with indicated MIC (mg/L)										
Imipenem	≤0.25	0.5	1	2	4	8	16	32	64	128	>128
ESBLs											
VEB (97)				3	3	3	9	32	47		
PER (9)				2		2	2	3			
GES-ESBL (7)				1	2	1	3				
SHV (2)			1					1			
CTX-M (1)					1						
Carbapenemases											
GES-5 (37)									15	22	
KPC (2)										2	
MBL (11)									1	1	9
OXA-48-like (4)									2	1	1
Susceptible (4)		3	1								
Meropenem											
ESBLs											
VEB (97)		4	2			2	4	47	24	14	
PER (9)						2	1	3		3	
GES ESBL (7)					1	3	3				
SHV (2)						1		1			
CTX-M (1)						1					
Carbapenemases											
GES-5 (37)										11	26
KPC (2)									1	1	
MBL (11)									1	2	8
OXA-48-like (4)									2	1	1
Susceptible (4)	3			1							
Imipenem/relebactam											
ESBLs											
VEB (97)		5	3	2	16	51	20				
PER (9)		1		1	3	3	1				
GES-ESBL (7)		1	1	2	2	1					
SHV (2)		1				1					
CTX-M (1)			1								
Carbapenemases											
GES-5 (37)								7	29	1	
KPC (2)			1	1							
MBL (11)									1	1	9
OXA-48-like (4)									2	2	
Susceptible (4)	3	1									

Ceftazidime											
ESBLs											
VEB (97)									1		96
PER (9)											9
GES ESBLs (7)									2		5
SHV (2)											2
CTX-M (1)										1	
Carbapenemases											
GES-5 (37)						3	14	17	2	1	1
KPC (2)											2
MBL (11)									1	3	7
OXA-48-like (4)				3							1
Susceptible (4)			3	1							
Ceftazidime/clavulanate											
ESBLs											
VEB (97)					5	20	36	21	8	3	4
PER (9)						1	2	1	2		3
GES-ESBL (7)								1	1	1	4
SHV (2)								1		1	
CTX-M (1)								1			
Carbapenemases											
GES-5 (37)						3	23	8	3		
KPC (2)										1	1
MBL (11)									3	2	6
OXA-48-like (4)				2	1						1
Susceptible (4)			2	2							
Ceftazidime/avibactam											
ESBLs											
VEB (97)					1	1	4	18	44	20	9
PER (9)					1		1	3	1		3
GES ESBLs (7)					2	1		3		1	
SHV (2)							1		1		
CTX-M (1)								1			
Carbapenemases											
GES-5 (37)				3	26	6	2				
KPC (2)						1	1				
MBL (11)									3	2	6
OXA-48-like (4)				3				1			
Susceptible (4)			3	1							
Ceftolozane/tazobactam											

ESBLs											
VEB (97)					1		2	1	1	8	84
PER (9)								1		2	5
GES ESBLs (7)							2		2	1	2
SHV (2)								1	1		
CTX-M (1)								1			
Carbapenemases											
GES-5 (37)					3	17	15	2			
KPC (2)										2	
MBL (11)											11
OXA-48-like (4)			3								1
Susceptible (4)	1	3									
Piperacillin/tazobactam											
ESBLs											
VEB (97)							7	15	35	20	20
PER (9)								2	2	1	4
GES ESBLs (7)							2	1	3		1
SHV (2)											2
CTX-M (1)											1
Carbapenemases											
GES-5 (37)								3	26	7	1
KPC (2)											2
MBL (11)									1	1	9
OXA-48-like (4)								2	1	1	
Susceptible (4)		1	1		2						
Tobramycin											
ESBL											
VEB (97)			1	2	1	5	10		12	32	34
PER (9)			2	1		1		1			4
GES ESBL (7)									1		6
SHV (2)								2			
CTX-M (1)											1
Carbapenemases											
GES-5 (37)						1	17	6	1		12
KPC (2)			1							1	
MBL (11)								2			9
OXA-48-like (4)										3	1
Susceptible (4)	1	1		2							
Gentamicin											
ESBLs											
VEB (97)				3	7	6	9	19	4	3	46
PER (9)					1	2			2		4

GES ESBL (7)									1		6
SHV (2)									1	1	
CTX-M (1)											1
Carbapenemases											
GES-5 (37)					1		17	4		1	14
KPC (2)					1						1
MBL (11)					1					1	9
OXA-48-like (4)								2	1		1
Susceptible (4)			1	1	1	1					
Amikacin											
ESBLs											
VEB (97)						10	6	3	19	34	25
PER (9)					1	1	1	1		1	4
GES ESBLs (7)							1		4	1	1
SHV (2)							1			1	
CTX-M (1)										1	
Carbapenemases											
GES-5 (37)			1		14	7	2		2	7	4
KPC (2)						1				1	
MBL (11)								1	1		9
OXA-48-like (4)									1	2	1
Susceptible (4)				1	1	2					
Colistin											
ESBLs											
VEB (97)			2	87	4	1	3				
PER (9)			1	7	1						
GES ESBLs (7)				7							
SHV (2)		1		1							
CTX-M (1)			1								
Carbapenemases											
GES-5 (37)				34	2		1				
KPC (2)				2							
MBL (11)			2	9							
OXA-48-like (4)				4							
Susceptible (4)				4							

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Table 2. Potentiation of imipenem by relebactam for *P. aeruginosa* isolates with different modes of resistance

Mechanism	No. of cases with indicated fold reduction in MIC							
	128	**64**	**32**	**16**	**8**	**4**	**2**	**No change**
ESBLs								
VEB (97)				1	41	52	3	
PER (9)					1	3	3	2
GES-ESBLs (7)						5	2	
SHV (2)						1	1	
CTX-M						1		
Carbapenemases								
GES-5 (37)							28	9
KPC (2)	1	1						
MBL(11)								11
OXA-48-like (4)						1		3
Susceptible (4)							4	

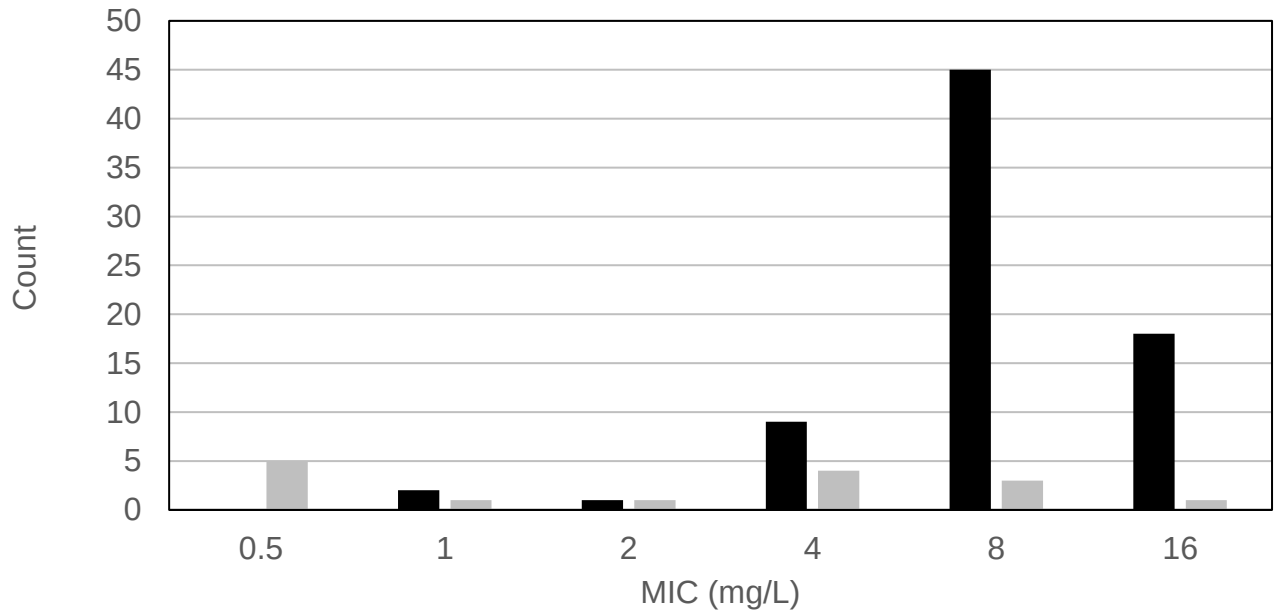
Table 3. Proportions of isolates susceptible to newer b-lactamase inhibitor combinations and their parent compounds

	Imipenem			Imipenem/relebactam			Ceftazidime			Ceftazidime/avibactam			Ceftolozane/tazobactam		
	EUC	CLSI-S	CLDI-I	EUC	CLSI-S[a]	CLDI-I[a]	EUC	CLSI-S	CLDI-I	EUC	CLSI-S	CLDI-I	EUC	CLSI-S	CLDI-I
Definition, mg/L	≤4	≤2	4	≤2	≤2	4	≤8	≤8	16	≤8	≤8	NA	≤4	≤4	8
ESBLs															
VEB (97)	6.2%	3.1%	3.1%	10.3%	10.3%	16.5%	0%	0%	0%	2.1%	2.1%	NA	1.0%	1.0%	0%
PER (9)	2/9	2/9	0/9	2/9	2/9	3/9	0/9	0/9	0/9	1/9	1/9	NA	0/9	0/9	0/9
GES-ESBL (7)	3/9	1/9	3/9	4/7	4/7	6/7	0/7	0/7	0/7	3/7	3/7	NA	0/7	0/7	0/7
SHV (2)	1/2	1/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2	1/2	1/2	NA	0/2	0/2	0/2
CTX-M (1)	1/1	0/1	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/2	0/2	NA	0/1	0/1	0/1
Carbapenemases															
GES-5 (37)	0%	0%	0%	0%	0%	0%	8.1%	8.1%	45.9%	94.6%	94.6%	NA	8.1%	8.1%	45.9%
KPC (2)	0/2	0/2	0/2	2/2	2/2	0/2	0/2	0/2	0/2	1/2	1/2	NA	0/2	0/2	0/2
MBL (11)	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	NA	0%	0%	0%
OXA-48-like (4)	0/4	0/4	0/4	0/4	0/4	0/4	3/4	3/4	0/4	3/4	3/4	NA	3/4	3/4	0/4
Susceptible (4)	4/4	4/4	0/4	4/4	4/4	0/4	4/4	4/4	0.4	4/4	4/4	NA	4/4	4/4	0/4

Abbreviations: - EUC, EUCAST susceptible (S) and, if applicable, high-dose susceptible (I) pooled, on the basis that those agents with an I category should ordinarily be used at highest licensed doses against *P. aeruginosa*; CLSI-S and CLSI, CLSI susceptible and intermediate, respectively. NA, not applicable

[a] FDA breakpoint, now adopted also by CLSI, although publication of this remains pending

Figure 1. MICs of imipenem/relebactam for *P. aeruginosa* with VEB ESBLs, according to whether these belonged or not to the widespread ST357 and its single locus variants



Black: ST357 and single locus variants (n=75); grey, non-ST357 isolates (n=17); 8 untyped isolates are excluded.

369 **Table S1**. PCR primers used to seek ESBL and carbapenemase genes

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2012-2015

PER-F	GGGACARTCSKATGAATGTCA	966-bp
PER-R	GGYSGCTTAGATAGTGCTGAT	966-bp
VEB-F	CGACTTCCATTTCCCGATGC	615-bp
VEB-R	GGACTCTGCAACAAATACGC	615-bp
GES-F	ATGCGCTTCATTCACGCAC	860-bp
GES-R	CTATTTGTCCGTGCTCAGG	860-bp
KPC-F	TGTCACTGTATCGCCGTC	c. 1-kb
KPC-R	CTCAGTGCTCTACAGAAAACC	c. 1-kb
OXA-48-F	TTGGTGGCATCGATTATCGG	743-bp
OXA-48-R	GAGCACTTCTTTTGTGATGGC	743-bp
NDM-F	GGGCAGTCGCTTCCAACGGT	475bp
NDM-R	GTAGTGCTCAGTGTCGGCAT	475bp
IMP-F	GGAATAGAGTGGCTTAATTCTC	188-bp
IMP-R	CCAAACCACTACGTTATCT	188-bp
VIM-F	GATGGTGTTTGGTCGCAT A	390-bp
VIM-R	CGAATGCGCAGCACCAG	390-bp
SPM-F	AAAATCTGGGTACGCAAACG	271-bp
SPM-R	ACATTATCCGCTGGAACAGG	271-bp
GIM-F	TCGACACACCTTGGTCTGAA	477-bp
GIM-R	AACTTCCAACTTTGCCATGC	477-bp
SIM-F	TACAAGGGATTCGGCATCG	570-bp
SIM-R	TAATGGCCTGTTCCCATGTG	570-bp

2015-2017

PER-F	GGGACARTCSKATGAATGTCA	966-bp
PER-R	GGYSGCTTAGATAGTGCTGAT	966-bp
VEB-F	CGACTTCCATTTCCCGATGC	615-bp
VEB-R	GGACTCTGCAACAAATACGC	615-bp
GES-F	ATGCGCTTCATTCACGCAC	860-bp
GES-R	CTATTTGTCCGTGCTCAGG	860-bp
KPC-F	GCAGCGGCAGCAGTTTGTTGATT	RT-PCR
KPC-R	GTAGACGGCCAACACAATAGGTGC	RT-PCR
KPC_probe	CAGTCGGAGACAAAACCGGAACCTGC	RT-PCR
NDM-F	CCAGCAAATGGAAACTGGCGAC	RT-PCR
NDM-R	ATCCAGTTGAGGATCTGGGCG	RT-PCR
NDM_probe	ACCGAATGTCTGGCAGCACACTTC	RT-PCR
OXA-48-F	GATTATGGTAATGAGGACATTTCGGGC	RT-PCR
OXA-48-R	CATATCCATATTCATCGCAAAAAACCACAC	RT-PCR
OXA-48_ probe	CCATTGGCTTCGGTCAGCATGGCTTGTTT	RT-PCR

VIM-F	TTGCTTTTGATTGATACAGCGTGGGG	RT-PCR
VIM-R	GTACGTTGCCACCCCAGCC	RT-PCR
VIM_II_probe	TCTCGCGGAGATTGAAAAGCAAATTGGACTTCC	CY5
IMP-F	GGAATAGAGTGGCTTAATTCTC	188-bp
IMP-R	CCAAACCACTACGTTATCT	188-bp
VIM-F	GATGGTGTTTGGTCGCAT A	390-bp
VIM-R	CGAATGCGCAGCACCAG	390-bp
SPM-F	AAAATCTGGGTACGCAAACG	271-bp
SPM-R	ACATTATCCGCTGGAACAGG	271-bp
GIM-F	TCGACACACCTTGGTCTGAA	477-bp
GIM-R	AACTTCCAACTTTGCCATGC	477-bp
SIM-F	TACAAGGGATTCGGCATCG	570-bp
SIM-R	TAATGGCCTGTTCCCATGTG	570-bp
DIM MP F	CCGAGATACAGAAACGCTCG	391-bp
DIM MP R	AGCTGATCGGGACCATTGAT	391-bp

2018-2019

PER-F	GGGACARTCSKATGAATGTCA	966-bp
PER-R	GGYSGCTTAGATAGTGCTGAT	966-bp
VEB-F	CGACTTCCATTTCCCGATGC	615-bp
VEB-R	GGACTCTGCAACAAATACGC	615-bp
GES-F	ATGCGCTTCATTCACGCAC	860-bp
GES-R	CTATTTGTCCGTGCTCAGG	860-bp
KPC-F	AusDiagnostics assay – commercial no primers available	
KPC-R	AusDiagnostics assay – commercial no primers available	
OXA-48-F	AusDiagnostics assay – commercial no primers available	
OXA-48-R	AusDiagnostics assay – commercial no primers available	
NDM-F	AusDiagnostics assay – commercial no primers available	
NDM-R	AusDiagnostics assay – commercial no primers available	
IMP-F	AusDiagnostics assay – commercial no primers available	
IMP-R	AusDiagnostics assay – commercial no primers available	
SPM-F	AusDiagnostics assay – commercial no primers available	
SPM-R	AusDiagnostics assay – commercial no primers available	
GIM-F	AusDiagnostics assay – commercial no primers available	
GIM-R	AusDiagnostics assay – commercial no primers available	
SIM-F	AusDiagnostics assay – commercial no primers available	
SIM-R	AusDiagnostics assay – commercial no primers available	
IMP-F	GGAATAGAGTGGCTTAATTCTC	188-bp
IMP-R	CCAAACCACTACGTTATCT	188-bp
SPM-F	AAAATCTGGGTACGCAAACG	271-bp
SPM-R	ACATTATCCGCTGGAACAGG	271-bp
GIM-F	TCGACACACCTTGGTCTGAA	477-bp
GIM-R	AACTTCCAACTTTGCCATGC	477-bp
SIM-F	TACAAGGGATTCGGCATCG	570-bp

SIM-R	TAATGGCCTGTTCCCATGTG	570-bp
DIM MP F	CCGAGATACAGAAACGCTCG	391-bp
DIM MP R	AGCTGATCGGGACCATTGAT	391-bp

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