WCK 4234, a novel diazabicyclooctane potentiating carbapenems against Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* with class A, C and D β-lactamases

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Running head: Diazabicyclooctane WCK 4234 *versus* class A, C and D β–lactamases

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Background. Several diazabicyclooctanes (DBOs) are under development as inhibitors of Class A and C β-lactamases. Inhibition of OXA (Class D) carbapenemases is variable, with those of *Acinetobacter* spp. remaining notably resistant. We describe a novel DBO, WCK 4234 (Wockhardt), with distinctive activity against OXA carbapenemases. **Methods**. MICs of imipenem and meropenem were determined by CLSI agar dilution with WCK 4234 added at 4 or 8 mg/L. Test organisms were clinical Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* with carbapenemases or carbapenem resistance via porin loss plus AmpC or ESBL activity. AmpC mutants were also tested. **Results**. WCK 4234, which lacked direct antibacterial activity, strongly potentiated imipenem and meropenem against Enterobacteriaceae with OXA-48/181, KPC enzymes, or with combinations of impermeability and AmpC or ESBL activity, with MICs reduced to ≤2 mg/L in almost all cases. Carbapenems likewise were potentiated against *P. aeruginosa* (n=2) with OXA-181 enzyme, with MICs reduced from 64-128 mg/L to 2-8 mg/L and against *A. baumannii* with OXA carbapenemases, particularly OXA-23 or hyperproduced OXA-51, with MICs reduced to ≤2 mg/L for 9/10 acinetobacters with OXA-23 enzyme. Carbapenems were not potentiated against Enterobacteriaceae or non-fermenters with metallo-β-lactamases. **Conclusion**. WCK 4234 distinctively overcame resistance mediated by OXA-type carbapenemases, including in *A. baumannii*. It behaved similarly to other DBOs against strains with KPC carbapenemases or combinations of impermeability and ESBL or AmpC activity.

Introduction

β-Lactam-β-lactamase inhibitor combinations offer one of the best prospects for overcoming the diversity of potent β–lactamases now challenging patient management. Interest centres on diazabicyclooctanes (DBOs) and boronates.[1]

The first DBO to be commercialised, avibactam, is licensed in combination with ceftazidime and under Phase II trials with aztreonam.[2] A second analogue, relebactam, is in phase III trials combined with imipenem-cilastatin.[1] Two further analogue – zidebactam (combined with cefepime)[3] and OP0595/RG6080[4]– are in earlier-stage clinical development. All these DBOs inhibit class A and C β-lactamases, including ESBLs, KPC and AmpC types, whereas none inhibits metallo- (Class B) β-lactamases (MBL), though these may be out-flanked by using aztreonam, which is stable to MBLs, as the partner β-lactam,[5] or where the DBO itself has antibacterial activity, as with RG6080 (which is active *versus* Enterobacteriaceae) or zidebactam (which is active against *P. aeruginosa* as well as Enterobacteriaceae).[3,4] Class D carbapenemases are variably overcome by DBOs. Avibactam inhibits OXA-48-like enzymes[6] and is combined with ceftazidime and aztreonam,[6,7] which anyway are stable; zidebactam does not inhibit these β-lactamases, but is combined with cefepime, which likewise is stable;[8] relebactam does not inhibit and does not potentiate imipenem against Enterobacteriaceae with OXA-48-like β-lactamases.[9] None of the published analogues protects against the OXA-23, -40, -51 and -58 type carbapenemases that cause most carbapenem resistance in *Acinetobacter* spp.

We describe here a novel DBO, WCK 4234 which is being developed for combination with meropenem as WCK 5999 (figure 1), with distinctive activity against Class D β–lactamases, including those of *Acinetobacter* spp., as well as class A and C types.

Materials and methods

Isolates

Clinical isolates (n=348) were referred by UK diagnostic laboratories to PHE for investigation of resistance or were collected in during resistance surveys. They were identified using API20E or API20NE strips (bioMerieux, Marcy l'Etoile, France) or by MALDI-ToF mass spectroscopy (Maldi-Biotyper, Bruker, Bremen, Germany), except for *A. baumannii* isolates, which were identified by PCR detection of *bla*$_{\text{OXA-51-like}}$.[10] Carbapenemase genes were identified by PCR or sequencing. The species split among Enterobacteriaceae with different resistance mechanisms is shown in Table 1. We also tested previously-described AmpC inducibility mutant series of Enterobacteriaceae and *P. aeruginosa* mutants with different combinations of AmpC inducibility and porin OprD expression. [11,12]

Antibiotics and susceptibility testing

MICs of imipenem (Wockhardt, Aurangabad, India), meropenem (Sequoia Research Products, Pangbourne, UK) and (as a control) ciprofloxacin combined with WCK 4234, (Wockhardt) at 0, 4 or 8 mg/L were determined by CLSI agar dilution[13] using Mueller-Hinton agar from Oxoid (Thermofisher, Basingstoke, UK). Ceftazidime (Sigma-Aldrich, Poole, UK), with and without avibactam 4 mg/L (Wockhardt), was tested in parallel as a comparator, as was ertapenem (Wockhardt). Tests were run once with the control strains advised by CLSI and further AMRHAI internal controls with ESBLs and carbapenemases.

Results

Enterobacteriaceae

Susceptibility phenotypes of the clinical isolates to established β–lactams were as expected. Thus, control Enterobacteriaceae without acquired resistance were susceptible to all three carbapenem analogues, as were isolates with only ESBL or AmpC activity, whereas carbapenem resistance was seen in isolates with KPC, MBL, OXA-48 and -181 enzymes and - particularly to ertapenem - in those with combinations of porin loss and AmpC or ESBL activity (Table 2). Carbapenem MICs were widely scattered for isolates with OXA-48-like (i.e. OXA-48 or 181) carbapenemases whereas isolates with KPC enzymes or MBLs more consistently had high-level resistance. Ceftazidime resistance was universal in all groups except for the controls, which were all susceptible, and those with OXA-48-

like enzymes, where ceftazidime MICs were bimodally distributed, probably reflecting the co-production or not of ESBLs. Ciprofloxacin resistance was widespread but variable within groups, as reflected by bimodal MIC distributions.

WCK 4234 lacked direct antibacterial activity at up to 128 mg/L and did not potentiate ciprofloxacin (not shown). We therefore consider that its interactions with carbapenems reflected β–lactamase inhibition, not PBP inhibition or permeabilisation. Summary MIC data for the clinical isolates, illustrating these interactions with imipenem and meropenem, are shown in Table 2, with full MIC distributions for isolates with KPC and OXA-48-like enzymes in Table 3.

WCK 4234, at 4 or 8 mg/L, caused four-fold or greater reductions in the geometric mean MICs of imipenem and meropenem for (i) Enterobacteriaceae isolates with combinations of high-level AmpC or ESBL activity and impermeability and (ii) Enterobacteriaceae with KPC, OXA-48 and OXA-181 carbapenemases. In all these cases the geometric mean MICs of imipenem and meropenem fell from the intermediate or (generally) resistant range to the susceptible (i.e. $\leq$1 mg/L) and, except for a few isolates with KPC carbapenemases, the top-most MICs remained $\leq$2+4 mg/L, corresponding to intermediate for the unprotected carbapenems on CLSI criteria (Table 3). This 'target' of four-fold reduction of geometric mean MIC was only narrowly missed for the carbapenem-susceptible AmpC isolates (Table 2), and four- to eight- fold potentiation of imipenem and meropenem was widely seen for the AmpC-inducible and -derepressed Enterobacteriaceae organisms in the isogenic mutant series, though not for the corresponding AmpC-deficient mutants (not shown). These data support the view that AmpC enzymes have a weak protective effect against carbapenems, but only confer resistance if permeability is reduced.[14]

Little potentiation of carbapenems, in terms of geometric mean MICs, was seen for control Enterobacteriaceae, lacking potent β–lactamases, or for carbapenem-susceptible ESBL or AmpC producers, all of which were anyway susceptible to imipenem and meropenem. Nevertheless, for reasons not understood, WCK 4234, 4 or 8 mg/L, engendered a *c.* two-fold lowering in geometric mean MICs for imipenem, but not meropenem. No potentiation of carbapenems by WCK 4234 was

seen for MBL-producing Enterobacteriaceae, which were consistently resistant to carbapenems and their WCK 4234 combinations.

Ceftazidime-avibactam achieved similarly broad activity to carbapenem-WCK 4234 combinations against the Enterobacteriaceae groups. At its CLSI and EUCAST breakpoint of 8+4 mg/, and asides from MBL producers, resistance was confined to 1/35 carbapenem-resistant AmpC strains and 1/31 with a KPC carbapenemase. Geometric mean MICs of ceftazidime-avibactam nevertheless were higher than those of the carbapenem-WCK 4234 combinations for virtually all test groups of Enterobacteriaceae.

P. aeruginosa

MICs of carbapenems for AmpC-hyperproducing, OprD-deficient *P. aeruginosa* isolates were reduced four-fold or more by WCK 4234 at 4 or 8 mg/L (Table 2) though the potentiated values often only fell into the intermediate range (4-8 mg/L, on CLSI criteria). Weaker dose-dependent potentiation, with two- or three-fold reductions in geometric mean MICs, stronger for imipenem than meropenem, was seen for all other *P. aeruginosa* groups except MBL producers, which remained highly resistant regardless of WCK 4234. These small generalised MIC reductions for imipenem, seen also with relebactam, accord with the view that inducible or derepressed AmpC ordinarily gives a small degree of protection against imipenem.[9,15]

With only two representatives it is impossible to be definitive about *P. aeruginosa* with OXA-48-like carbapenemases; nevertheless, the imipenem and meropenem MICs for these two organisms, both with OXA-181 enzyme, were reduced from $\geq$64 mg/L to 2-8 mg/L by WCK 4234 at 4 or 8 mg/L (Table 3). Potentiation was also seen for AmpC-inducible and –derepressed laboratory strains, including those deficient for porin OprD, but not for AmpC-deficient mutants (Table 4).

Avibactam reduced the geometric mean MIC of ceftazidime by four-fold or more for the AmpC-derepressed *P. aeruginosa* groups, with a two-fold effect for cystic fibrosis isolates and little

or no effect for other groups. The two OXA-181 isolates were anyway susceptible to ceftazidime at 2-4 mg/L, with these values unaltered by avibactam (Table 2).

A. baumannii

WCK 4234 achieved strong potentiation of both imipenem and meropenem against isolates with class D carbapenemases, with geometric mean MICs reduced 8- to 40- fold (Table 2). For isolates with OXA-23 – the commonest carbapenemase in *A. baumannii* in much of the world[16] – imipenem and meropenem MICs were reduced to the 2 mg/L CLSI breakpoint, or below, by WCK 4234 at 8 mg/L for 9 of 10 cases (Table 3), with this target also achieved by 4 mg/L WCK 4234 in the case of meropenem. Similarly-good potentiation was seen also for isolates with hyper-produced chromosomal OXA-51 enzymes, whereas WCK 4234-potentiated MICs mostly remained in the intermediate and resistant ranges for isolates with OXA-24/40 β-lactamases and, for meropenem only, for those with OXA-58 (Table 3). WCK 4234 had minimal effect on the carbapenem MICs for control *Acinetobacter* isolates and for those with AmpC and metallo carbapenemases. Avibactam did not potentiate ceftazidime against any group of *A. baumannii* isolates (Table 2).

Discussion

Carbapenemases present a growing clinical problem, which is compounded by their biochemical and structural diversity and by the geographic localisation of particular types.[17] These factors complicate the design of both hydrolysis-evading molecules and inhibitors. KPC enzymes (Class A) dominate among Enterobacteriaceae in the Americas, China, Israel, Italy and Greece[18] but OXA-48-like enzymes (Class D) are increasingly prominent in much of Europe as well as Africa and the Middle East.[19] NDM types (Class B) predominate in the Indian subcontinent,[20] but OXA-181, a sequence variant of OXA-48, is also frequent. Globalisation is eroding these geographic patterns and the UK, as an international crossroads, sees similar, albeit small, proportions of Enterobacteriaceae isolates – principally *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. with NDM, KPC and OXA-48-like

carbapenemases and a few with VIM and IMP MBLs (PHE, data on file). Also frequent are Enterobacteriaceae with low-level carbapenem resistance via combinations of AmpC or ESBL activity together with impermeability caused by porin loss,[21] though these seem rarely to be implicated in outbreaks. Different carbapenemases, principally the acquired OXA-23, -24/-40, and -58 types, dominate in *A. baumannii*, though some isolates instead have IS*Aba1*-mediated upregulation of chromosomal OXA-51 and a few have acquired metallo-enzymes.[22,23] *P. aeruginosa* differs from other species in that most carbapenem resistance does not involve acquired carbapenemases. Rather, it arises via mutational loss of porin OprD, a mechanism that requires continued production of AmpC β–lactamase. Efflux augments resistance to meropenem, not imipenem.[24] Minorities of carbapenem-resistant *P. aeruginosa* isolates have acquired carbapenemases, principally MBLs.

As outlined in the introduction, all the DBOs under development protect partner β–lactams against AmpC enzymes and class A β–lactamases, including KPC types. OXA-48-like enzymes are less reliably inhibited, but can be circumvented by combining the DBO with an OXA-48-stable β–lactam (e.g ceftazidime, cefepime or aztreonam)[5,7,8] or where the DBO has direct antibacterial activity, whilst none of the developmental combinations had activity, at accepted breakpoints of the unprotected carbapenems, against *A. baumannii* with OXA carbapenemases. WCK 4234 thus represents a further advance in DBO development, potentiating carbapenems against Enterobacteriaceae with OXA-48 and -181 enzymes, *P. aeruginosa* with OXA-181 and, crucially, *Acinetobacter* spp. with OXA-23, -24/-40, upregulated -51-like, and -58 enzymes.

Besides this unique activity against strains with Class D carbapenemases, WCK 4234 behaved like other DBOs,[1,25] in potentiating its partner drugs against Enterobacteriaceae (i) with combinations of AmpC or ESBL activity and impermeability, (ii) *P. aeruginosa* with AmpC and (iii) Enterobacteriaceae with KPC carbapenemases. In most cases the MICs of imipenem and meropenem were reduced below those of ceftazidime-avibactam, though this advantage may be offset by the high breakpoint (8+4 mg/L) assigned to ceftazidime-avibactam. Generalised weak potentiation of imipenem –less so meropenem– was seen for imipenem against *P. aeruginosa*,

probably reflecting the fact that chromosomal AmpC in this species, whether inducible or derepressed, gives some protection against this carbapenem.[15] Similar generalised potentiation of imipenem against *P. aeruginosa* is seen with relebactam[9] and AmpC-inhibiting penems and monobactams.[26,27]

In many cases – exceptions were a few Enterobacteriaceae with combinations of AmpC and impermeability or KPC enzymes, several *P. aeruginosa* groups and *A. baumannii* with OXA-24/40 or, for meropenem only, with OXA-58 enzymes – even the highest MICs of carbapenems-WCK 4234 combinations were within the current CLSI and EUCAST breakpoints for the unprotected molecules, suggesting significant potential utility. Except in the case of *A. baumannii* with OXA-58 enzymes, which are uncommon, there was little difference in performance between the imipenem and meropenem combinations, meaning that partner decisions will best be predicated on chemical stability, easy of formulation, dosing flexibility, scope for prolonged infusion and resistance to inactivation renal dehydropeptidase, rather than microbiological spectrum. These factors support meropenem as a partner.

Note.

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Table 1. Species distribution in relation to resistance mechanism among test isolates of Enterobacteriaceae

Mechanism	*Enterobacter* spp.	*E. coli*	*Klebsiella* spp.	Grand Total
AmpC	5	5	6	16
AmpC + Impermeability	13	11	11	35
ESBL	5	6	5	16
ESBL + Impermeability	11	11	10	32
KPC	11	9	11	31
MBL[a]	5	5	5	15
OXA-181		6	5	11
OXA-48	10	5	5	20
Susceptible control	5	5	5	15
Grand Total	65	63	63	191

[a] Six isolates with VIM enzymes, 9 with NDM types

Table 2: Summary MIC parameters (mg/L) for WCK 4234 combinations and comparators

	n	IMP	IMP + WCK 4	IMP + WCK 8	MEM	MEM + WCK 4	MEM + WCK 8	CIP	CAZ	CAZ + AVI4	ERT
Enterobacteriaceae											
Controls GM	15	0.26	0.14	0.15	0.02	0.02	0.02	0.03	0.43	0.16	0.04
Range		0.12-0.5	0.12-0.25	0.12-0.25	≤0.03	≤0.03	≤0.03	0.008-0.25	0.12-1	0.03-0.5	≤0.06
AmpC Carb S GM	16	0.57	0.19	0.18	0.07	0.02	0.02	BM	86.7	0.37	0.38
Range		0.25-2	0.12-0.5	0.12-0.25	0.06-0.12	≤0.06	≤0.06	0.016->128	64->128	0.12-1	0.12-1
AmpC + Imperm. GM	35	3.2	0.42	0.32	1.5	0.12	0.10	BM	107.2	0.83	15.7
Range		1-32	0.12-2	0.12-2	0.12-16	0.016-2	0.016-2	0.03->128	32->128	≤0.03-32	2-128
ESBL Carβ−S GM	16	0.28	0.19	0.16	0.05	0.03	0.02	BM	43.34	0.23	0.13
Range		0.12-1	0.12-0.5	0.12-0.5	0.03-0.5	≤0.016-0.5	≤0.016-0.06	0.03->128	2->128	≤0.03-1	≤0.016-2
ESBL + Imperm. GM	32	1.3	0.33	0.28	2.0	0.11	0.09	BM	74.5	0.69	12.1
Range		0.25-4	0.12-2	0.12-1	0.25-16	≤0.016-1	≤0.016-0.5	≤0.016->128	2->128	0.12-8	2-64
KPC GM	31	64.0	0.86	0.60	53.5	0.15	0.10	BM	83.7	0.95	97.9
Range		16->128	0.12-4(16) [a]	0.12-8 (32)	8->128	0.03-4(64)	≤0.016-2(64)	≤0.016->128	16->128	0.03-4 (>128)	64->128
OXA-48 GM	20	15.5	0.32	0.27	9.85	0.06	0.06	BM	BM	0.54	38.1
Range		4-128	0.12-1	0.12-0.5	2-128	≤0.016-0.25	≤0.016-0.5	≤0.016->128	1->128	0.25-1	4->128

	n	IMP	IMP + WCK 4	IMP + WCK 8	MEM	MEM + WCK 4	MEM + WCK 8	CIP	CAZ	CAZ + AVI4	ERT
OXA-181 GM	11	19.0	0.35	0.31	17.0	0.13	0.10	67.8	53.9	1.12	48.0
Range		4-128	0.12-2	≤0.03-2	1-128	≤0.016-0.5	≤0.016-0.5	1-128	0.5-128	0.12-4	4-128
MBL GM	15	67.0	67.0	67.0	70.2	58.4	58.4	BM	122.3	97.1	84.5
Range		16->128	16->128	16>128	8->128	8->128	8->128	≤0.016->128	64->128	16->128	16->128
P. aeruginosa											
Controls GM	20	1.0	0.36	0.26	0.21	0.10	0.08	0.26	1.6	1.2	-
Range		0.25-2	≤0.06-1	0.12-0.5	0.06-2	≤0.016-1	≤0.016-1	0.03-4	0.5-8	0.25-2	-
OprD loss GM	12	19.0	8.0	6.0	4.8	4.0	4.0	0.28	2.4	2.2	-
Range		8-32	4-16	2-8	4-8	2-8	2-8	0.12-2	2-4	1-4	-
AmpC Carβ–S GM	10	1.7	0.71	0.57	0.81	0.46	0.35	BM	55.7	5.3	-
Range		0.5-4	0.25-2	0.12-1	0.12-2	0.06-2	0.03-1	0.12-32	16->128	4-16	-
AmpC + OprD loss GM	10	26.0	6.1	3.7	13.9	3.0	1.7	1.9	84.5	4.0	-
Range		16-32	1-32	0.5-16	8-16 (128)	0.5-8	0.12-8	0.12-32	32-128	2-8	-
Efflux GM	15	2.5	1.6	1.3	3.0	2.6	2.2	0.41	4.8	4.2	-
Range		0.12-32	0.12-16	0.06-16	0.06-32	≤0.016-32	≤0.016-32	0.03-1	0.03-64	0.03-16	-

	n	IMP	IMP + WCK 4	IMP + WCK 8	MEM	MEM + WCK 4	MEM + WCK 8	CIP	CAZ	CAZ + AVI4	ERT
	16										
Cystic fibrosis GM		26.9	9.1	6.4	21.7	8.0	6.4	4.0	83.1	38.1	-
Range		2->128	0.12-128	≤0.06-128	0.25-128	0.12-128	0.03-128	1-32	8->128	4->128	-
OXA ESBLs GM[b]	6	3.2	1.3	1.0	2.2	1.6	1.6	2.2	BM	BM	-
Range		2-32	0.5-8	0.5-8	1-8	1-4	1-4	0.25-16	4->128	2->128	-
OXA-48 GM	2	too few	too few	too few	too few	too few	too few	too few	too few	too few	-
Range		≥128	8-16	4-8	64-128	2-4	2-4	16	2-4	2-4	-
MBL GM	5	73.5	73.5	73.5	42.2	42.2	42.2	BM	64.0	73.5	-
Range		32-128	32-128	32-128	16-128	16-128	16-128	0.25-128	16->128	32->128	
Acinetobacter											
Controls GM	5	0.29	0.25	0.25	0.25	0.22	0.22	0.33	4.00	4.59	3.03
Range		*0.12-0.5*	*0.12-1*	*0.12-1*	*0.12-0.5*	*0.12-0.5*	*0.12-0.5*	*0.25-0.5*	*2-8*	*2-16*	*2-4*
AmpC	10	1.1	0.54	0.44	1.2	0.81	0.87	BM	73.5	29.9	12.1
Range		0.5-2	*0.25-1*	*0.25-1*	*0.25-2*	*0.25-2*	*0.25-2*	*0.5-128*	*8->128*	*8-64*	*8-32*
OXA-23	10	48.50	2.14	1.62	45.25	1.74	1.15	103.98	128.07	51.98	>128
Range		*16-128*	*0.25-4*	*0.5-4*	*32-128*	*0.5-8*	*0.5-4*	*32->128*	*≥128*	*8-128*	*>128*

	n	IMP	IMP + WCK 4	IMP + WCK 8	MEM	MEM + WCK 4	MEM + WCK 8	CIP	CAZ	CAZ + AVI4	ERT
OXA-24/40	10	59.7	4.9	2.8	78.8	5.7	3.0	BM	48.5	19.7	128.1
Range		*16-128*	*1-32*	*0.5-16*	*32->128*	*1-32*	*0.5-16*	*2->128*	*8-128*	*4-32*	*>128*
OXA-51–ISAba1	10	16.0	0.87	0.62	36.8	1.2	1.2	90.5	104.0	34.3	>128
Range		8-32	0.5-4	0.25-2	16->128	0.25.4	0.5-4	4-128	16->128	16->128	≥128
OXA-58	10	39.4	2.1	1.4	19.7	3.0	2.6	68.6	>128	90.56	104.00
Range		32-64	1-4	0.5-4	8-32	0.5-16	0.5-16	16->128	≥128	16->128	64->128
MBL (NDM)	5	>128	>128	>128	>128	>128	>128	BM	>128	>128	>128
Range		≥128	≥128	≥128	≥128	≥128	≥128	0.25-128	>128	>128	>128

Abbreviations: AVI, avibactam; BM, bimodal distribution, invalidating geometric mean; Carb S, susceptible to carbapenems at CLSI breakpoints; CAZ, ceftazidime; CIP, ciprofloxacin; ERT, ertapenem; GM, geometric mean; IMP, imipenem; MEM, meropenem, WCK 4/WCK 8, WCK 4234 at 4 or 8 mg/L respectively.

[a] Single isolate with MIC far outside the general range; [b] Isolates with ESBL variants belonging to the OXA-2 and -10 families

Underlining highlights cases where the geometric mean MIC for an inhibitor combination was at least four-fold lower than for the unprotected β-lactam

Table 3. MIC distributions of carbapenems and their WCK 4234 combinations for groups of isolates with KPC and Class D carbapenemases

	No. isolates with indicated MIC (mg/L)														
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Enterobacteriaceae KPC															
Imipenem											2	9	7	8	5
IMP+WCK 4234, 4 mg/L				1	8	6	7	4	3		2				
IMP+WCK 4234, 8 mg/L				2	12	6	5	3	1	1		1			
Meropenem										2	3	8	6	6	6
MEM+WCK 4234, 4 mg/L		11	6	1	4	3	2	2	1				1		
MEM+WCK 4234, 8 mg/L	1	13	5	2	4	3		1					1		
Enterobacteriaceae OXA-48															
Imipenem									6	4	2	4	1	3	
IMP+WCK 4234, 4 mg/L				4	6	9	1								
IMP+WCK 4234, 8 mg/L				5	8	7									
Meropenem								5	3	2	5	2	2	1	
MEM+WCK 4234, 4 mg/L	1	6	7	4	2										
MEM+WCK 4234, 8 mg/L	1	7	5	5	1	1									
Enterobacteriaceae OXA-181															
Imipenem									4	1		4	1	1	
IMP+WCK 4234, 4 mg/L				3	4	2		2							
IMP+WCK 4234, 8 mg/L			1	3	3	2		2							
Meropenem							2		3			2	2	2	

	No. isolates with indicated MIC (mg/L)														
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
MEM+WCK 4234, 4 mg/L	1	2		3	3	2									
MEM+WCK 4234, 8 mg/L	1	2	1	5	1	1									
P. aeruginosa OXA-48															
Imipenem														1	1
IMP+WCK 4234, 4 mg/L										1	1				
IMP+WCK 4234, 8 mg/L									1	1					
Meropenem													1	1	
MEM+WCK 4234, 4 mg/L								1	1						
MEM+WCK 4234, 8 mg/L								1	1						
Acinetobacter OXA-23															
Imipenem											1	3	5	1	
IMP+WCK 4234, 4 mg/L					1		1	3	5						
IMP+WCK 4234, 8 mg/L						2		7	1						
Meropenem												6	3	1	
MEM+WCK 4234, 4 mg/L						1	2	6		1					
MEM+WCK 4234, 8 mg/L						1	7	1	1						
Acinetobacter OXA-24/40															
Imipenem											1	2	4	3	
IMP+WCK 4234, 4 mg/L							3	1	1	1	3	1			

	No. isolates with indicated MIC (mg/L)														
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
IMP+WCK 4234, 8 mg/L						2	2		2	3	1				
Meropenem												2	3	3	2
MEM+WCK 4234, 4 mg/L							3	1		2	2	2			
MEM+WCK 4234, 8 mg/L						2	2		2	2	2				
*Acinetobacter IS*Aba1-OXA-51															
Imipenem										4	2	4	4		
IMP+WCK 4234, 4 mg/L						6	1	2	1						
IMP+WCK 4234, 8 mg/L					1	6	2	1							
Meropenem											4	1	4		1
MEM+WCK 4234, 4 mg/L					1	1	4	3	1						
MEM+WCK 4234, 8 mg/L						1	7	1	1						
Acinetobacter OXA-58															
Imipenem												7	3		
IMP+WCK 4234, 4 mg/L							2	5	3						
IMP+WCK 4234, 8 mg/L						1	5	2	2						
Meropenem										2	3	5			
MEM+WCK 4234, 4 mg/L						1	1	1	6		1				
MEM+WCK 4234, 8 mg/L						1	2	1	5		1				

Table 4. MICs of carbapenem-WCK 4234 combinations and comparators against AmpC and OprD mutants of *P. aeruginosa*

		MIC (mg/L)									
	Designation and phenotype	IMI	IMI + WCK 4	IMI + WCK 8	MEM	MEM + WCK 4	MEM + WCK 8	CIP	CAZ	CAZ + AVI4	ERT
P. aeruginosa	1405-con	4	1	1	1	0.5	0.25	0.25	128	8	32
	1405 -con OprD$^-$	32	8	8	16	8	8	0.5	128	8	>128
	1405 -def	0.5	0.25	0.25	0.25	0.25	0.25	0.5	8	2	4
	1405 -def OprD$^-$	1	1	1	4	4	4	0.25	2	2	16
P. aeruginosa	2297	2	1	0.5	0.5	0.25	0.25	0.25	2	2	4
	2297 -con	2	0.5	0.5	0.5	0.25	0.125	0.25	64	4	8
	2297 -con OprD$^-$	16	8	4	8	4	4	0.25	128	4	128
	2297 -def	0.25	0.25	0.25	0.25	0.125	0.125	0.25	2	2	2
	2297 –def OprD$^-$	4	2	2	4	2	2	0.25	2	2	32

Strain numbers suffixed –CON, derepressed for AmpC; -DEF, deficient for AmpC; OprD-, deficient for porin OprD; un-suffixed numbers inducible for AmpC. Other abbreviations are as in Table 2

Figure 1. Structure of WCK 4234

Figure 1