ACTIVITY OF OP0595-β-LACTAM COMBINATIONS AGAINST GRAM-NEGATIVE BACTERIA WITH EXTENDED-SPECTRUM, AMPC AND CARBAPENEM-HYDROLYSING β-LACTAMASES

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Background. OP0595 is a diazabicyclooctane which (i) acts as PBP2-active antibacterial, (ii) inhibits Class A and C β-lactamases, and (iii), like mecillinam, gives β-lactamase-independent potentiation of β-lactams targeting other PBPs. We tested its behaviour against β-lactam-resistant Enterobacteriaceae and non-fermenters. **Methods**. Organisms were UK clinical isolates; MICs were determined by CLSI agar dilution for OP0595 alone or combined at 1-4 mg/L with aztreonam, biapenem, cefepime or piperacillin. **Results.** MICs of OP0595 for *Escherichia coli, Enterobacter, Citrobacter* and *Klebsiella* spp. were mostly 1-4 mg/L but values >4 mg/L were seen for minorities of isolates irrespective of other resistances, and for 50-60% of those with ertapenem resistance involving porin loss plus ESBL or AmpC activity. OP0595 MICs for *Serratia,* Proteeae and non-fermenters mostly were >4 mg/L. When its MIC was ≤4 mg/L, OP0595's antibacterial activity dominated combination activity. For OP0595-'resistant' (MIC >4 mg/L) isolates with Class A or C β-lactamases OP0595 achieved strong potentiation of substrate β–lactams, contingent on β-lactamase inhibition. β–Lactamase-independent potentiation was evident with aztreonam, cefepime and piperacillin – less so for biapenem – for many OP0595-resistant Enterobacteriaceae with Class B carbapenemases, which are not inhibited by OP0595. OP0595 acted solely as a β-lactamase inhibitor for non-fermenters. **Conclusions**. OP0595 inhibited Enterobacteriaceae, not non-fermenters; its combinations had broad activity *versus* Enterobacteriaceae, largely contingent on OP0595's antibacterial activity but also on inhibition of class A and C β-lactamases and on the β-lactam-enhancer effect, which allowed activity against many OP0595-resistant metallo-β-lactamase-producing Enterobacteriaceae. For non-fermenters OP0595 acted only as a β-lactamase inhibitor.

Introduction

The proliferation of extended-spectrum and carbapenem-hydrolysing β–lactamases challenges the continued dominance of β–lactam-based therapies, which are 'Standard-of-Care' for most severe infections in non-allergic patients.

Developing a single β–lactam that evades all the now-prevalent β–lactamases is challenging, and a more realistic prospect is to combine a β–lactam that evades some β–lactamases with an inhibitor that inactivates others, thereby achieving overall breadth of spectrum. Aztreonam/avibactam exemplifies this approach, with aztreonam being stable to MBL- and OXA-48 carbapenemases and with avibactam protecting against aztreonam-hydrolysing extended-spectrum, AmpC and KPC β–lactamases.[1,2] This strategy might be extended by employing a β–lactamase inhibitor with secondary activities. Avibactam has only marginal antibacterial activity, with MICs around 16 mg/L for *Escherichia coli* and higher for other Gram-negatives, but other diazabicyclooctanes have greater activity. OP0595, which was discovered independently by Meiji and Fedora,[3] in particular, binds strongly to PBP2 of Enterobacteriaceae, thereby achieving antibacterial activity as well as acting as an inhibitor of class A and C β–lactamases. In addition, and like mecillinam,[4-6] which also binds to PBP2,[7] OP0595 seems able to potentiate PBP-3-active β–lactams via a β–lactamase-independent 'enhancer' effect, hypothesised to reflect concurrent attack on different PBPs by the two molecules.[3]

In the present study we characterised the activity of OP0595 combined with aztreonam, biapenem, cefepime and piperacillin against Enterobacteriaceae and non-fermenters with potent and clinically-frequent β-lactamases.

Materials and methods

Antibiotics

OP0595, avibactam, biapenem and cefepime powders were provided by Meiji (Yokohama, Japan); aztreonam, ceftazidime and piperacillin were purchased from Sigma (Poole, UK).

Bacteria

Bacteria were recent reference submissions to Public Health England's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit from UK diagnostic laboratories. Numbers of isolates by species and resistance mechanism are detailed in Tables 1 and 2. Carbapenemase genes were sought by PCR and sequencing; outer membrane protein expression was previously characterised by gene sequencing and protein profiles, as described previously.[8] Isolates included as carbapenemase producers may have had additional extended-spectrum β-lactamases (ESBLs) or AmpC enzymes; isolates included as ESBL and AmpC producers lacked carbapenemases but may have had additional penicillinases; those included as controls lacked ESBLs, AmpC enzymes or carbapenemases but may have produced penicillinases

Susceptibility testing

Susceptibility testing was performed by CLSI agar dilution,[9] using aztreonam, biapenem, cefepime and piperacillin combined with OP0595 at 1, 2 and 4 mg/L for Enterobacteriaceae and with OP0595 at 4 mg/L, only, for non-fermenters. Ceftazidime was tested alone and in combination with avibactam at 4 mg/L.

Results

Antibacterial activity of OP0595

MIC distributions of OP0595, by species and irrespective of resistance mechanisms, are shown in Table 1. MICs for *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. were mostly 1-4 mg/L, though values exceeding 4 mg/L were recorded for 9/50 Enterobacter spp. and 26/118 *Klebsiella* spp. MICs of OP0595 for all the *Morganella morganii* and 12/15 *Serratia* spp. isolates exceeded 4 mg/L, as did those for 29/30 *Acinetobacter* and the *P. aeruginosa* isolates tested MICs were largely unrelated to known resistance mechanisms, except that values were >4 mg/L for 15/28 ertapenem-resistant, porin-deficient *E. coli*, *Klebsiella* and *Enterobacter* spp. with ESBL or AmpC enzymes *versus* 6/100 ertapenem-susceptible isolates of *E. coli*, *Klebsiella* and *Enterobacter* spp. or *Citrobacter* spp.) with the same β–lactamases (p <0.001, chi-squared test). These data suggest that porin loss can restrict entry of OP0595.

OP0595 in combination with β–lactams versus Enterobacteriaceae

Susceptibility data for OP0595 in combination with aztreonam, biapenem, cefepime and piperacillin are summarised in Table 2. For convenient review, results are graded against the more stringent of CLSI[9] or EUCAST (http://www.eucast.org) criteria for the partner β-lactam. These values were considered to represent the lowest breakpoints that any regulatory agency might reasonably adopt. They should *not* be construed as definitive breakpoints, particularly for ceftazidime/avibactam, which has recently been given a breakpoint of 8+4 mg/L by the FDA, based upon a 2+0.5 g every 8h regimen, and where CLSI and EUCAST reviews are pending.

On this rationale, we graded susceptible $\leq$1 mg/L and resistant >4 mg/L for aztreonam, ceftazidime and cefepime against Enterobacteriaceae, matching EUCAST values for these β-lactams alone. For piperacillin we graded susceptible as MIC $\leq$8 mg/L and resistant as MIC >16 mg/L again adopting EUCAST values. In all these cases CLSI breakpoints are equal or higher. For biapenem, which has no CLSI or EUCAST

breakpoints, we graded susceptible ≤1 mg/L and resistant >4 mg/L, matching CLSI criteria for imipenem and meropenem and both CLSI and EUCAST criteria for doripenem; EUCAST breakpoints for imipenem and meropenem are higher.

Combination performance against Enterobacteriaceae was largely dominated by the antibacterial activity of OP0595 itself, since the concentrations used (1-4 mg/L) were equalled or exceeded the molecule's MIC for most of the test strains (Table 2). With OP0595 at 4 mg/L, >80% 'susceptibility' was achieved by all combinations *versus* all resistance groups, including MBL producers. Susceptibility rates ≥90% were achieved for all combinations except (i) biapenem/OP0595 against AmpC-derepressed strains, where performance was constrained by the inclusion of *M. morganii*, a species that was inherently less susceptible than other Enterobacteriaceae to both biapenem and OP0595; (ii) piperacillin/OP0595 against isolates that combined ESBL or AmpC activity with porin loss, and (iii) cefepime/OP0595 against MBL producers – where the more striking point is that 35/40 isolates were susceptible and that the aztreonam/, biapenem/ and piperacillin/ OP0595 combinations did achieve 90% activity, even though only 31/40 isolates (77.5%) were inhibited by OP0595 itself at 4 mg/L.

Ceftazidime/avibactam 4 mg/L, tested as a comparator, had MICs ≤1 mg/L ('susceptible' on the criteria used) for >90% of isolates in the ertapenem-susceptible ESBL and AmpC groups, and for those with KPC and OXA-48 carbapenemases. However ceftazidime/avibactam MICs were ≤1 mg/L for only 19/28 (67.8%) of the ertapenem-resistant isolates that combined AmpC or ESBL activity with impermeability, with intermediate activity (MIC 2-4 mg/L) for eight of the remaining nine. MICs of ceftazidime/avibactam exceeded 4+4 mg/L for isolates with MBLs in 39/40 cases.

OP0595 combinations versus Enterobacteriaceae resistant to OP0595 4 mg/L

MIC data for Enterobacteriaceae isolates with OP0595 MICs >4 mg/L were further explored, to gain insight into the molecule's secondary activities. MIC data for OP0595-resistant isolates with ESBLs and AmpC enzymes are summarised in Table 3, and those for OP0595-resistant isolates with carbapenemases are line-listed in Table 4.

Strong potentiation was seen for ESBL substrates - i.e. aztreonam, cefepime and piperacillin - against OP0595-resistant ESBL producers. Similarly, strong potentiation was seen for AmpC substrates (aztreonam, piperacillin) against AmpC-hyperproducing OP0595-resistant *E. coli, Enterobacter* and *Klebsiella* spp. Weaker potentiation was seen for cefepime against AmpC producers, doubtless because cefepime is a weaker substrate for AmpC enzymes; nevertheless, and notably, the geometric mean MIC of cefepime for ertapenem-resistant porin-deficient AmpC hyperproducers was reduced from 6.4 mg/L to 0.89 with OP0595 at 1 mg/L and to 0.076 mg/L with OP0595 at 4 mg/L. More generally, higher concentrations of OP0595 were needed to potentiate partner agents against ertapenem-resistant than against ertapenem susceptible-strains with ESBL and AmpC β-lactamases, again implying that porin loss restricts uptake of the diazobicyclooctane

Potentiation by OP0595 was seen for piperacillin against AmpC-hyper-producing *M. morganii* and *Serratia* spp., which were inherently less susceptible to the antibacterial activity of OP0595, with most MICs >4 mg/L. Derepressed AmpC gave less protection against aztreonam in these species than in *E. coli*, *Klebsiella* or *Enterobacter* spp. but, 18- to 55-fold reductions in geometric mean MIC were achieved when the diazabicyclooctane was incorporated in the agar at 4 mg/L, with 3.3- to 17- fold MIC reductions for cefepime.

Potentiation against ESBL and AmpC groups was weaker for biapenem, which, like other carbapenems, is a poor substrate for these enzymes.[10] Even so, OP0595 at 4 mg/L achieved $\geq$5-fold reductions in geometric mean biapenem MICs both for ertapenem-resistant ESBL producers and – irrespective of ertapenem resistance – for AmpC-hyperproducing *E. coli, Enterobacter* and *Klebsiella* spp., though with little potentiation against AmpC-derepressed *M. morganii*. Avibactam similarly potentiated ceftazidime

against ESBL and AmpC producers with the distinction, compared with aztreonam/OP0595 and cefepime/OP0595,that derepressed *M. morganii* AmpC gave greater (avibactam-reversed) resistance to ceftazidime than to either aztreonam or cefepime. For almost all groups with ESBLs or AmpC enzymes, the geometric MICs of aztreonam-, cefepime- and piperacillin/OP0595 4 mg/L were lower than those of ceftazidime/avibactam 4 mg/L for the same isolates. For ertapenem-resistant, porin-deficient organisms with ESBL and AmpC, the geometric mean MICs of aztreonam-, cefepime/OP0595 and piperacillin/OP0595 were <1 mg/L, thus falling into the 'susceptible' range, whereas values for ceftazidime/avibactam were 1.2 - 2.5 mg/L, falling into EUCAST's intermediate MIC category for ceftazidime.

OP0595 gave strong potentiation of all four partner agents against the six OP0595-resistant isolates with KPC carbapenemases. In all cases the partner MICs were reduced below their target values when the inhibitor was present at 4 mg/L and, except for *E. cloacae* H401 and aztreonam this objective was achieved with 2 mg/L OP0595. The fact that potentiation was seen with biapenem, which is unlikely to be affected by any co-produced β-lactamase, supports the view that it was mediated by inhibition of the KPC enzyme, which OP0595 have been shown to inactivate in direct assays.[3]

Aztreonam/OP0595, cefepime/OP0595 and ceftazidime/avibactam had good activity against the OP0595-resistant strains with OXA-48 β-lactamase, with MICs below 1 mg/L when 2-4 mg/L of inhibitor was present. Three of the four OP0595-resistant strains with OXA-48 carbapenemase were highly resistant to the cephalosporins and aztreonam and, given that these oxyimino aminothiazolyl molecules are weak substrates for OXA-48 enzyme or are stable[1,11] potentiation is inferred largely to reflect inhibition of co-produced ESBLs or AmpC enzymes. The fourth OXA-48 strain (*K. pneumoniae* H483) was susceptible or intermediate to aztreonam, cefepime and ceftazidime, with MICs of 0.25-2 mg/L, implying the absence of ESBLs or AmpC enzymes. For this organism, OP0595 at 2 or 4 mg/L still reduced aztreonam and cefepime MICs by eight-fold or more, suggesting an enhancer effect, whereas avibactam 4 mg/L reduced the ceftazidime MIC by only two-fold.

Also notable were the comparative behaviours of biapenem (unlikely to be affected by any β-lactamase besides OXA-48) and piperacillin (a substrate for OXA-48 as well as co-produced ESBLs and penicillinases). Potentiation of biapenem by OP0595 was weak, with only two- to four-fold MIC reductions with the inhibitor at 2-4 mg/L. For piperacillin, by contrast, MICs consistently exceeded 256 mg/L in the absence of OP0595 (including for the aztreonam-susceptible strain H483) and were reduced to ≤8 mg/L with OP0595 at 2-4 mg/L. It is difficult to reconcile the poor protection of biapenem and the good potentiation of piperacillin with a solely β-lactamase-mediated mechanism (see Discussion).

Turning, lastly, to the MBL producers: these comprise five aztreonam- susceptible or intermediate (MIC 0.25-2 mg/L) Enterobacteriaceae that were deduced to lack secondary ESBL or AmpC activity and four aztreonam-resistant organisms (MIC ≥64 mg/L) that putatively had ESBL or AmpC activity. Strong potentiation of aztreonam by OP0595 was seen for the aztreonam-resistant organisms, probably reflecting inhibition of these ESBLs or AmpC enzymes; more interestingly, however, and even at 2 mg/L, OP0595 reduced aztreonam MICs by eight-fold or more for 3/5 aztreonam-susceptible organisms; at 4 mg/L it achieved this effect, which cannot readily be explained by β-lactamase inhibition, for all five organisms.

More generally, OP0595 at 2 mg/L achieved ≥8-fold potentiation of piperacillin, cefepime and biapenem for 8/9, 6/9, and 3/9 OP0595-resistant MBL producers, respectively. Corresponding proportions with OP0595 at 4 mg/L were 9/9, 9/9 and 4/9, respectively. Given that the MBLs must have been the major contributors to biapenem resistance and to cefepime resistance in the low-aztreonam-MIC strains, it is again difficult to explain these results in terms of a β-lactamase-inhibition alone, supporting the contribution of an enhancer effect (see Discussion). Potentiation of ceftazidime by avibactam was seen for just one of the nine OP0595-resistant MBL producers; for the remainder, ceftazidime/avibactam MICs remained above 256 mg/L.

P. aeruginosa and A. baumannii

Results for non-fermenters are shown in Table 5. Except for one *P. aeruginosa* all were resistant to OP0595 at 4 mg/L (Table 1).

OP0595 potentiated aztreonam, cefepime and piperacillin against *P. aeruginosa* with derepressed AmpC or acquired PER or VEB β-lactamases, but not against those with MBLs, nor against the β-lactam-susceptible control strains. Similar behaviour was seen between ceftazidime and avibactam, with avibactam 4 mg/L tending to achieve slightly greater fold potentiation of ceftazidime for AmpC-derepressed organisms than OP0595 4 mg/L achieved for aztreonam and cefepime; neverthless the geometric mean MICs of aztreonam/OP0595, cefepime/OP0595 and ceftazidime/avibactam all remained similar to one another

OP0595 4 mg/L achieved *c.* 2.4 fold-potentiation of biapenem for the control *P. aeruginosa* strains, 4.3-fold potentiation for imipenem-susceptible (putatively OprD-expressing) AmpC-derepressed organisms and 5.4-fold potentiation for imipenem/biapenem-resistant (OprD-deficient) AmpC derepressed isolates. Previous experience shows that biapenem, like imipenem, is weakly affected even by inducible *P. aeruginosa* AmpC, and that this protection confers significant resistance if the organism also becomes impermeable via loss of OprD.[12] The present results are compatible with these earlier findings, and with the fact that OP0595 inhibits the activity of purified pseudomonal AmpC enzyme.[3] Similar potentiation is seen between imipenem and another diazabicyclooctane, MK-7655;[13] also between imipenem and AmpC-inhibitory penems, such as BRL42715[14] and bridged monobactams, e.g. Ro-48-1256.[15]

In the case of *A. baumannii*, OP0595 achieved no significant potentiation of aztreonam, biapenem or cefepime against the control strains nor those with OXA or metallo carbapenemases. Similarly, avibactam did not potentiate ceftazidime against these groups. OP0595 did give weak potentiation of aztreonam, cefepime and piperacillin for isolates with AmpC-associated cephalosporin resistance, though geometric mean MICs remained high,

with the lowest value (7.9 mg/L) recorded for cefepime/OP0595; similar behaviour was seen between ceftazidime and avibactam. OP0595 potentiated piperacillin against the control *A. baumannii* strains, probably reflecting inhibition of co-produced penicillinases.

Discussion

These findings, for sizeable panels of multi-resistant organisms, extend the data reported Morinaka *et al*.[3] for OP0595 combinations. They support Morinaka's conclusions that OP0595 has a triple activity, acting as an antibiotic, inhibitor of Class A and C β-lactamases, and as a β-lactamase-inhibition-independent enhancer of partner β-lactams that bind to PBP3.

The antibacterial activity of OP0595, which is associated with inhibition of PBP2,[3] is the simplest of these activities to define and was strongest against *E. coli, Klebsiella* and *Enterobacter* spp. As with mecillinam,[16] which also exclusively attacks PBP2,[3] activity was weaker against *Morganella* and *Serratia* spp. than against other Enterobacteriaceae. *P. aeruginosa* and *A. baumannii* were more resistant. This behaviour seems likely largely to reflect target insensitivity, or dispensability[17] since OP0595 continued to act as an inhibitor of AmpC enzymes in all these species proving that it must be able to permeate them and evade efflux.

This antibacterial activity of OP0595 dominated combination behaviour against Enterobacteriaceae (Table 2). However, OP0595's β-lactamase-inhibitory activity became apparent in the potentiation seen between OP0595 and substrate β-lactams against those AmpC-, ESBL- and KPC- β-lactamase-producing strains that were resistant to the antibacterial activity of OP0595 itself. Major reductions in the MIC of the partner β-lactam were evident even with OP0595 at 1 mg/L, equating to $\leq$0.125 x MIC OP0595 for these organisms. Although it is impossible to completely disentangle the contributions of β-lactamase inhibition and the enhancer effect in this potentiation, a major contribution by β-

lactamase inhibition is supported by: (i) potentiation being stronger with substrate β-lactams than non-substrates, (ii) potentiation being stronger against organisms with Class A and C enzymes, which OP0595 inhibits,[3] than against those with Class B enzymes, which are not inhibited and (iii) by potentiation extending to aztreonam-, cefepime- and piperacillin-combinations against AmpC-derepressed *P. aeruginosa*, whereas there was no potentiation of these β-lactams by OP0595 against wild-type *P. aeruginosa* without derepression of AmpC.

Evidence for the enhancer effect, which was unique to Enterobacteriaceae, came from OP0595's frequent potentiation of β-lactams that were not substrates for the organism's β-lactamase, particularly for the common, though not universal, potentiation of aztreonam and cefepime against aztreonam-susceptible (i.e. ESBL- and AmpC-negative) OP0595-resistant MBL-producing Enterobacteriaceae (Table 4). Potentiation was less consistent or extensive for biapenem and these findings are in keeping with the view that the enhancer effect arises when the PBP2-directed activity of OP0595 is combined with PBP3-targeted agents[3] rather than those, like biapenem, that strongly bind PBP2.[18] This view also accommodates the stronger potentiation of piperacillin than biapenem for strains with OXA-48 carbapenemase, even though both β-lactams are substrates for this enzyme.[12,19]

Given that the enhancer activity was demonstrable in OP0595-resistant strains, and was retained in an OP0595-resistant mutant,[3] it clearly does not require the antibacterial activity of OP0595. A plausible explanation lies in the observation that resistance to mecillinam commonly reflects compensatory mutations up-regulating FtsQAZ[20-22] or increasing cellular levels of the regulatory molecule ppGpp,[23] not to changes to PBP2 itself. It may be that these mutations prevent inhibition of PBP2 leading to cell death but that, with PBP2 still present and inhibited, the enhancer activity remains. This hypothesis is compatible with the observation that an OP0595-selected *E. coli* mutant, lacked sequence changes to the PBP2 gene, *pbp2*.[3]

Compared with ceftazidime/avibactam the major difference was that the various OP0595 combinations remained active, even at the very stringent definitions adopted, owing either to the antimicrobial activity of OP0595 or to its enhancer effect, against the great majority of MBL-producing Enterobacteriaceae (40/40 for aztreonam-OP0595, 36/40 for cefepime-OP0595, 35/40 for biapenem-OP0595 and 39/40 for piperacillin-OP0595, all based on tests in the presence of 4 mg/L OP0595), whereas organisms were consistently resistant to ceftazidime/avibactam, as also found by others.[24] Deeper comparison is difficult because (i) OP0595 and avibactam were tested in combination with different β-lactams, (ii) the final partner agent(s) for OP0595 remain to be decided, and (iii) final CLSI and EUCAST breakpoints may differ from the 'most stringent' values adopted here. Despite these caveats it is notable that OP0595 combinations more often retained full activity against ertapenem-resistant Enterobacteriaceae with ESBLs or derepressed AmpC than did ceftazidime/avibactam. For AmpC-derepressed *P. aeruginosa*, the performance of ceftazidime/avibactam, cefepime/OP0595 and aztreonam/avibactam was similar.

In summary, and combined with Morinaka's data,[3] these results support the complex tripartite activity of OP0595, and indicate the potential for diazabicyclooctane combinations with a broader activity than ceftazidime/avibactam. Major challenges remain, most obviously in the choice of partner agent for OP0595 and in assessing vulnerabilities to mutational resistance, both (i) for OP0595's own activities and (ii) for combinations where, as with ceftazidime/avibactam, vulnerability can involve the β-lactamase increasing its substrate specificity rather than developing inhibitor resistance *per se*.[25]

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Transparency declaration

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Table 1. MIC distribution of OP0595 by species

	MIC (mg/L)			
	≤1	2	4	>4
E. coli	10	32	4	3
Enterobacter spp.	9	29	3	9
Citrobacter spp.		9	1	
Klebsiella spp.		48	44	26
M. morganii				15
Serratia spp.			3	12
P. aeruginosa				40
A. baumannii			1	29

Table 2. Activity of β–lactam OP0595 combinations, and ceftazidime/avibactam *versus* Enterobacteriaceae groups

	Count of isolates																	
	Aztreonam+OP0595 at (mg/L)				Biapenem+OP0595 at (mg/L)				Cefepime+OP0595 at (mg/L)				Piperacillin+OP0595 at (mg/L)				Ceftazidime+ avibactam at (mg/L)	
	0	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**4**
ESBL-producing Enterobacteriaceae, ertapenem susceptible (n=60)[a]																		
R at target MIC[b]	54	3	0	0	0	0	0	0	25	0	0	0	60	4	0	0	57	0
I at target MIC[b]	6	16	0	0	0	0	0	0	24	0	0	0	0	3	0	0	1	1
S at target MIC[b]	0	41	**60**	**60**	**60**	**60**	**60**	**60**	11	**60**	**60**	**60**	0	53	**60**	**60**	2	**59**
Inhibited by OP0595 alone, cumulative	-	6	49	57	-	6	49	57	-	6	49	57	-	6	49	57	-	1[c]
AmpC-producing Enterobacteriaceae, ertapenem susceptible (n=60)[d]																		
R at target MIC[b]	32	0	0	0	0	0	0	0	1	0	0	0	56	0	0	0	47	0
I at target MIC[b]	15	4	0	0	7	6	5	5	4	0	0	0	2	1	0	0	7[e]	0
S at target MIC[b]	13	**56**	**60**	**60**	53	54	55	55	**55**	**60**	**60**	**60**	2	**59**	**60**	**60**	6[e]	**60**
Inhibited by OP0595 alone, cumulative	-	4	28	37	-	4	28	37	-	4	28	37	-	4	28	37	-	0[c]
ESBL and AmpC-producing Enterobacteriaceae, ertapenem-resistant via porin loss (n=28)[f]																		
R at target MIC[b]	28	15	3	1	4	0	0	0	22	8	0	0	28	13	5	2	28	1
I at target MIC[b]	0	5	5	1	14	11	6	1	6	4	4	1	0	6	1	1	0	8
S at target MIC[b]	0	8	20	**26**	10	17	22	**27**	0	16	24	**27**	0	9	22	25	0	19
Inhibited by OP0595 alone, cumulative	-	1	8	13	-	1	8	13	-	1	8	13	-	1	8	13	-	0[c]
Enterobacteriaceae isolates with KPC carbapenemases (30)[g]																		
R at target MIC[b]	30	4	1	0	28	1	0	0	29	0	0	0	30	12	0	0	29	2

I at target MIC[b]	0	2	1	0	2	0	0	0	1	1	0	0	0	5	0	0	1	1
S at target MIC[b]	0	24	**28**	**30**	0	**29**	**30**	**30**	0	**29**	**30**	**30**	0	**13**	**30**	**30**	0	**27**
Inhibited by OP0595 alone, cumulative	-	2	17	23	-	2	17	23	-	2	17	23	-	2	17	23	-	0 [c]
***K. oxytoca* hyperproducing K1 β–lactamase (n=10)**																		
R at target MIC[b]	10	0	0	0	0	0	0	0	0	0	0	0	10	1	0	0	0	0
I at target MIC[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	8	0
S at target MIC[b]	0	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	0	5	9	**10**	2	**10**
Inhibited by OP0595 alone, cumulative	-	0	4	9	-	0	4	9	-	0	4	9	-	0	4	9	-	0 [c]
Enterobacteriaceae isolates with MBLs (n=40)[h]																		
R at target MIC[b]	26	1	0	0	20	10	4	3	39	20	8	3	40	15	4	1	40	39
I at target MIC[b]	1	3	1	0	10	5	3	1	1	5	1	2	0	5	1	0	0	1
S at target MIC[b]	13	**36**	**39**	**40**	10	25	33	**36**	0	15	31	35	0	20	**35**	**39**	0	0
Inhibited by OP0595 alone, cumulative	-	5	19	31	-	5	19	31	-	5	19	31	-	5	19	31	-	0 [c]
Enterobacteriaceae (all *K. pneumoniae*) isolates with OXA-48 carbapenemases (n=10)[i]																		
R at target MIC[b]	8	1	0	0	5	4	1	1	8	1	0	0	10	4	0	0	8	0
I at target MIC[b]	0	0	0	0	3	2	3	1	2	1	0	0	0	1	0	0	0	0
S at target MIC[b]	2	**9**	**10**	**10**	2	4	6	8	0	8	**10**	**10**	0	5	**10**	**10**	2	**10**
Inhibited by OP0595 alone, cumulative	-	0	3	6	-	0	3	6	-	0	3	6	-	0	3	6	-	0 [c]
ESBL, AmpC, carbapenemase negative Enterobacteriaceae control isolates (n=40)[j]																		
R at target MIC[b]	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0
I at target MIC[b]	0	0	0	0	3	2	2	2	0	0	0	0	9	0	0	0	0	0
S at target MIC[b]	**40**	**40**	**40**	**40**	**37**	**38**	**38**	**38**	**40**	**40**	**40**	**40**	22	**40**	**40**	**40**	**40**	**40**

Inhibited by OP0595 alone, cumulative	-	8	24	30	-	8	24	30	-	8	24	30	-	8	24	30	-	0 [c]

a 20 *E. coli*, 20 *Enterobacter* spp., 20 *K. pneumoniae*

b Aztreonam, biapenem, cefepime and ceftazidime all categorised as S ≤1 mg/L, I 2-4 mg/L, R >4 mg/L; piperacillin as S <8, I = 16 mg/L and R >16 mg/L; these values were adopted as the more stringent of EUCAST or CLSI breakpoints for the partner β-lactams and therefore the lowest likely to be adopted for any future combination. As such they should be seen as a 'worst case scenario' and the US Food and Drug Administration has recently granted a higher (8+4 mg/L susceptibility) breakpoint to ceftazidime/avibactam. Numbers in the susceptible category are shown in bold font wherever 90% or more of the isolates are inhibited.

c Inhibited by 4 mg/L avibactam alone

d 10 *E. coli* and 10 *Klebsiella* spp. with plasmid AmpC; 10 *Enterobacter* spp. 10 Citrobacter spp., 10 *M. morganii* and 10 *Serratia* spp. with derepressed chromosomal AmpC. Among 23 isolates with OP0595 MICs >4 mg/L, 20 were *Morganella* and *Serratia.* All the biapenem 'intermediate' isolates were *M. morganii.*

e 10/13 Ceftazidime-susceptible or intermediate isolates were *Serratia* spp.

f 9 *E. coli* and 9 *Klebsiella* spp. with ESBLs and porin loss; 10 *Enterobacter* spp. with derepressed AmpC + porin loss

g 5 *E. coli*, 20 *Klebsiella* spp. and 5 *Enterobacter* spp.

h 10 *E. coli*, 20 *Klebsiella* spp 10 *Enterobacter*; 13 with IMP, 9 with VIM, 18 and with NDM carbapenemases.

i 10 *Klebsiella* spp.

j 10 *E. coli*, 10 *Klebsiella* spp., 10 *Enterobacter* spp. 5 *M. morganii* and 5 *Serratia* spp. All the 10 with OP0595 MICs >4 mg/L were *M. morganii* and *Serratia* spp.;
all biapenem 'intermediate' isolates were *M. morganii*

\- No diazabicyclooctane present, therefore no activity attributable to it.

457 **Table 3.** Response of carbapenemase-negative Enterobacteriaceae isolates with OP0595 MICs >4 mg/L to OP0595 combinations
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	MICs (mg/L)																	
	Aztreonam+OP0595 at (mg/L)				Biapenem+OP0595 at (mg/L)				Cefepime+OP0595 at (mg/L)				Piperacillin+OP0595 at (mg/L)				Ceftazidime+ avibactam (mg/L)	
	0	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**1**	**2**	**4**	**0**	**4**
ESBL producing Enterobacteriaceae; ertapenem susceptible (n=3)																		
Range	256	2-4	0.12-1	≤0.02-0.06	0.25-1	0.06-0.5	≤0.02-0.5	0.03-0.5	4	0.03-0.12	≤0.02-0.06	≤0.02	>256	8	0.5-4	≤0.12-0.25	64-256	0.25-0.5
Geom. mean	256	2.5	0.32	0.037	0.40	0.20	0.12	0.12	4	0.078	0.023	0.014	>256	8.0	1.0	0.16	101.5	0.40
ESBL-producing Enterobacteriaceae; ertapenem resistant (n=9)																		
Range	64->256	1-128	0.03-8	≤0.02-4	1-8	0.5-4	0.25-2	0.03-2	16->256	1-64	0.03-4	≤0.02-1	>256	8->256	0.25->256	≤0.12-32	16->256	0.5-2
Geom. mean	219.5	12.7	0.62	0.075	2.5	1.3	0.79	0.39	188.2	8.6	0.36	0.069	>256	109.7	8.0	1.0	138.3	1.2
AmpC-hyperproducing producing Enterobacteriaceae, *E. coli, Enterobacter* spp., *Klebsiella* spp.; ertapenem susceptible (n = 6)																		
Range	0.5-64	0.03-2	≤0.02-0.25	≤0.02-0.25	0.12-1	0.03-1	≤0.02-0.5	≤0.02-0.5	0.12-1	≤0.02-0.06	≤0.02-0.06	≤0.02-0.06	32->256	2-8	≤0.12-2	≤0.12-2	16-256	0.25-0.5
Geom. Mean MIC (mg/L)	12.7	0.31	0.026	0.023	0.40	0.097	0.060	0.047	0.28	0.042	0.018	0.018	114.0	2.8	0.25	0.25	50.8	0.40
AmpC-hyperproducing *M. morganii* (n=10)																		
Range	0.03-32	0.03-2	≤0.02-1	≤0.02-1	1-4	0.5-2	1-2	0.5-2	0.03-1	≤0.02-0.25	≤0.02-0.25	≤0.02-0.25	8->256	0.25-16	0.25-4	≤0.12-2	2->256	0.03-1
Geom. mean	1.1	0.14	0.099	0.059	1.7	1.4	1.4	1.2	0.12	0.048	0.042	0.036	111.4	1.4	0.81	0.38	24.3	0.18
AmpC-hyperproducing *S. marcescens* (n=7)																		
Range	0.12-8	0.12-1	0.03-0.5	≤0.02-0.12	012-1	0.12-1	0.12-1	≤0.02-1	0.06-2	0.06-0.5	≤0.02-0.5	≤0.02-0.12	4-128	1-8	≤0.12-2	≤0.12-0.5	0.12-2	0.06-1
Geom. mean	1.6	0.37	0.14	0.029	0.41	0.31	0.28	0.12	0.41	0.17	0.073	0.024	29.0	3.6	0.91	0.20	0.67	0.23
***E. coli, Enterobacter* spp., *Klebsiella* spp. with AmpC and porin loss, ertapenem resistant (n=6)**																		
Range	64->256	4-256	0.12-128	0.03-16	1-8	1-4	0.5-4	0.06-1	4-16	0.25-8	0.06-4	0.03-2	128->256	4->256	1-256	≤0.12-32	128->256	1-32
Geom. mean	128.0	16.0	2.5	0.31	2.8	2.2	1.4	0.44	6.4	0.89	0.35	0.076	203.2	14.3	5.0	0.63	203.2	2.5

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Table 4. MICs (mg/L) of OP0595 combinations *versus* carbapenemase-producing Enterobacteriaceae isolates with OP0595 MICs >4 mg/L.

			Aztreonam+OP0595 at (mg/L)				Biapenem+OP0595 at (mg/L)				Cefepime+OP0595 at (mg/L)				Piperacillin+OP0595 at (mg/L)				Ceftazidime+ avibactam (mg/L)	
			0	1	2	4	0	1	2	4	0	1	2	4	0	1	2	4	0	4
H587	*E. cloacae*	KPC	>256	4	0.03	≤0.02	8	0.5	0.03	≤0.02	32	0.5	≤0.02	≤0.02	>256	128	≤0.12	≤0.12	128	1
H401	*E. cloacae*	KPC	>256	32	8	0.06	4	0.5	0.5	0.25	32	1	0.25	≤0.02	256	16	4	≤0.12	>256	8
H451	*K. pneumoniae*	KPC	>256	1	0.12	≤0.02	>256	8	1	0.06	>256	0.25	≤0.02	≤0.02	>256	32	0.5	≤0.12	>256	1
H316	*K. pneumoniae*	KPC	>256	1	≤0.02	≤0.02	32	0.25	≤0.02	≤0.02	32	0.12	≤0.02	≤0.02	>256	32	≤0.12	≤0.12	32	0.5
H467	*K. pneumoniae*	KPC	>256	16	0.06	≤0.02	32	1	0.12	0.03	256	1	≤0.02	≤0.02	>256	32	≤0.12	≤0.12	256	0.5
H538	*K. pneumoniae*	KPC	256	2	0.12	0.06	32	1	0.5	0.5	8	0.25	≤0.02	≤0.02	>256	32	1	0.5	32	0.5
H458	*K. pneumoniae*	KPC	>256	1	0.03	≤0.02	32	0.5	0.03	≤0.02	128	0.25	≤0.02	≤0.02	>256	64	0.25	≤0.12	64	1
H483	*K. pneumoniae*	OXA-48	0.25	0.06	0.03	≤0.02	32	16	16	8	2	0.25	0.03	0.03	>256	32	2	2	0.5	0.25
H386	*K. pneumoniae*	OXA-48	>256	1	0.12	0.06	8	8	4	4	>256	2	0.12	0.06	>256	32	8	4	256	1
H329	*K. pneumoniae*	OXA-48	128	0.5	≤0.02	≤0.02	4	2	1	0.06	>256	0.5	0.12	0.03	>256	64	0.5	≤0.12	64	0.5
H706	*K. pneumoniae*	OXA-48	128	0.25	0.03	0.03	4	2	2	1	>256	0.5	0.03	0.03	>256	16	2	0.5	64	0.25
H373	*E. cloacae*	IMP	0.5	0.5	0.12	≤0.02	16	32	16	8	128	256	256	16	64	128	32	2	>256	>256
H555	*K. pneumoniae*	IMP	0.25	0.12	0.03	0.03	2	2	1	1	16	4	4	2	64	16	0.5	0.5	>256	>256
H370	*K. pneumoniae*	IMP	64	0.25	≤0.02	≤0.02	8	16	2	2	128	128	16	2	>256	32	2	0.25	>256	>256
H538	*Klebsiella* spp.	IMP	0.5	0.25	0.25	≤0.02	128	128	128	128	128	16	64	8	64	16	8	0.5	>256	>256
H459	*K. pneumoniae*	VIM	2	0.03	≤0.02	≤0.02	1	0.5	0.5	0.06	4	0.12	0.03	≤0.02	>256	8	≤0.12	≤0.12	128	2
H744	*K. pneumoniae*	VIM	0.25	0.03	≤0.02	≤0.02	32	4	4	0.5	64	2	0.5	0.03	>256	0.5	≤0.12	≤0.12	>256	>256
H254	*K. pneumoniae*	NDM	128	0.5	≤0.02	≤0.02	4	4	0.03	0.03	64	128	0.03	0.03	>256	>256	≤0.12	≤0.12	>256	>256
H282	*K. pneumoniae*	NDM	128	1	0.03	≤0.02	128	64	1	≤0.02	>256	256	32	≤0.02	>256	>256	16	≤0.12	>256	>256
H519	*K. pneumoniae*	NDM	>256	0.25	0.06	0.06	16	16	16	16	256	64	16	16	>256	>256	32	32	>256	>256

Table 5. Interactions between OP0595 and partner antibiotics for groups of non-fermenters

Group	n	Geometric mean MIC (mg/L)									
		Aztreonam		Biapenem		Cefepime		Piperacillin		Ceftazidime	
		Alone	+OP0595 4 mg/L	Alone	+OP0595 4 mg/L	Alone	+OP0595 4 mg/L	Alone	+OP0595 4 mg/L	Alone	+avibactam 4 mg/L
P. aeruginosa											
Controls, fully susceptible	10	2.3	2.0	0.38	0.16	2.6	2.5	6.9	3.0	1.6	1.3
Derepressed AmpC, Imipenem S	9	27.4	4.0	0.73	0.17	17.3	5.0	161.3	8.0	40.3	2.3
Derepressed AmpC, Imipenem NS	11	68.2	4.8	9.7	1.8	34.1	4.3	240.4	12.4	82.3	3.8
PER or VEB ESBLs	5	222.9	10.6	0.57	0.22	84.5	3.0	36.8	4.6	>256	7.0
IMP or VIM MBLs	5	13.9	10.6	97.1	97.1	64.0	64.0	168.9	97.0	111.5	73.5
A. baumannii											
Controls, full susceptible	10	13.9	12.1	0.14	0.14	3.0	3.7	14.9	4.6	3.5	3.2
AmpC-mediated cephalosporin resistance	10	34.3	17.0	0.31	0.16	14.9	7.9	222.9	13.0	39.4	10.6
OXA carbapenemases	5	55.7	64	12.1	10.6	27.8	32	>256	194.0	111.4	32.0
IMP or NDM MBLs	5	36.8	48.5	16.0	7.0	128.0	111.5	168.9	128.0	194.1	168.9