Potentiation of imipenem by relebactam for *Pseudomonas aeruginosa* from bacteraemia and respiratory infections

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Short running title: Imipenem/relebactam *versus P. aeruginosa*

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Synopsis

Background. Imipenem resistance in *Pseudomonas aeruginosa* most often entails loss of the 'carbapenem-specific' porin OprD; more rarely it reflects acquired carbapenemases. Loss of OprD only confers resistance to imipenem if AmpC β-lactamase is expressed, and we investigated whether this mechanism was overcome by relebactam, a developmental diazabicyclooctane β-lactamase inhibitor.

Methods. Consecutive *P. aeruginosa* isolates causing bacteraemia or hospital-onset lower respiratory tract infections were collected between 2014 and 2016 under the aegis of the BSAC Resistance Surveillance Programme. Imipenem MICs were determined centrally by BSAC agar dilution, with relebactam at a fixed concentration (4 mg/L).

Results. For most imipenem-susceptible *P. aeruginosa* (726/759, 95.6%) the MICs of imipenem alone were 0.5-2 mg/L and were decreased 3- to 4-fold by addition of relebactam, as based on geometric means or modes. For most imipenem-non-susceptible *P. aeruginosa* (82/92, 89%), imipenem MICs were 8-16 mg/L, and were reduced to 1-2 mg/L by relebactam. These patterns applied regardless of whether the isolates were susceptible to penicillins and cephalosporins or had phenotypes suggesting derepressed AmpC or upregulated efflux. Imipenem MICs for five *P. aeruginosa* with MBLs remained high (≥16 mg/L) regardless of relebactam.

Conclusions. Potentiation of imipenem by relebactam was almost universal, according with the view that endogenous pseudomonal AmpC ordinarily protects against this carbapenem to a small degree. Imipenem MICs were reduced to the current breakpoint, or lower, except for MBL-producers. Potentiation was not compromised by derepression of AmpC or upregulation of efflux.

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Introduction

Pseudomonas aeruginosa is the third-most frequent Gram-negative agent of bacteraemia, with 8.1 reports/100000 population for England, Wales and Northern Ireland,[1] and with this rate increasing by around 16% since 2009.[1] *P. aeruginosa* is also among the most prevalent agents of hospital-acquired and ventilator-associated pneumonia, accounting for up to a quarter of cases.[2]

As a species, *P. aeruginosa* is less susceptible than Enterobacteriaceae to most antibiotics[3] reflecting inherent impermeability and efflux-based mechanisms. Moreover, it can readily mutate to become resistant to those β-lactams, fluoroquinolones and aminoglycosides that ordinarily are active. In the case of imipenem, mutational resistance almost always arises via functional loss of OprD, a 'carbapenem-specific' porin.[4] This 'impermeability-mediated resistance' requires continued inducible or derepressed expression of the endogenous AmpC β-lactamase,[5] which has a feeble activity against imipenem that becomes significantly protective once entry of the drug is restricted by porin loss. The involvement of AmpC is demonstrated by imipenem MICs being reduced if the AmpC enzyme is lost by mutation[5] or is inactivated with penems (e.g. BRL42715)[6] or bridged monobactams (e.g. Ro 48-1256).[7]

Relebactam (MK-7655, Merck) is a diazabicyclooctane β-lactamase inhibitor,[8] being developed in combination with imipenem. The combination's spectrum includes Enterobacteriaceae with KPC carbapenemases and combinations of AmpC β-lactamase and impermeability as well as *P. aeruginosa*.[9, 10] Using isolates submitted to the BSAC Resistance Surveillance Programme,[11] we examined its activity against *P. aeruginosa* isolates in relation to their phenotypic resistance to imipenem and other agents.

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Materials and methods

Consecutive *P. aeruginosa* isolates causing bacteraemia and hospital-onset lower respiratory tract infection (HO-LRTI) were submitted to the BSAC Resistance Surveillance Programme from sentinel laboratories in the UK and Ireland (see http://www.bsacsurv.org). Bloodstream isolates were collected from Jan 2015 to Dec 2015 and HO-LRTI isolates from Oct 2014 to Sept 2016. Thirty-eight laboratories participated in the first 12-month period, collecting seven isolates per infection type, whereas 24 laboratories participated in the latter 12-month period, collecting 10 isolates per infection type. HO-LRTIs were defined as arising >48 h after hospital admission. Respiratory isolates from cystic fibrosis patients were excluded, as were repeat isolates from the same patient within 14 days.

Isolates were re-identified centrally by MALDI-TOF and BSAC agar dilution was used to determine MICs,[12] with relebactam combined with imipenem at a fixed concentration of 4 mg/L. Breakpoints followed EUCAST criteria (v9.0, 2019)[13] and it was assumed that imipenem/relebactam breakpoints would broadly follow those of imipenem (S ≤4 mg/L, R >4 mg/L for *P. aeruginosa*).

Isolates were categorised according to their susceptibility to piperacillin/tazobactam 16 mg/L, ceftazidime 8 mg/L and carbenicillin 128 mg/L; these values correspond to EUCAST breakpoints for the first two agents,[13] whilst the value for carbenicillin corresponds to the BSAC legacy breakpoint;[14] more importantly all three values correspond to ECOFFS.[15] Isolates susceptible to all three agents on these criteria were categorised as ‘wild-type’; those non-susceptible to either or both of ceftazidime and piperacillin but susceptible to carbencillin were categorised as likely AmpC derepressed and those with carbenicillin resistance and proportionate rises in ceftazidime and piperacillin/tazobactam MICs (irrespective of

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whether or not these conferred non-susceptibility) were categorised as having upregulated efflux. The principles of this interpretive reading were outlined previously,[16] although the present analysis was constrained by a limited range of antibiotics. These wild type, AmpC-derepressed and upregulated efflux groups, also a final cluster of unclassifiable isolates, were then categorised as imipenem-susceptible (MIC ≤4 mg/L) and or imipenem non-susceptible (MIC >8 mg/L). Lastly, isolates with broad resistance to all β-lactams and with ceftazidime MIC ≥128 mg/L or imipenem ≥64 mg/L were subjected to PCR for carbapenemase (*bla*$_{IMP}$, *bla*$_{NDM}$ and *bla*$_{VIM}$)[17] and ESBL (*bla*$_{VEB}$[18] and *bla*$_{PER}$[19]) genes.

Results

In total, 851 *P. aeruginosa* isolates were tested; 433 from bacteraemia and 418 from HO-LRTI. MIC distributions of imipenem alone and with relebactam for this entire collection are depicted in Figure 1, which shows that addition of relebactam reduced imipenem MICs for almost all isolates. To better understand this behaviour, we divided the collection based on their resistance phenotypes to agents other than imipenem itself, using the interpretive reading principles outlined previously.[16]

Most (706/851, 83%) isolates were susceptible to all of piperacillin/tazobactam, ceftazidime and carbenicillin, both in relation to breakpoints and to ECOFFs: 669 of these 706 wild-type isolates were susceptible also to imipenem, whereas 37 were non-susceptible, implying OprD loss in isolation (Table 1). Twenty-two isolates had profiles suggesting de-repression of AmpC, with non-susceptibility to either or (mostly) both of piperacillin/tazobactam and ceftazidime but retained susceptibility to carbenicillin 128 mg/L: 13 of these were susceptible to imipenem 4 mg/L and nine were non-susceptible, implying OprD loss (Table 1). Next, 102 isolates had non-susceptibility to carbenicillin 128 mg/L, without evidence

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of ESBL or MBL activity, and with broadly proportionate rises in ceftazidime and piperacillin/tazobactam MICs, implying upregulated efflux: 73 of these remained susceptible to imipenem 4 mg/L whereas 29 were non-susceptible (Table 1), implying that they also lacked functional OprD. Two highly carbenicillin-resistant isolates were identified, by PCR, as having ESBLs (1 PER, 1 VEB) and five had MBLs (4 VIM and 1 NDM); all of these seven were non-susceptible to imipenem (Table 1). Finally, after defining these groups, we were left with 14 isolates, all carbenicillin resistant, that had anomalous profiles, mostly with disproportionately high resistance to ceftazidime relative to carbenicillin or with high-level resistance to carbenicillin and piperacillin/tazobactam, but not ceftazidime (Table 1); 4 were imipenem susceptible and 10 were resistant. These isolates may have mixed, or unsuspected resistance mechanisms.

Modal MICs of all agents for all groups are shown in Table 1, along with ranges and geometric means for imipenem and imipenem/relebactam only. Addition of relebactam 4 mg/L typically reduced the MIC of imipenem by around 3-fold (based on geometric means) or 4-fold (based on modes) for isolates in the imipenem-susceptible groups, from around 1 mg/L to 0.25 mg/L. MIC reduction for imipenem-resistant groups, excepting the carbapenemase producers, were larger, typically from 16 mg/L to around 1 -2 mg/L, based on modes, and 7- to 14-fold, based on geometric means.

No synergy was seen between imipenem and relebactam for the five MBL producers, where imipenem MICs remained almost equally high when relebactam was present (geometric mean 64 mg/L) or absent (geometric mean 55.7 mg/L).

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Discussion

Potentiation of imipenem by relebactam was almost universal for *P. aeruginosa*, supporting the view that endogenous AmpC, whether inducible or de-repressed, protects against the action of imipenem for this species to some degree.[5] Addition of relebactam to imipenem typically resulted in a 3- to 4-fold reduction of imipenem MIC for imipenem-susceptible *P. aeruginosa,* regardless of their other resistance, and an 8- to 16-fold reduction for imipenem-non-susceptible *P. aeruginosa* lacking MBLs. Relebactam 4 mg/L brought imipenem MICs to the EUCAST breakpoint (4 mg/L), or below, for 87/92 (95%) imipenem-non-susceptible *P. aeruginosa*, the exceptions being the five MBL producers. Continued potentiation against carbenicillin-resistant isolates, inferred to have up-regulated efflux, is notable insofar as it implies that this trait does not impede periplasmic accumulation of sufficient relebactam to inhibit AmpC activity. Also interesting, albeit based on just two isolates, is the activity of imipenem/relebactam, at 2-4 mg/L, against the isolates with ESBLs. ESBL-producing *P. aeruginosa*, mostly with VEB enzymes (as in one of the present two) are increasingly seen in the UK.[20] Many are imports but PHE is aware of at least one UK-based outbreak. Most of these ESBL-positive *P. aeruginosa* are also (as here) resistant to carbapenems, presumably owing to OprD loss, and are highly resistant to all other β-lactams, including ceftolozane/tazobactam[16] and (usually) ceftazidime/avibactam.[20] They present treatment challenges as great as for carbapenemase producers. The continued resistance of MBL-producing *P. aeruginosa* to imipenem/relebactam is predictable; MBLs are not inhibited by relebactam or other diazabicyclooctane β-lactamase inhibitors.[10]

While the work presented here represents *P. aeruginosa* isolated from the UK and Ireland only, our findings corroborate with those of a European study: *P.*

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aeruginosa isolates (n=1705) collected from intra-abdominal, urinary tract, and lower respiratory tract infections from patients from 17 European countries, including three laboratories (n=121 isolates) from the UK. This surveillance found that, whereas 72% (1228/1705) of isolates were susceptible to unprotected imipenem, this proportion rose to 94.7% (1615/1705) for imipenem/relebactam, with 81% (387/477) of the imipenem-non-susceptible isolates were rendered susceptible.[21] Similar surveillance of *P. aeruginosa* isolates (n=845) from patients located across 21 US found that 70.3% (594/845) were susceptible to imipenem whereas 94.2% (796/845) were susceptible to imipenem/relebactam. Of the imipenem-non-susceptible isolates, 80.5% (202/251) were rendered susceptible by the addition of relebactam.[22]

A limitation of our studies is that the isolates were only categorised in terms of phenotype and not (except for the few ESBL and carbapenemase producers) genotype. Diversity may exist among the up-regulated efflux group in terms of (i) the particular pumps affected,[23] (ii) whether pump expression or specificity is altered, and (iii) whether up-regulation reflects changes to direct or pleiotropic regulators. Likewise, there may be sequence variation among AmpC enzymes, affecting substrate specificity,[24] and the genetic lesions causing inactivation of OprD are extremely variable.[25] Such variations does not however negate the fact that the great majority of *P. aeruginosa* isolates (except those from cystic fibrosis, which are more complex) can readily be grouped by phenotype.[16, 26-28] Isolates with anomalous, difficult-to-assign profiles comprised only 14 of the 851 studied here, forming the 'mixed/uncertain' group in Table 1.

We conclude that the addition of relebactam gave a generalised potentiation of the carbapenem against *P. aeruginosa* and provides a potentially valuable option against those with 'impermeability-type' resistance to imipenem, regardless of their

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AmpC and efflux status. Its potential against ESBL-producing *P. aeruginosa* deserves further exploration, given their growing importance.

Acknowledgements

The authors thank those who have provided sponsorship for the BSAC Resistance Surveillance Programme over the years; staff in the sentinel laboratories submitting isolates, and at the Central Testing Laboratory, PHE, London. The BSAC Standing Committee on Resistance Surveillance, and Sponsor Representatives at the time of manuscript preparation: Alasdair MacGowan (Chair), Derek Brown (formerly EUCAST), Alan Johnson (PHE), Neil Woodford (PHE), Nowell Redder (Cardiome), Adela Alvárez Buylla (MSD), Mike Allen (MSD), Susanne Paukner (Nabriva), James Campling (Pfizer), Jan Chesham (Pfizer), and Chris Longshaw (Shionogi).

Funding

The BSAC Resistance Surveillance Programme was funded by Basilea, Bayer, MSD, Nabriva and Pfizer during the period of surveillance (October 2014 to December 2016) when the isolates reviewed here were collected.

Transparency Declaration

DML: Advisory Boards or ad-hoc consultancy: Accelerate, Achaogen, Adenium, Allecra, AstraZeneca, Auspherix, Basilea, BioVersys, Centauri, Discuva, Meiji, Merck, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx, Wockhardt, Zealand, Paid lectures: AstraZeneca, Beckman-Coulter, Cardiome, Merck and Nordic. Relevant shareholdings in Dechra, GSK, Merck, Perkin Elmer, Pfizer amounting to <10% of portfolio. Contract research: Achaogen, Allecra, AstraZeneca, Melinta, Meiji, Merck, Roche, Wockhardt. Other authors have no conflicts of interest.

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Table 1. Summary of MIC parameters according to resistance phenotype.

Phenotype	n	Modal MIC (mg/L)					MIC range (mg/L)		Geometric mean MIC (mg/L)	
Antimicrobial Agent		CAR	TZP	CAZ	IMP	IMP/REL	IMP	IMP/REL	IMP	IMP/REL
CAR-S, TZP-S, CAZ-S (wild-type)										
Imipenem S	669	64	4	2	1	0.25	0.06-4	0.06-2	0.77	0.27
Imipenem N/S	37	64	4	2	16	1	8-32	0.5-4	11.9	0.93
CAR-S, NS to either or both of TZP/CAZ (derepressed for AmpC)										
Imipenem S	13	128	32	8	1	0.25	0.5-2	0.125-0.5	1.0	0.33
Imipenem N/S	9	128	32	16	16	1	8-32	0.5-2	13.7	0.93
CAR-NS, proportionately raised TZP/CAZ (upregulated efflux)										
Imipenem S	73	256	16	4	0.5-1	0.25	0.25-4	0.125-2	0.99	0.35
Imipenem N/S	29	>256	16-32	8	16	2	8-32	1-4	12.0	1.8
ESBL-positive	2	Too few					8-16	2-4	11.3	2.8
Carbapenemase-positive*	5	>256	32	16	32	32	32 ->256	16->256	64.0	55.7
Uncertain/mixed										
Imipenem S	4	Too few					0.5-4	0.25-4	2	0.59
Imipenem N/S	10	>256	256/>256	64	16	1-4	8-32	0.5-4	16	1.7

Key: CAR, carbenicillin; CAZ, ceftazidime; IMP, imipenem; IMP/REL, imipenem with 4 mg/L relebactam; TZP, piperacillin/tazobactam; S, susceptible; NS, non-susceptible. *All five carbapenemase-positive isolates were MBL-positive: four have *bla*$_{VIM}$ and one *bla*$_{NDM}$. Isolates with a MIC ≥128mg/L to carbenicillin and susceptibility to the other agents were categorised within the wild-type category and may include those with very minor up-regulations of efflux or acquired penicillinase.

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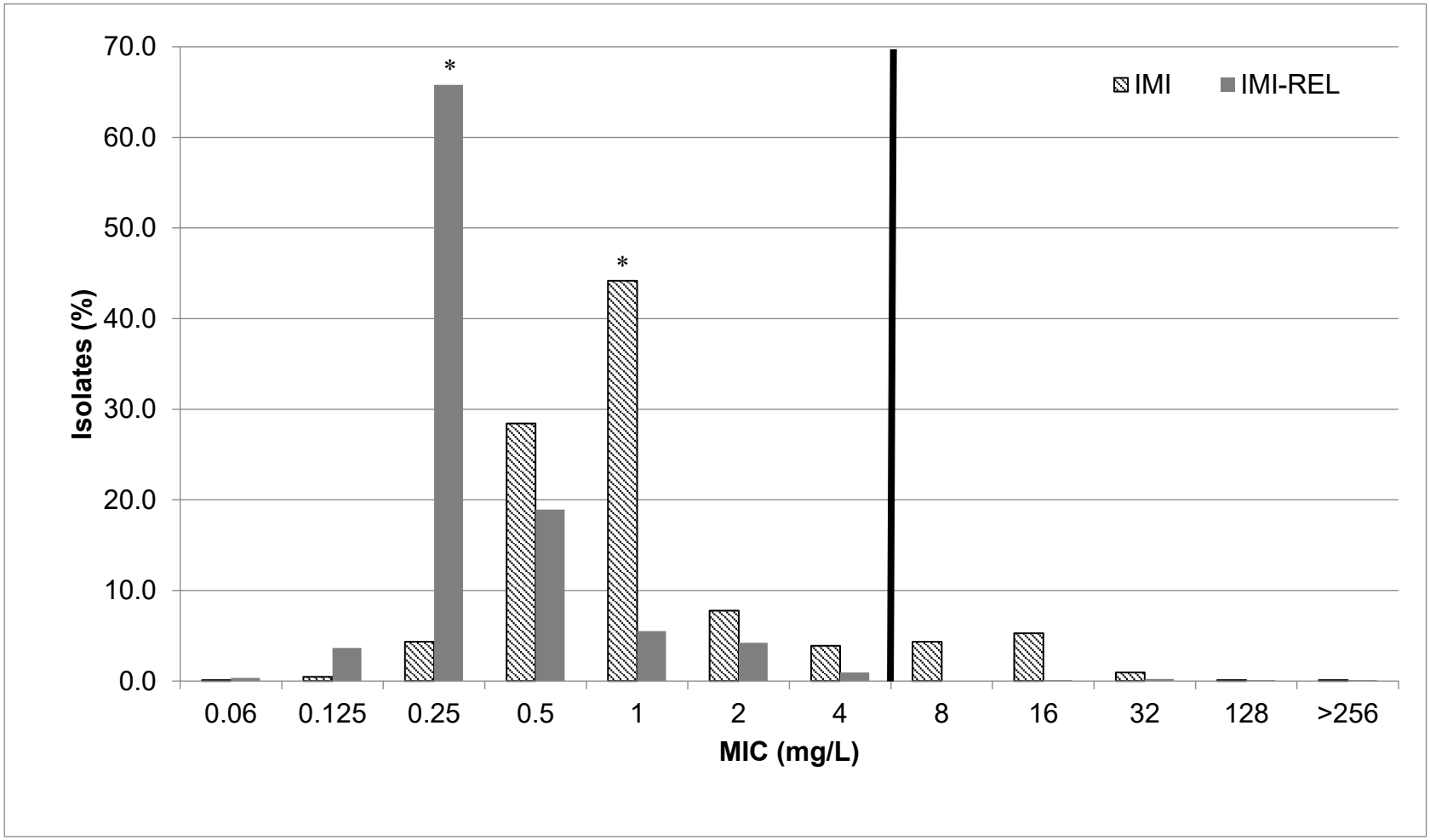
296 Figure 1. Susceptibility of all *P. aeruginosa* tested against imipenem and imipenem with relebactam (n=851).



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298 The solid black line indicates the EUCAST breakpoint for imipenem and *P. aeruginosa* (S ≤4 mg/L; R >4 mg/L).

299 * indicates the modal MIC distribution for each agent

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