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

5

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19 **Running head:** OP0595 combinations versus β -lactamase producers

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29 **Background.** OP0595 is a diazabicyclooctane which (i) acts as PBP2-active antibacterial,
30 (ii) inhibits Class A and C β -lactamases, and (iii), like mecillinam, gives β -lactamase-
31 independent potentiation of β -lactams targeting other PBPs. We tested its behaviour
32 against β -lactam-resistant Enterobacteriaceae and non-fermenters. **Methods.** Organisms
33 were UK clinical isolates; MICs were determined by CLSI agar dilution for OP0595 alone or
34 combined at 1-4 mg/L with aztreonam, biapenem, cefepime or piperacillin. **Results.** MICs
35 of OP0595 for *Escherichia coli*, *Enterobacter*, *Citrobacter* and *Klebsiella* spp. were mostly
36 1-4 mg/L but values >4 mg/L were seen for minorities of isolates irrespective of other
37 resistances, and for 50-60% of those with ertapenem resistance involving porin loss plus
38 ESBL or AmpC activity. OP0595 MICs for *Serratia*, Proteeae and non-fermenters mostly
39 were >4 mg/L. When its MIC was ≤ 4 mg/L, OP0595's antibacterial activity dominated
40 combination activity. For OP0595-'resistant' (MIC >4 mg/L) isolates with Class A or C β -
41 lactamases OP0595 achieved strong potentiation of substrate β -lactams, contingent on β -
42 lactamase inhibition. β -Lactamase-independent potentiation was evident with aztreonam,
43 cefepime and piperacillin – less so for biapenem – for many OP0595-resistant
44 Enterobacteriaceae with Class B carbapenemases, which are not inhibited by OP0595.
45 OP0595 acted solely as a β -lactamase inhibitor for non-fermenters. **Conclusions.** OP0595
46 inhibited Enterobacteriaceae, not non-fermenters; its combinations had broad activity
47 *versus* Enterobacteriaceae, largely contingent on OP0595's antibacterial activity but also
48 on inhibition of class A and C β -lactamases and on the β -lactam-enhancer effect, which
49 allowed activity against many OP0595-resistant metallo- β -lactamase-producing
50 Enterobacteriaceae. For non-fermenters OP0595 acted only as a β -lactamase inhibitor.

51

52 **Introduction**

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

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

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

74

75 **Materials and methods**

76 *Antibiotics*

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

79

80 *Bacteria*

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

91

92 *Susceptibility testing*

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

97

98 **Results**

99 *Antibacterial activity of OP0595*

100 MIC distributions of OP0595, by species and irrespective of resistance mechanisms, are
101 shown in Table 1. MICs for *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. were
102 mostly 1-4 mg/L, though values exceeding 4 mg/L were recorded for 9/50 *Enterobacter*
103 spp. and 26/118 *Klebsiella* spp. MICs of OP0595 for all the *Morganella morganii* and
104 12/15 *Serratia* spp. isolates exceeded 4 mg/L, as did those for 29/30 *Acinetobacter* and the
105 *P. aeruginosa* isolates tested. MICs were largely unrelated to known resistance
106 mechanisms, except that values were >4 mg/L for 15/28 ertapenem-resistant, porin-
107 deficient *E. coli*, *Klebsiella* and *Enterobacter* spp. with ESBL or AmpC enzymes versus
108 6/100 ertapenem-susceptible isolates of *E. coli*, *Klebsiella* and *Enterobacter* spp. or
109 *Citrobacter* spp.) with the same β -lactamases ($p < 0.001$, chi-squared test). These data
110 suggest that porin loss can restrict entry of OP0595.

111

112 *OP0595 in combination with β -lactams versus Enterobacteriaceae*

113 Susceptibility data for OP0595 in combination with aztreonam, biapenem, cefepime and
114 piperacillin are summarised in Table 2. For convenient review, results are graded against
115 the more stringent of CLSI⁹ or EUCAST (<http://www.eucast.org>) criteria for the partner β -
116 lactam. These values were considered to represent the lowest breakpoints that any
117 regulatory agency might reasonably adopt. They should *not* be construed as definitive
118 breakpoints, particularly for ceftazidime/avibactam, which has recently been given a
119 breakpoint of 8+4 mg/L by the FDA, based upon a 2+0.5 g every 8h regimen, and where
120 CLSI and EUCAST reviews are pending.

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

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

129 Combination performance against Enterobacteriaceae was largely dominated by the
130 antibacterial activity of OP0595 itself, since the concentrations used (1-4 mg/L) were
131 equalled or exceeded the molecule's MIC for most of the test strains (Table 2). With
132 OP0595 at 4 mg/L, $>80\%$ 'susceptibility' was achieved by all combinations *versus* all
133 resistance groups, including MBL producers. Susceptibility rates $\geq 90\%$ were achieved for
134 all combinations except (i) biapenem/OP0595 against AmpC-derepressed strains, where
135 performance was constrained by the inclusion of *M. morgani*, a species that was inherently
136 less susceptible than other Enterobacteriaceae to both biapenem and OP0595; (ii)
137 piperacillin/OP0595 against isolates that combined ESBL or AmpC activity with porin loss,
138 and (iii) cefepime/OP0595 against MBL producers – where the more striking point is that
139 35/40 isolates were susceptible and that the aztreonam/, biapenem/ and piperacillin/
140 OP0595 combinations did achieve 90% activity, even though only 31/40 isolates (77.5%)
141 were inhibited by OP0595 itself at 4 mg/L.

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

149

150 *OP0595 combinations versus Enterobacteriaceae resistant to OP0595 4 mg/L*

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

155 Strong potentiation was seen for ESBL substrates - i.e. aztreonam, cefepime and
156 piperacillin - against OP0595-resistant ESBL producers. Similarly, strong potentiation was
157 seen for AmpC substrates (aztreonam, piperacillin) against AmpC-hyperproducing
158 OP0595-resistant *E. coli*, *Enterobacter* and *Klebsiella* spp. Weaker potentiation was seen
159 for cefepime against AmpC producers, doubtless because cefepime is a weaker substrate
160 for AmpC enzymes; nevertheless, and notably, the geometric mean MIC of cefepime for
161 ertapenem-resistant porin-deficient AmpC hyperproducers was reduced from 6.4 mg/L to
162 0.89 with OP0595 at 1 mg/L and to 0.076 mg/L with OP0595 at 4 mg/L. More generally,
163 higher concentrations of OP0595 were needed to potentiate partner agents against
164 ertapenem-resistant than against ertapenem susceptible-strains with ESBL and AmpC β -
165 lactamases, again implying that porin loss restricts uptake of the diazobicyclooctane

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

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

178 against ESBL and AmpC producers with the distinction, compared with aztreonam/OP0595
179 and cefepime/OP0595, that derepressed *M. morganii* AmpC gave greater (avibactam-
180 reversed) resistance to ceftazidime than to either aztreonam or cefepime. For almost all
181 groups with ESBLs or AmpC enzymes, the geometric MICs of aztreonam-, cefepime- and
182 piperacillin/OP0595 4 mg/L were lower than those of ceftazidime/avibactam 4 mg/L for the
183 same isolates. For ertapenem-resistant, porin-deficient organisms with ESBL and AmpC,
184 the geometric mean MICs of aztreonam-, cefepime/OP0595 and piperacillin/OP0595 were
185 <1 mg/L, thus falling into the 'susceptible' range, whereas values for ceftazidime/avibactam
186 were 1.2 - 2.5 mg/L, falling into EUCAST's intermediate MIC category for ceftazidime.

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

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

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

213 Turning, lastly, to the MBL producers: these comprise five aztreonam- susceptible
214 or intermediate (MIC 0.25-2 mg/L) Enterobacteriaceae that were deduced to lack
215 secondary ESBL or AmpC activity and four aztreonam-resistant organisms (MIC ≥ 64 mg/L)
216 that putatively had ESBL or AmpC activity. Strong potentiation of aztreonam by OP0595
217 was seen for the aztreonam-resistant organisms, probably reflecting inhibition of these
218 ESBLs or AmpC enzymes; more interestingly, however, and even at 2 mg/L, OP0595
219 reduced aztreonam MICs by eight-fold or more for 3/5 aztreonam-susceptible organisms; at
220 4 mg/L it achieved this effect, which cannot readily be explained by β -lactamase inhibition,
221 for all five organisms.

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

231

232 *P. aeruginosa* and *A. baumannii*

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

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

243 OP0595 4 mg/L achieved c. 2.4 fold-potentiation of biapenem for the control *P.*
244 *aeruginosa* strains, 4.3-fold potentiation for imipenem-susceptible (putatively OprD-
245 expressing) AmpC-derepressed organisms and 5.4-fold potentiation for
246 imipenem/biapenem-resistant (OprD-deficient) AmpC derepressed isolates. Previous
247 experience shows that biapenem, like imipenem, is weakly affected even by inducible *P.*
248 *aeruginosa* AmpC, and that this protection confers significant resistance if the organism
249 also becomes impermeable via loss of OprD.¹² The present results are compatible with
250 these earlier findings, and with the fact that OP0595 inhibits the activity of purified
251 pseudomonal AmpC enzyme.³ Similar potentiation is seen between imipenem and another
252 diazabicyclooctane, MK-7655;¹³ also between imipenem and AmpC-inhibitory penems,
253 such as BRL42715¹⁴ and bridged monobactams, e.g. Ro-48-1256.¹⁵

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

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

262

263 **Discussion**

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

269 The antibacterial activity of OP0595, which is associated with inhibition of PBP2,³ is
270 the simplest of these activities to define and was strongest against *E. coli*, *Klebsiella* and
271 *Enterobacter* spp. As with mecillinam,¹⁶ which also exclusively attacks PBP2,³ activity was
272 weaker against *Morganella* and *Serratia* spp. than against other Enterobacteriaceae. *P.*
273 *aeruginosa* and *A. baumannii* were more resistant. This behaviour seems likely largely to
274 reflect target insensitivity, or dispensability¹⁷ since OP0595 continued to act as an inhibitor
275 of AmpC enzymes in all these species proving that it must be able to permeate them and
276 evade efflux.

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

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

292 Evidence for the enhancer effect, which was unique to Enterobacteriaceae, came
293 from OP0595's frequent potentiation of β -lactams that were not substrates for the
294 organism's β -lactamase, particularly for the common, though not universal, potentiation of
295 aztreonam and cefepime against aztreonam-susceptible (i.e. ESBL- and AmpC-negative)
296 OP0595-resistant MBL-producing Enterobacteriaceae (Table 4). Potentiation was less
297 consistent or extensive for biapenem and these findings are in keeping with the view that
298 the enhancer effect arises when the PBP2-directed activity of OP0595 is combined with
299 PBP3-targeted agents³ rather than those, like biapenem, that strongly bind PBP2.¹⁸ This
300 view also accommodates the stronger potentiation of piperacillin than biapenem for strains
301 with OXA-48 carbapenemase, even though both β -lactams are substrates for this
302 enzyme.^{12,19}

303 Given that the enhancer activity was demonstrable in OP0595-resistant strains, and
304 was retained in an OP0595-resistant mutant,³ it clearly does not require the antibacterial
305 activity of OP0595. A plausible explanation lies in the observation that resistance to
306 mecillinam commonly reflects compensatory mutations up-regulating FtsQAZ²⁰⁻²² or
307 increasing cellular levels of the regulatory molecule ppGpp,²³ not to changes to PBP2 itself.
308 It may be that these mutations prevent inhibition of PBP2 leading to cell death but that, with
309 PBP2 still present and inhibited, the enhancer activity remains. This hypothesis is
310 compatible with the observation that an OP0595-selected *E. coli* mutant, lacked sequence
311 changes to the PBP2 gene, *pbp2*.³

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

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

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338

339 **Transparency declaration**

340 **DML:** Advisory Boards or ad hoc consultancy – Accelerate, Achaogen, Adenium, Alere,
341 Allecra, Altermune, Astellas, AstraZeneca, Auspherix, Basilea, Bayer, BioVersys, Cubist,
342 Curetis, Cycle, Discuva, Forest, GSK, Meiji, Pfizer, Roche, Shionogi, Tetrphase,
343 VenatoRx, Wockhardt; Paid lectures – AOP Orphan, Astellas, AstraZeneca, Bruker,
344 Curetis, Merck, Pfizer, Leo; shareholdings in– Dechra, GSK, Merck, Perkin Elmer,
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350 Cubist, UK Department of Health, UK Food Standards Agency, Glaxo SmithKline, Henry
351 Stewart Talks, IHMA, Melinta, Merck Sharpe & Dohme, Meiji, Momentum Biosciences,
352 Nordic, Norgine, Rempex, Rokitan, Smith & Nephew, Trius , VenatoRx, Wockhardt.

353

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Table 1. MIC distribution of OP0595 by species

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

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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

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- a 20 *E. coli*, 20 *Enterobacter* spp., 20 *K. pneumoniae*
- b Aztreonam, biapenem, cefepime and ceftazidime all categorised as S \leq 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.

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Table 3. Response of carbapenemase-negative Enterobacteriaceae isolates with OP0595 MICs >4 mg/L to OP0595 combinations

	Aztreonam+OP0595 at (mg/L)				Biapenem+OP0595 at (mg/L)				MICs (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, <i>E. coli</i>, <i>Enterobacter</i> spp., <i>Klebsiella</i> 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 <i>M. morgani</i> (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 <i>S. marcescens</i> (n=7)																		
Range	0.12- 8	0.12 -1	0.03- 0.5	≤0.02 -0.12	0.12 -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
<i>E. coli</i>, <i>Enterobacter</i> spp., <i>Klebsiella</i> 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	<i>E. cloacae</i>	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	<i>E. cloacae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>E. cloacae</i>	IMP	0.5	0.5	0.12	≤0.02	16	32	16	8	128	256	256	16	64	128	32	2	>256	>256
H555	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	IMP	64	0.25	≤0.02	≤0.02	8	16	2	2	128	128	16	2	>256	32	2	0.25	>256	>256
H538	<i>Klebsiella</i> 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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	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	<i>K. pneumoniae</i>	NDM	>256	0.25	0.06	0.06	16	16	16	16	256	64	16	16	>256	>256	32	32	>256	>256

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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	Alone	+OP0595	Alone	+OP0595	Alone	+OP0595	Alone	+avibactam
			4 mg/L		4 mg/L		4 mg/L		4 mg/L		4 mg/L
<i>P. aeruginosa</i>											
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
<i>A. baumannii</i>											
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

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