1 **Activity of ceftazidime/avibactam against problem Enterobacteriaceae and**

2 ***Pseudomonas aeruginosa* in the UK, 2015-2016**

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20 Running head: Ceftazidime/avibactam versus gram-negatives

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23   **Abstract**

24   **Introduction.** Ceftazidime/avibactam combines an established oxyimino-cephalosporin with

25   the first diazabicyclooctane β-lactamase inhibitor to enter clinical use. We reviewed activity

26   against Gram-negative isolates, predominantly from the UK, referred for resistance

27   investigation in the first year of routine testing, beginning July 2015. **Methods.** Isolates were

28   as received from referring laboratories; there is a bias to submit those with suspected

29   carbapenem resistance. Identification was by MALDI-ToF mass spectroscopy, and

30   susceptibility testing by BSAC agar dilution. Carbapenemase genes were sought by PCR;

31   other resistance mechanisms were inferred using genetic data and interpretive reading.

32   **Results.** Susceptibility   rates   to   ceftazidime/avibactam   exceeded   95%   for: (i)

33   Enterobacteriaceae   with   KPC,   GES   or   other   Class   A   carbapenemases,   (ii)

34   Enterobacteriaceae with OXA-48-like enzymes and (iii) for ESBL or AmpC producers, even

35   when these had impermeability-mediated ertapenem resistance. Almost all isolates with

36   metallo-carbapenemases were resistant. Potentiation of ceftazidime by avibactam was seen

37   for 87% of ceftazidime-resistant Enterobacteriaceae with 'unassigned' ceftazidime resistance

38   mechanisms, including two widely referred groups of *Klebsiella pneumoniae* where no synergy

39   was seen between cephalosporins and established β-lactamase inhibitors. Potentiation here

40   may be a diazabicyclooctane/cephalosporin enhancer effect. Activity was seen against

41   *Pseudomonas aeruginosa* with derepressed AmpC, but not for those with efflux-mediated

42   resistance. **Conclusions.** Of   available   β-lactams   or   inhibitor   combinations,

43   ceftazidime/avibactam has the widest activity spectrum against problem Enterobacteriaceae,

44   covering all major types except metallo-carbapenemase producers; against *P. aeruginosa* it

45   has a slightly narrower spectrum than ceftolozane/tazobactam, which also covers efflux-type

46   resistance.

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Introduction

Ceftazidime/avibactam is the first β-lactam/diazabicyclooctane β-lactamase inhibitor combination to enter clinical use.[1] Avibactam inhibits most ceftazidime-hydrolysing Class A and C β-lactamases, including KPC carbapenemases as well as ESBLs and AmpC enzymes;[2,3] ceftazidime is anyway stable to OXA-48-like carbapenemases[4] and has good antipseudomonal activity. Consequently, the combination has the potential for wide activity against Enterobacteriaceae with these problem β-lactamases and against *Pseudomonas aeruginosa* with derepressed AmpC.[5,6] β-Lactamases that evade inhibition by avibactam include metallo-carbapenemases and the OXA carbapenemases of *Acinetobacter* spp.[2,3]

PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit added ceftazidime/avibactam to its antibiotic panel, tested against all referred Gram-negative submissions, in July 2015. We review here our experience over the subsequent 12 months.

Materials and methods

Isolates

Bacteria were as referred: around 90% were from English diagnostic laboratories, 9% from other parts of the UK and 1% from overseas, principally the Republic of Ireland. Most were submitted owing to unusual resistance and there was a strong current bias towards referral of isolates suspected of carbapenem resistance, though a few were sent because they were unusually susceptible, were resistant to non-β-lactam agents or because the sender had obtained discrepant results between different test methods. We excluded isolates tested or re-tested for internal and external quality assurance and repeat/multiple tests on the same isolate from the same submission.

Data were reviewed for one year starting from July 2015, when we began to test ceftazidime/avibactam routinely; the drug was not licensed or in significant use during this

75 period.   Numbers of isolates are slightly lower than in a similar analysis for

76 ceftolozane/tazobactam[7] owing to a test failure with ceftazidime/avibactam in one week.

77

78 *Identification and resistance investigation*

79 Bacteria were identified by MALDI-ToF mass spectroscopy (Bruker Daltonics, Bremen,

80 Germany) and MICs determined by BSAC agar dilution.[8] Asides from ceftazidime/avibactam

81 4 mg/L, we tested clinically-used $\beta-$lactams alone or in combination with fixed concentrations

82 of inhibitors as follows: ampicillin, amoxicillin/clavulanate 2 mg/L, aztreonam, carbenicillin,

83 cefepime, cefotaxime, cefoxitin, ceftazidime, ceftolozane/tazobactam 4 mg/L, ertapenem,

84 imipenem, meropenem, piperacillin/tazobactam 4 mg/L and temocillin. To help predict

85 $\beta-$lactamase   types,   we   additionally   tested   cefotaxime/clavulanate   2   mg/L,

86 cefotaxime/cloxacillin 100 mg/L, ceftazidime/clavulanate 2 mg/L, cefepime/clavulanate 2 mg/L

87 and imipenem/EDTA 320 mg/L.

88   Genes for KPC, VIM, NDM and OXA-48-like carbapenemases were sought by multiplex

89 PCR[9] in all Enterobacteriaceae submitted owing to suspected carbapenem resistance and in

90 those submitted for other reasons, but found to have phenotypes suggesting carbapenemase

91 production. Enterobacteriaceae found negative for these commonest carbapenemases, but

92 with phenotypes suggesting carbapenemase production were examined with further multiplex

93 PCRs seeking (i) bla_{IMP}, bla_{SPM}, bla_{GIM}, bla_{SIM}[10] or (ii) bla_{FRI}, bla_{GES}, bla_{IMI}, and bla_{SME}.[11] The first

94 of these multiplexes was also used for *P. aeruginosa* isolates showing imipenem/EDTA

95 synergy together with broad resistance to penicillins and cephalosporins.

96   The genomes of carbapenemase producers with unusual behaviour were sequenced,

97 using Illumina methodology, as were representatives of two unusual phenotypes of *Klebsiella*

98 *pneumoniae* (see Results). Sequenced genomes were searched against our locally-curated

99 database of antimicrobial resistance determinants using AMRHAI's GeneFinder algorithm.[12]

100 Searches for new β–lactamases were performed on assembled-contigs translated in the six

101 possible reading frames using PSI-BLAST (position-specific iterated BLAST) and the HMM-

102 based (Hidden Markov Models) method in the HMMer software suite (v3.1).[13] HMMER

103 searches were performed at increasingly stringent thresholds using the β-lactamase-related

104 pfam domains obtained from public databases.[14] Clover leaf/Hodge tests were performed on

105 selected organisms, seeking to detect hydrolysis of carbapenems (using 10 μg ertapenem,

106 imipenem and meropenem discs) or oxyimino-cephalosporins (using 30 μg cefepime,

107 cefotaxime and ceftazidime discs); *Escherichia coli* ATCC 25922 was the indicator organism

108 throughout.

109

110 *Categorisation of isolates by resistance mechanisms*

111 Molecular detection of a carbapenemase gene was considered definitive. Mechanisms in

112 isolates lacking carbapenemase genes were assigned based on interpretive reading[15,16] of

113 phenotypes, using an in-house algorithm. Two levels of match were allowed: 'Hard', where the

114 phenotype was a perfect match and 'Soft', where the phenotype was less perfect, but the

115 mechanism remained the most likely.[7] Some isolates did not match any well-recognised

116 phenotype considered and were left as 'unassigned'.

117

118 **Results**

119 *Distribution of resistance mechanisms by species group*

120 Among the 3144 referred Enterobacteriaceae isolates tested, 907 (28.8%) had

121 carbapenemase genes, predominantly bla_{OXA-48}-like, bla_{NDM} or bla_{KPC}, while 898 (28.6%)

122 had AmpC phenotypes and lacked carbapenemase genes and 655 (20.8%) had

123 ESBL phenotypes, again lacking carbapenemase genes (Table 1). Fully 80% of the

124 AmpC producers and 58.5% of the ESBL producers were non susceptible to ertapenem at

125 EUCAST's 0.5 mg/L breakpoint, whilst 13.7% and 6.3%, respectively were non-susceptible to

126 meropenem at 2 mg/L. These proportions considerably exceed those for AmpC and ESBL

127 producers in general [17,18] and we infer that many of these organisms also had reduced

128 permeability, which is a general correlate of ertapenem resistance among AmpC and ESBL

129 producers.[19]

130   Smaller numbers of isolates had phenotypes suggesting: (i) co-production of AmpC

131 and ESBL enzymes, with clavulanate potentiating cefepime, but not ceftazidime or cefotaxime

132 (n=71, 2.3%); (ii) hyper-production of K1 enzyme (in *K. oxytoca* isolates, n=8, 0.25%), or (iii)

133 reduced permeability alone (n=85, 2.7%). One hundred and forty-one referrals (4.5%) had

134 wild-type phenotypes with respect of β−lactams; these mostly had been submitted owing to

135 resistance to other antibiotic classes. Finally, 379 (12.1%) had resistance patterns that were

136 not predictive of any particular mechanisms: these varied widely in their phenotypes of

137 resistance to different β-lactams, but universally lacked cephalosporin/clavulanate or

138 cefotaxime/cloxacillin synergy (see below).

139

140 *Isolates with carbapenemases*

141 Modal ceftazidime MICs for isolates with KPC enzymes fell from 16 mg/L to 0.5 mg/L when

142 avibactam was added, and those for isolates with GES enzymes from 256 to 1 mg/L (Table

143 2). Only two isolates with KPC carbapenemases – an *Enterobacter* sp. and a *K. pneumoniae*,

144 were resistant to ceftazidime/avibactam at its 8+4 mg/L breakpoint. Resistance was stable in

145 the *K. pneumoniae* isolate, where genome sequencing revealed classical *bla*KPC-2, without the

146 mutations associated with ceftazidime/avibactam resistance.[20,21] Resistance in the

147 *Enterobacter* was lost on subculture, precluding investigation. Eleven isolates had other class

148 A carbapenemases – specifically IMI, SME and FRI types. These were resistant to ertapenem

149 (MICs 4->16 mg/L) and non-susceptible to either or both of imipenem (MICs 8->128 mg/L)

150 and meropenem (MICs 4->32 mg/L, except one IMI isolate, 0.12 mg/L); all except one were

151 susceptible or borderline resistant to unprotected ceftazidime (MICs 0.25-2 mg/L), with only

152 limited avibactam synergy, e.g. for the *E. cloacae* strain with FRI-2,[22] where the ceftazidime

153 fell from 0.5 to 0.25 mg/L.

154   The MIC distribution of ceftazidime for OXA-48 Enterobacteriaceae was bimodal, with

155 peaks at 0.5 and >256 mg/L; 34.8% of isolates inhibited by unprotected ceftazidime at

156 EUCAST's 1 mg/L susceptible breakpoint and 45.0% at the 4 mg/L resistance breakpoint.

157 With avibactam added, this distribution became unimodal, with a peak at 0.25 mg/L and 94%

158 of MICs between 0.12 and 2 mg/L. Potentiation was ≤4-fold for isolates with ceftazidime MICs

159 ≤1 mg/L, but 128- to 1024-fold for those with high-level ceftazidime resistance. Five OXA-48

160 isolates (two *K. pneumoniae* from separate hospitals and single *K. oxytoca*, *E. coli* and *C.*

161 *freundii*) tested as resistant to ceftazidime/avibactam, with MICs >32+4 mg/L but this was not

162 confirmed on retesting and was not pursued further.

163   Isolates with metallo-carbapenemases consistently were resistant to ceftazidime and

164 remained so with avibactam added. The few exceptions to this generalisation were *E. coli* that

165 were inhibited by avibactam alone at 4 mg/L (Table 2).

166

167 *Isolates with ESBLs, AmpC and other mechanisms*

168 As already stressed, referred AmpC and ESBL producers are biased towards those with

169 reduced susceptibility to carbapenems. To accommodate this bias, ceftazidime/avibactam

170 MICs for these isolates are shown, ESBL producers (Table 3) and AmpC

171 hyperproducers(Table 4), in relation to those of ertapenem, as a proxy for impermeability. The

172 AmpC isolates mostly were *Enterobacter* spp., where ertapenem MICs of 1-2 mg/L are typical

173 for AmpC-derepressed strains; the ESBL producers were mostly *E. coli* and *K. pneumoniae*

174 (Table 1).

175   Among the ESBL producers, 96.2% were non-susceptible to ceftazidime 1 mg/L and

176 77.8% were highly resistant, with MICs 32->256 mg/L; corresponding proportions among the

177   AmpC producers were 93.9% and 74.1%, respectively. With avibactam added, the ceftazidime

178   MICs were reduced to <8+4 mg/L (i.e. susceptible) for 99.7% of ESBL producers and 98.3%

179   with AmpC. The MICs of ceftazidime/avibactam for ESBL producers trended upwards as the

180   ertapenem MIC increased from 0.12 to 1 mg/L, but with little further rise for highly-ertapenem

181   resistant isolates. This behaviour contrasted to ceftazidime/clavulanate (not shown) and

182   ceftolozane/tazobactam,[7] where MICs rose progressively with the ertapenem MIC. MICs of

183   ceftazidime/avibactam for AmpC producers did rise in parallel with ertapenem MICs but the

184   combination remained active against 109/115 isolates with ertapenem MICs >16 mg/L. Fifteen

185   of the 898 AmpC producers were resistant to ceftazidime/avibactam 8+4 mg/L; four of these

186   were *Hafnia alvei* (*versus* 12 *H. alvei* among the whole 898) and eight were 'Soft

187   matches'(*versus* 65 Soft matches among the 898) implying a greater risk that they were mis-

188   categorisations or had secondary mechanisms. Two Soft-matched ESBL *K. pneumoniae* were

189   resistant to ceftazidime/avibactam; both were among the most–highly-ertapenem resistant

190   (MICs >16 mg/L) and probably represent extreme examples of impermeability.

191   Among isolates with both AmpC and ESBL activity, 69/71 (97.2%) were susceptible to

192   ceftazidime/avibactam 8+4 mg/L whereas MICs of unprotected ceftazidime were >128 mg/L

193   in 66/71 cases.. Only eight K1 β-lactamase-hyperproducing *K. oxytoca* were included: these

194   had characteristic resistance to piperacillin/tazobactam and aztreonam, but with MICs around

195   EUCAST breakpoints for oxyimino-cephalosporins and 4- to 32-fold cefepime/ and

196   cefotaxime/clavulanate synergy.[15,16] MICs of unprotected ceftazidime were from 0.25-2 mg/L,

197   falling to 0.12-1 mg/L with avibactam added. Last, among characterised groups, 85 isolates

198   were inferred solely to have reduced permeability, with cefoxitin MICs >32 mg/L and

199   ertapenem MICs (>0.5 mg/L in 64/85 cases). Oxyimino-cephalosporin MICs remained around

200   breakpoints (0.5-4 mg/L) with (i) no differential between cefepime and other oxyimino-agents,

201   and (ii) no cephalosporin synergy with cloxacillin or clavulanate. MICs of unprotected

202   ceftazidime were 0.5-4 mg/L and remained in this range with ceftazidime/avibactam in 71/85

203   cases, falling slightly for the remaining 14.

204

205   *Unassigned isolates*

206   The 379 organisms with unassigned mechanisms were dominated by *K. pneumoniae* (n=203)

207   and *E. coli* (n=124) (Table 1). The major common feature, along with some degree of

208   cephalosporin resistance, was the absence of synergy between cephalosporins and

209   clavulanate or cloxacillin, and between imipenem and EDTA. The lack of

210   ceftazidime/clavulanate synergy is illustrated in fig 1a. Prior to adding ceftazidime/avibactam

211   to the test panel, we believed that these isolates mostly had β-lactamase-independent, modes

212   of resistance but subsequently were surprised by the large proportion with potentiation was

213   seen. Thus, among all 379 isolates, 199 were resistant to ceftazidime 8 mg/L and 195 to

214   ceftazidime/clavulanate 8+2 mg/L but only 26 to ceftazidime/avibactam 8+4 mg/L (fig 1b).

215   Two regularly-seen *K. pneumoniae* phenotypes ('Type I' and 'Type II') accounted for

216   many of these isolates, and MIC data are illustrated in Table 5. Type I isolates were resistant

217   to cefepime and ceftazidime, with MICs 8-64 mg/L, but remained borderline susceptible to

218   cefotaxime, with MICs 1-4 mg/L. Type II isolates were resistant to all three oxyimino-

219   cephalosporins, with MICs 32->256 mg/L.   Both types were resistant to cefoxitin,

220   piperacillin/tazobactam, and amoxicillin/clavulanate. Temocillin MICs were raised above the

221   4-8 mg/L values typical for *K. pneumoniae*, but mostly remained ≤64 mg/L. Carbapenem MICs

222   were raised, with almost all non-susceptible to ertapenem at EUCAST's 0.5 mg/L breakpoint;

223   many, particularly among Type II isolates, were highly resistant, with MICs >16 mg/L. Both

224   types have been referred from multiple hospitals over the past 3-4 years and are non-clonal,

225   based on Variable Number Tandem Repeat typing.[22] They varied in fluoroquinolone and

226   aminoglycoside susceptibility. Crucially, while cephalosporin MICs were not reduced by

227   clavulanate or cloxacillin, those of ceftazidime were reduced by avibactam, mostly falling to

228   1-4 mg/L.

229   Whole genome sequencing of 10 Type I representatives, mostly pre-dating the present

230   series, confirmed clonal diversity and found seven to have only the SHV-1 β-lactamase typical

231   of *K. pneumoniae*, without mutations to the coding or promoter sequences; single

232   representatives had SHV-27 (an ESBL), SHV-36 (unknown spectrum) or SHV-1 plus TEM-10

233   (an ESBL). Increased read depth, relative to *gyrA* and *parC*, suggested that bla_{SHV} was

234   amplified in most cases whilst *ompK35* was inactivated by an identical frame shift mutation in

235   all isolates and *ompK36* was inactivated in most by various mutations or insertions. The genes

236   encoding the essential PBPs (1, 2 and 3) were conserved, without mutations. Sequencing of

237   four Type II isolates variously revealed CTX-M-15 plus OXA-1, CMY-42 plus OXA-1, CTX-M-

238   15, OXA-1 plus SHV-53 and CTX-M-33, OXA-1, SHV-11 and TEM-1.

239   None of the genetic changes seen for Type I isolates adequately explains their phenotypes

240   (see Discussion). Further bioinformatic analysis failed to find motifs suggesting additional

241   β−lactamase genes, and clover leaf (Hodge) tests were negative for both carbapenems and

242   oxyimino-cephalosporins.

243

244   *Pseudomonas aeruginosa*

245   Data were obtained for 1384 *P. aeruginosa.* Analysis must be cautious because, unlike for

246   Enterobacteriaceae, we used ceftazidime/avibactam in categorising these isolates,[7]

247   distinguishing those with derepressed AmpC (carbenicillin MIC $\leq$128 mg/L, cefotaxime MIC >

248   carbenicillin MIC and ceftazidime MIC > 4x ceftazidime/avibactam MIC) from those with up-

249   regulated efflux (carbenicillin, piperacillin/tazobactam and ceftazidime MICs raised in

250   approximate proportion, without ceftazidime/avibactam potentiation).

251   Among 147 putative AmpC-derepressed *P. aeruginosa*, 94.6% were susceptible to

252   ceftazidime/avibactam 8+4 mg/L versus 21.0% to ceftazidime 8 mg/L and 96.6% to

253   ceftolozane/tazobactam 4+4 mg/L. Among 388 with moderately raised efflux (carbenicillin

254   MICs 256-512 mg/L), 86.1% were susceptible to ceftazidime/avibactam, 65.7% to ceftazidime

255   and 99.7% to ceftolozane/tazobactam. Among 149 with highly raised efflux (carbenicillin MICs

256   >512 mg/L), 41.6% were susceptible to ceftazidime/avibactam, 27.5% to ceftazidime and

257   95.3% to ceftolozane/tazobactam. The gain versus AmpC-derepressed isolates doubtless

258   reflects β-lactamase inhibition of; that versus 'efflux isolates' was largely a thresholding effect,

259   with the ceftazidime MIC reduced from 16 to 8 mg/L thus crossing the breakpoint but remaining

260   within one doubling dilution of the ceftazidime value. Four hundred and ten *P. aeruginosa*

261   isolates were non-susceptible to *all* of carbenicillin, piperacillin/tazobactam, ceftazidime,

262   imipenem and meropenem at EUCAST breakpoints. Of these, 28.7% were susceptible to

263   ceftazidime/avibactam 8+4 mg/L and 52.6% to ceftolozane/tazobactam 4+4 mg/L, rising to

264   43.3% and 81.6%, respectively, if isolates with metallo-carbapenemases (n = 118, mostly VIM

265   types), ESBLs (n = 31 mostly VEB) or GES enzymes (n = 4) were excluded.

266

267   **Discussion**

268   These data are for 'problem' isolates sent to PHE's reference laboratory, and therefore with a

269   heavy bias to resistance. They show ceftazidime/avibactam broadly active against: (i)

270   Enterobacteriaceae with KPC, GES and other class A carbapenemases, (ii)

271   Enterobacteriaceae with OXA-48-like enzymes, irrespective of susceptibility to ceftazidime

272   alone, and (iii) Enterobacteriaceae with ESBLs or AmpC enzymes, irrespective of the

273   impermeability traits that confer resistance to ertapenem. Lastly, ceftazidime/avibactam 8+4

274   mg/L remained active against 87% (fig. 1b) of the 199 Enterobacteriaceae with unassigned

275   mechanisms, but which were resistant to ceftazidime alone at 8 mg/L, including members of

276   the widely encountered Type I and II phenotypes of *K. pneumoniae* illustrated in Table 5.

277   Activity against KPC-, ESBL- and AmpC- producers is in keeping with the known ability

278   of avibactam to inhibit these enzymes.[2,3] Ceftazidime itself remains active against a sizeable

279   minority of Enterobacteriaceae with OXA-48-like enzymes, whereas others are highly

280   resistant, as illustrated by the bi-modal MIC distribution in Table 2. The explanation is that

281   OXA-48-like enzymes do not, themselves,[4] attack ceftazidime, but that many producers also

282   have further mechanisms - most often ESBLs[23] - that confer resistance. Avibactam gave weak

283   potentiation of ceftazidime against ceftazidime-susceptible isolates with OXA-48-like

284   enzymes, but strongly potentiated ceftazidime against those with high-level resistance,

285   presumably via inhibition of these secondary β-lactamases.

286   The only major gaps in ceftazidime/avibactam's spectrum, as is well recognised,[2,3]

287   were metallo-carbapenemase producers. These accounted for a little over one-third of

288   carbapenemase-producing Enterobacteriaceae referred to AMRHAI (302/873 = 34.6% in the

289   period reviewed). Their actual proportion may be lower since: (i) isolates with KPC

290   carbapenemases are concentrated in a few hospitals in Northwest England, which no longer

291   refer all producers, and (ii) isolates with metallo-carbapenemases, particularly NDM, are highly

292   resistant and unlikely to be missed, whereas many with OXA-48-like enzymes have marginal

293   carbapenem resistance, likely leading to under-detection. Proportions of non-metallo- versus

294   metallo-carbapenemases vary globally, with KPC types predominating in the Americas, Italy,

295   Greece and China; OXA-48 in Turkey, Romania and Spain, and NDM in South Asia; strains

296   with both OXA-48 and NDM appear prevalent in the Middle East.[24,25]

297   A few isolates with KPC and OXA-48 enzymes were resistant to ceftazidime/avibactam

298   on primary testing, but resistance was only confirmed for one *K. pneumoniae* with a KPC

299   carbapenemase. It is impossible to ascertain whether initial results for the others were in error

300   or whether unstable resistance had been lost. Sequencing revealed that the stably-resistant

301   *K. pneumoniae* isolate produced KPC-2 carbapenemase and its behaviour possibly reflected

302   the activity of this enzyme together with impermeability. It lacked the bla_{KPC} mutations

303   associated with emerging ceftazidime/avibactam resistance during therapy, and found also in

304   mutants generated *in vitro*; these cluster around the Ω-loop and increase affinity for

305   ceftazidime, protecting against binding of avibactam.[20,21,26] Emerging resistance to

306   ceftazidime/avibactam in an isolate with an OXA-48 enzyme was associated with Pro170Ser

307 and Thr264Ile substitutions to a co-produced CTX-M-14 ESBL, without changes to OXA-48

308 itself.[27]

309 Retained activity against isolates with combinations of ESBL or AmpC and

310 impermeability was striking. Although such strains rarely cause outbreaks and often are

311 unstable, they are not infrequent and can be selected during carbapenem therapy,

312 complicating treatment .[28,29]

313 The broad activity of ceftazidime/avibactam against ceftazidime-resistant isolates with

314 unassigned mechanisms is intriguing, especially as these were almost all resistant to

315 ceftazidime/clavulanate (fig. 1). The obvious explanation is that these isolates have

316 unsuspected β–lactamases, inhibited by avibactam, but not by clavulanate, cloxacillin or

317 tazobactam. However, for the two largest groups, i.e. the Type I and II *K. pneumoniae* in

318 Table 5 – we have been unable to find any such enzyme: the Type I isolates largely have an

319 increased copy number classical bla_{SHV-1}, which is chromosomal and ubiquitous in *K.*

320 *pneumoniae*,[30] along with inactivation of *ompK35* and *ompK36,* whilst the Type II unknowns

321 had various ESBL or AmpC enzymes. Further analysis has concentrated on the Type I isolates

322 as the simpler case. A quarter century ago, Petit *et al.*[31] cautiously associated increased

323 expression of SHV-1 enzyme with resistance to ceftazidime but not cefotaxime in *K.*

324 *pneumoniae*, as in our Type I isolates. However, (i) their strains, unlike ours, had

325 ceftazidime/clavulanate synergy, as would be expected, and (ii) they did not seek non-β-

326 lactamase-mediated mechanisms. It may be that the porin mutations in our isolates excluded

327 clavulanate more effectively that avibactam, reconciling this discrepancy. But, if so, the

328 distinction was remarkably clear cut, whereas significant cephalosporin/clavulanate synergy

329 typically is retained for impermeable, ertapenem-resistant, ESBL producers of the type

330 detailed in the bottom rows of Table 3 (see also ref. 19). An alternative hypothesis,

331 speculative but plausible, is that these organisms have some perturbation (in the broadest

332 sense) of cell wall synthesis that simultaneously confers (a) reduced susceptibility to multiple

333 β-lactams and (b) vulnerability ceftazidime/avibactam synergy by a mechanism other than β-

334 lactamase inhibition. Potentiation of cephalosporins independently of β-lactamase inhibition

335 is a common feature of other DBOs, notably nacubactam (RG6080/OP0595) or zidebactam

336 and seems to depend on the DBO interacting with PBP2 whilst the partner β-lactam attacks

337 PBP3.[32] The absence of PBP gene changes in *K. pneumoniae* with the Type I and II

338 phenotypes does not refute these speculations, for it is established that the consequence of

339 DBO- and mecillinam- mediated inhibition of PBP2 are modulated by mutations to genes

340 involved in the stringent response rather than directly in peptidoglycan biogenesis. The threat

341 posed by these phenotypes is debatable: on the one hand they are widely scattered and

342 regularly referred, moreover the Type II isolates are very broadly resistant to β-lactams other

343 than ceftazidime/avibactam; on the other hand we have not seen outbreaks, and susceptibility

344 rates to non-β-lactams are high, particularly for Type I isolates, meaning that treatment options

345 remain (Table 5).

346 We have only included a limited analysis for *P. aeruginosa* because we used

347 ceftazidime/avibactam MICs to help categorise resistance mechanisms.[7] Nevertheless the

348 findings are entirely compatible with the view, inherently plausible and supported by previous

349 work, that avibactam substantially overcomes AmpC-mediated ceftazidime resistance,[6] but

350 not that due to efflux. Ceftolozane/tazobactam, by contrast, retains activity against >95% of

351 isolates with either of these mechanisms.[7] Neither inhibitor combination overcomes metallo-

352 carbapenemases nor VEB-type ESBL-mediated resistance in the species, but these

353 mechanisms are uncommon in the UK.

354 In summary, these data show that ceftazidime/avibactam has activity against most

355 problem Enterobacteriaceae groups seen in the UK, as referred to the national reference

356 laboratory. Its activity extends to two frequently-referred *K. pneumoniae* phenotypes where

357 ceftazidime resistance is not obviously β−lactamase-mediated; these remain under active

358 investigation. The isolates studied here pre-date clinical use of ceftazidime/avibactam in the

359 UK and, as the drug enters use, attention will need to be paid to any emergence of resistance.

360 Shields and colleagues, in Pittsburgh, saw emerging resistance in 3/31 cases where

361 ceftazidime/avibactam was used to treat severe infections due to *K. pneumoniae* ST258 with

362 KPC carbapenemases.[21] These mutations –and similar ones selected by ourselves *in vitro*-

363 make KPC enzymes into 'better' ceftazidimases,[20,26] but also reduce carbapenemase activity.

364 An interesting possibility is that co-administration of meropenem might block this route to

365 resistance, counter-selecting against any mutation that degraded carbapenemase activity and

366 thus 'forcing' the KPC enzyme to remain vulnerable to avibactam.

367

370

371 **Transparency declaration**

386 Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd,

387 Smith & Nephew UK Ltd, Trius Therapeutics, VenatoRx Pharmaceuticals and Wockhardt Ltd.

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483 **Table 1.** Referred isolates, by detected or inferred resistance mechanism

	Carbapenemases						Non-carbapenemases				Other, uncertain			
	KPC	GES	Other class A[a]	OXA-48-like	MBL[b]	NDM + OXA-48	AmpC	ESBL	ESBL+ AmpC	K1	Imperm-eable	Wild-type	Unas-signed	Grand Total
Citrobacter spp.	4			13	12		45	2	1			2	4	83
E. coli	33	4		127	93	4	116	352	42		35	33	124	963
Enterobacter spp.	26[c]	1	7	40	28		633	47	20			45	25	872
H. alvei							12						0	12
K. oxytoca	4	15		6	3			3		8	1	2	13	55
K. pneumoniae	130	3		142	160	28	49	248	8		49	18	203	1038
M. morganii					2		8					14	0	24
Providencia spp.					4		1					2	1	8
Rare fermenters	2	1		1				2				6	2	14
Serratia spp.	4	1	4	4	1		34	1				19	7	75
Grand Total	203	25	11	333	303	32	898	655	71	8	85	141	379	3144
Hard match[d]	Not applicable; molecular identification of mechanism(s)						833	599	53	8	85	141	N/A	
Soft match[d]							65	56	18	0	0	0	N/A	

484

485 [a] 6 IMI, 4 SME and 1 FRI-2.

486 [b] MBL, metallo-β-lactamases, 242 NDM, 36 VIM, 24 IMP and 1 with both IMP and NDM

487 [c] Includes one isolate also with an OXA-48 enzyme as well as a KPC type

488 [d] Hard match: phenotype perfectly matches that expected for the mechanism; Soft: phenotype best matches this mechanism, but with minor anomalies

489

Table 2. MICs of ceftazidime and ceftazidime/avibactam for carbapenemase-producing Enterobacteriaceae isolates

Enzyme	Ceftazidime +/-AVI	No isolates with indicated MIC, mg/L													
		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Class A															
KPC (202)	Alone						4	20	47	52	39	14	7	11	8
	+AVI		8	39	78	56	13	6				2*			
GES (25)	Alone								2	1		4	4	11	3
	+AVI	1			1	5	15	3							
IMI (6)	Alone			2	2		1		1						
	+AVI		1	1	4										
SME (4)	Alone					1	3								
	+AVI				2	2									
FRI-2 (1)	Alone				1										
	+AVI			1											
Class D															
OXA-48-like (333)	Alone		6	34	40	36	24	10	26	10	13	28	32	36	38
	+AVI	9	39	85	83	81	25	5	1			5*			
Class B															
NDM (242)	Alone											1			241
	+AVI	2			1						2	237*			

VIM (36)	Alone						1	6	11	13	5
	+AVI			1		7	9	19*			
IMP (24)	Alone							1	1	4	18
	+AVI						1	23*			
Multiple, no MBL											
KPC+OXA-48-like (1)	Alone						1				
	+AVI	1									
Multiple, inc. MBL											
NDM+OXA-48-like (32)	Alone				1		1				30
	+AVI		1		1			30*			
NDM+IMP (1)	Alone										1
	+AVI						1*				

491

Abbreviations: AVI, avibactam 4 mg/L; MBL, metallo-β-lactamase

493 * MIC > indicated value

494

495

496

497 **Table 3.** MICs of ceftazidime/avibactam in relation to ertapenem for referred ESBL producers

Ertapenem MIC (mg/L)	No. isolates with indicated ceftazidime/avibactam MIC (mg/L)										Grand Total
	$\leq$0.06	0.125	0.25	0.5	1	2	4	8	16	>16	
$\leq$0.12	13	**50**	**77**	**20**	3	1					164
0.25		5	**16**	**24**	9	2	1				57
0.5	3	1	**11**	**19**	6	**9**	2				51
1	3	4	3	**23**	**28**	**12**	3				76
2	7	5	4	**26**	**25**	**13**	4				84
4	3	7	11	**12**	**17**	**12**	1	2			65
8	2	2	5	**26**	**17**	**11**	1	2			66
16	2	1	4	**16**	**21**	**6**	2		1		53
>16				**5**	**23**	**5**	3	2	1		39
Grand Total	33	75	131	171	149	71	17	6	2		655
MICs of unprotected ceftazidime			3	6	16	16	36	45	80	429	

498

499 For each ertapenem MIC the three dilutions accounting for most ceftazidime/avibactam MICs are highlighted in bold

500

501 **Table 4.** MICs of ceftazidime/avibactam in relation to ertapenem for referred AmpC producers

Ertapenem MIC (mg/L)	No. isolates with indicated ceftazidime/avibactam MIC (mg/L)											Grand Total
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	>32	
<=0.12	6	**25**	**25**	**7**	1	2		1	1			68
0.25	3	2	**6**	**7**	**8**		1					27
0.5		3	**20**	**26**	**20**	9	3		1		2	84
1	1		**21**	**45**	**43**	15	3					128
2	2	6	12	**51**	**97**	**16**	4	1				189
4	2	1	3	**23**	**62**	**36**	5	1			1	134
8	1	3	6	**16**	**35**	**27**	5	2	1		1	97
16	1	2	2	**10**	**14**	**18**	6		1	1		55
>16			3	18	**28**	**26**	**22**	12	1	2	3	115
Grand Total	16	42	98	203	308	149	49	17	5	3	7	897[a]
MICs of unprotected ceftazidime		1	5	15	34	48	29	42	59	115	549	

502

503 For each ertapenem MIC the three dilutions accounting for most ceftazidime/avibactam MICs are highlighted in bold

504 [a] Total is 897 not 898 (see Table 1) owing to one test failure with ertapenem

Table 5. MICs of ceftazidime/avibactam and comparators against *K. pneumoniae* Types I and II, with unknown modes of resistance

	No isolates with indicated MIC (mg/L)													
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Type I														
Ceftazidime								3	8	17	10			
Ceftazidime/clavulanate							1	2	13	14	8*			
Ceftazidime/avibactam			2	2	9	14	5	5	1					
Cefepime								7	19	5	7			
Cefepime/clavulanate						1	3	10	12	9	3*			
Cefotaxime					12	14	12							
Cefotaxime/clavulanate				4	11	17	6							
Cefotaxime/cloxacillin				2	15	15	5		1					
Ceftolozane/tazobactam						8	15	10	5					
Piperacillin/tazobactam										1		37*		
Amoxicillin/clavulanate											1	37*		
Cefoxitin										1	3	12	22*	
Temocillin								3	18	10	7			
Aztreonam (1 nt)					1	9	16	7	16					
Ertapenem				2	2	4	3	3	10	14*				
Meropenem		4	3	4	3	10	6	6	2					
Imipenem		1	3	7	11	8	5	1	2					
Ciprofloxacin		15**	11	9	1		1		1*					
Gentamicin		3**	12	19	2		1							
Amikacin				8**	17	10	1	1			1			
Type II														

Ceftazidime									3	3	14	36	26*
Ceftazidime/clavulanate								1	14	67*			
Ceftazidime/avibactam			6	21	33	15	7						
Cefepime									3	2	77*		
Cefepime/clavulanate						1	2	6	73*				
Cefotaxime								5					77
Cefotaxime/clavulanate						3		2					77
Cefotaxime/cloxacillin						1	2	1	1			1	76
Ceftolozane/tazobactam						1		1	3	77*			
Piperacillin/tazobactam										82*			
Amoxicillin/clavulanate										82*			
Cefoxitin									2	27	53*		
Temocillin							1	5	34	38	13	1*	
Aztreonam (2 nt)							2	2			76*		
Ertapenem								16	66*				
Meropenem			1	3	12	22	34	8	2				
Imipenem		3	7	28	29	9	2	4					
Ciprofloxacin	6**	3	1	2	4	5	5	56*					
Gentamicin		10	16	4	1			1	1	48*			
Amikacin			4**	12	5	20	23	15	1	1	1*		

506 * MIC $\geq$ indicated value

507 ** MIC $\leq$ indicated value

508 nt, not tested

509 Because the mechanisms of resistance in these isolates remain unknown, precise definitions are difficult and the inclusion or exclusion of some isolates is

510 arguable; accordingly total numbers of isolates included should be viewed with caution

511

512   **Figure legends**

513

514   MIC distributions of (a) ceftazidime/clavulanate and (b) ceftazidime/avibactam in relation to those of unprotected ceftazidime for

515   Enterobacteriaceae (n=379) with unassigned resistance mechanisms

516

517

518 **Figure 1.**

519 Panel a)

Ceftazidime/ clavulanate MIC (mg/L)	MIC ceftazidime (mg/L)													Grand Total
	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	
≤0.125	3	4												7
0.25	3	16	10	1										30
0.5		1	28	10	1									40
1		1	2	21	9									33
2				3	21	7	3							34
4					5	12	5							22
8					2	4	7	3	2					18
16							1	13	10	1				25
32								1	19	8	16			44
>32									2	12	9	42	61	126
Grand Total	6	22	40	35	38	23	16	17	33	21	25	42	61	379

520

521

522

523   Panel b)

Ceftazidime/ avibactam MIC (mg/L)	MIC ceftazidime (mg/L)													Grand Total
	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	
≤0.06	2	3	5		3		1		2					16
0.125	3	11	7	1	3	3	1							29
0.25	1	8	20	6	4	1		1	2				1	44
0.5			6	17	12	1	2		3		4	2		47
1			2	11	11	4	5	8	11	6	14	12		84
2					4	12	2	6	10	3	3	23	15	78
4					1	2	4	1	3	5	1	3	14	34
8							1	1	1	5	1	1	11	21
16									1	1	1	1	9	13
32													3	3
>32										1	1		8	10
Grand Total	6	22	40	35	38	23	16	17	33	21	25	42	61	379

524