Activity of ceftazidime/avibactam against problem Enterobacteriaceae and *Pseudomonas aeruginosa* in the UK, 2015-2016

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Running head: Ceftazidime/avibactam versus gram-negatives

Abstract

Introduction. Ceftazidime/avibactam combines an established oxyimino-cephalosporin with the first diazabicyclooctane β-lactamase inhibitor to enter clinical use. We reviewed activity against Gram-negative isolates, predominantly from the UK, referred for resistance investigation in the first year of routine testing, beginning July 2015. **Methods.** Isolates were as received from referring laboratories; there is a bias to submit those with suspected carbapenem resistance. Identification was by MALDI-ToF mass spectroscopy, and susceptibility testing by BSAC agar dilution. Carbapenemase genes were sought by PCR; other resistance mechanisms were inferred using genetic data and interpretive reading. **Results.** Susceptibility rates to ceftazidime/avibactam exceeded 95% for: (i) Enterobacteriaceae with KPC, GES or other Class A carbapenemases, (ii) Enterobacteriaceae with OXA-48-like enzymes and (iii) for ESBL or AmpC producers, even when these had impermeability-mediated ertapenem resistance. Almost all isolates with metallo-carbapenemases were resistant. Potentiation of ceftazidime by avibactam was seen for 87% of ceftazidime-resistant Enterobacteriaceae with 'unassigned' ceftazidime resistance mechanisms, including two widely referred groups of *Klebsiella pneumoniae* where no synergy was seen between cephalosporins and established β-lactamase inhibitors. Potentiation here may be a diazabicyclooctane/cephalosporin enhancer effect. Activity was seen against *Pseudomonas aeruginosa* with derepressed AmpC, but not for those with efflux-mediated resistance. **Conclusions.** Of available β-lactams or inhibitor combinations, ceftazidime/avibactam has the widest activity spectrum against problem Enterobacteriaceae, covering all major types except metallo-carbapenemase producers; against *P. aeruginosa* it has a slightly narrower spectrum than ceftolozane/tazobactam, which also covers efflux-type resistance.

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

period. Numbers of isolates are slightly lower than in a similar analysis for ceftolozane/tazobactam[7] owing to a test failure with ceftazidime/avibactam in one week.

Identification and resistance investigation

Bacteria were identified by MALDI-ToF mass spectroscopy (Bruker Daltonics, Bremen, Germany) and MICs determined by BSAC agar dilution.[8] Asides from ceftazidime/avibactam 4 mg/L, we tested clinically-used β–lactams alone or in combination with fixed concentrations of inhibitors as follows: ampicillin, amoxicillin/clavulanate 2 mg/L, aztreonam, carbenicillin, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftolozane/tazobactam 4 mg/L, ertapenem, imipenem, meropenem, piperacillin/tazobactam 4 mg/L and temocillin. To help predict β–lactamase types, we additionally tested cefotaxime/clavulanate 2 mg/L, cefotaxime/cloxacillin 100 mg/L, ceftazidime/clavulanate 2 mg/L, cefepime/clavulanate 2 mg/L and imipenem/EDTA 320 mg/L.

Genes for KPC, VIM, NDM and OXA-48-like carbapenemases were sought by multiplex PCR[9] in all Enterobacteriaceae submitted owing to suspected carbapenem resistance and in those submitted for other reasons, but found to have phenotypes suggesting carbapenemase production. Enterobacteriaceae found negative for these commonest carbapenemases, but with phenotypes suggesting carbapenemase production were examined with further multiplex 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 of these multiplexes was also used for *P. aeruginosa* isolates showing imipenem/EDTA synergy together with broad resistance to penicillins and cephalosporins.

The genomes of carbapenemase producers with unusual behaviour were sequenced, using Illumina methodology, as were representatives of two unusual phenotypes of *Klebsiella pneumoniae* (see Results). Sequenced genomes were searched against our locally-curated database of antimicrobial resistance determinants using AMRHAI's GeneFinder algorithm.[12] Searches for new β–lactamases were performed on assembled-contigs translated in the six

possible reading frames using PSI-BLAST (position-specific iterated BLAST) and the HMM-based (Hidden Markov Models) method in the HMMer software suite (v3.1).[13] HMMER searches were performed at increasingly stringent thresholds using the β-lactamase-related pfam domains obtained from public databases.[14] Clover leaf/Hodge tests were performed on selected organisms, seeking to detect hydrolysis of carbapenems (using 10 μg ertapenem, imipenem and meropenem discs) or oxyimino-cephalosporins (using 30 μg cefepime, cefotaxime and ceftazidime discs); *Escherichia coli* ATCC 25922 was the indicator organism throughout.

Categorisation of isolates by resistance mechanisms

Molecular detection of a carbapenemase gene was considered definitive. Mechanisms in isolates lacking carbapenemase genes were assigned based on interpretive reading[15,16] of phenotypes, using an in-house algorithm. Two levels of match were allowed: 'Hard', where the phenotype was a perfect match and 'Soft', where the phenotype was less perfect, but the mechanism remained the most likely.[7] Some isolates did not match any well-recognised phenotype considered and were left as ‘unassigned’.

Results

Distribution of resistance mechanisms by species group

Among the 3144 referred Enterobacteriaceae isolates tested, 907 (28.8%) had carbapenemase genes, predominantly *bla*$_{\text{OXA-48}}$-like, *bla*$_{\text{NDM}}$ or *bla*$_{\text{KPC}}$, while 898 (28.6%) had AmpC phenotypes and lacked carbapenemase genes and 655 (20.8%) had ESBL phenotypes, again lacking carbapenemase genes (Table 1). Fully 80% of the AmpC producers and 58.5% of the ESBL producers were non susceptible to ertapenem at EUCAST’s 0.5 mg/L breakpoint, whilst 13.7% and 6.3%, respectively were non-susceptible to meropenem at 2 mg/L. These proportions considerably exceed those for AmpC and ESBL

producers in general [17,18] and we infer that many of these organisms also had reduced permeability, which is a general correlate of ertapenem resistance among AmpC and ESBL producers.[19]

Smaller numbers of isolates had phenotypes suggesting: (i) co-production of AmpC and ESBL enzymes, with clavulanate potentiating cefepime, but not ceftazidime or cefotaxime (n=71, 2.3%); (ii) hyper-production of K1 enzyme (in *K. oxytoca* isolates, n=8, 0.25%), or (iii) reduced permeability alone (n=85, 2.7%). One hundred and forty-one referrals (4.5%) had wild-type phenotypes with respect of β–lactams; these mostly had been submitted owing to resistance to other antibiotic classes. Finally, 379 (12.1%) had resistance patterns that were not predictive of any particular mechanisms: these varied widely in their phenotypes of resistance to different β-lactams, but universally lacked cephalosporin/clavulanate or cefotaxime/cloxacillin synergy (see below).

Isolates with carbapenemases

Modal ceftazidime MICs for isolates with KPC enzymes fell from 16 mg/L to 0.5 mg/L when avibactam was added, and those for isolates with GES enzymes from 256 to 1 mg/L (Table 2). Only two isolates with KPC carbapenemases – an *Enterobacter* sp. and a *K. pneumoniae*, were resistant to ceftazidime/avibactam at its 8+4 mg/L breakpoint. Resistance was stable in the *K. pneumoniae* isolate, where genome sequencing revealed classical bla_{KPC-2}, without the mutations associated with ceftazidime/avibactam resistance.[20,21] Resistance in the *Enterobacter* was lost on subculture, precluding investigation. Eleven isolates had other class A carbapenemases – specifically IMI, SME and FRI types. These were resistant to ertapenem (MICs 4->16 mg/L) and non-susceptible to either or both of imipenem (MICs 8->128 mg/L) and meropenem (MICs 4->32 mg/L, except one IMI isolate, 0.12 mg/L); all except one were susceptible or borderline resistant to unprotected ceftazidime (MICs 0.25-2 mg/L), with only

limited avibactam synergy, e.g. for the *E. cloacae* strain with FRI-2,[22] where the ceftazidime fell from 0.5 to 0.25 mg/L.

The MIC distribution of ceftazidime for OXA-48 Enterobacteriaceae was bimodal, with peaks at 0.5 and >256 mg/L; 34.8% of isolates inhibited by unprotected ceftazidime at EUCAST's 1 mg/L susceptible breakpoint and 45.0% at the 4 mg/L resistance breakpoint. With avibactam added, this distribution became unimodal, with a peak at 0.25 mg/L and 94% of MICs between 0.12 and 2 mg/L. Potentiation was ≤4-fold for isolates with ceftazidime MICs ≤1 mg/L, but 128- to 1024-fold for those with high-level ceftazidime resistance. Five OXA-48 isolates (two *K. pneumoniae* from separate hospitals and single *K. oxytoca*, *E. coli* and *C. freundii*) tested as resistant to ceftazidime/avibactam, with MICs >32+4 mg/L but this was not confirmed on retesting and was not pursued further.

Isolates with metallo-carbapenemases consistently were resistant to ceftazidime and remained so with avibactam added. The few exceptions to this generalisation were *E. coli* that were inhibited by avibactam alone at 4 mg/L (Table 2).

Isolates with ESBLs, AmpC and other mechanisms

As already stressed, referred AmpC and ESBL producers are biased towards those with reduced susceptibility to carbapenems. To accommodate this bias, ceftazidime/avibactam MICs for these isolates are shown, ESBL producers (Table 3) and AmpC hyperproducers(Table 4), in relation to those of ertapenem, as a proxy for impermeability. The AmpC isolates mostly were *Enterobacter* spp., where ertapenem MICs of 1-2 mg/L are typical for AmpC-derepressed strains; the ESBL producers were mostly *E. coli* and *K. pneumoniae* (Table 1).

Among the ESBL producers, 96.2% were non-susceptible to ceftazidime 1 mg/L and 77.8% were highly resistant, with MICs 32->256 mg/L; corresponding proportions among the

AmpC producers were 93.9% and 74.1%, respectively. With avibactam added, the ceftazidime MICs were reduced to ≤8+4 mg/L (i.e. susceptible) for 99.7% of ESBL producers and 98.3% with AmpC. The MICs of ceftazidime/avibactam for ESBL producers trended upwards as the ertapenem MIC increased from 0.12 to 1 mg/L, but with little further rise for highly-ertapenem resistant isolates. This behaviour contrasted to ceftazidime/clavulanate (not shown) and ceftolozane/tazobactam,[7] where MICs rose progressively with the ertapenem MIC. MICs of ceftazidime/avibactam for AmpC producers did rise in parallel with ertapenem MICs but the combination remained active against 109/115 isolates with ertapenem MICs >16 mg/L. Fifteen of the 898 AmpC producers were resistant to ceftazidime/avibactam 8+4 mg/L; four of these were *Hafnia alvei* (*versus* 12 *H. alvei* among the whole 898) and eight were 'Soft matches'(*versus* 65 Soft matches among the 898) implying a greater risk that they were mis-categorisations or had secondary mechanisms. Two Soft-matched ESBL *K. pneumoniae* were resistant to ceftazidime/avibactam; both were among the most–highly-ertapenem resistant (MICs ≥16 mg/L) and probably represent extreme examples of impermeability.

Among isolates with both AmpC and ESBL activity, 69/71 (97.2%) were susceptible to ceftazidime/avibactam 8+4 mg/L whereas MICs of unprotected ceftazidime were >128 mg/L in 66/71 cases.. Only eight K1 β-lactamase-hyperproducing *K. oxytoca* were included: these had characteristic resistance to piperacillin/tazobactam and aztreonam, but with MICs around EUCAST breakpoints for oxyimino-cephalosporins and 4- to 32-fold cefepime/ and cefotaxime/clavulanate synergy.[15,16] MICs of unprotected ceftazidime were from 0.25-2 mg/L, falling to 0.12-1 mg/L with avibactam added. Last, among characterised groups, 85 isolates were inferred solely to have reduced permeability, with cefoxitin MICs >32 mg/L and ertapenem MICs (>0.5 mg/L in 64/85 cases). Oxyimino-cephalosporin MICs remained around breakpoints (0.5-4 mg/L) with (i) no differential between cefepime and other oxyimino-agents, and (ii) no cephalosporin synergy with cloxacillin or clavulanate. MICs of unprotected ceftazidime were 0.5-4 mg/L and remained in this range with ceftazidime/avibactam in 71/85 cases, falling slightly for the remaining 14.

Unassigned isolates

The 379 organisms with unassigned mechanisms were dominated by *K. pneumoniae* (n=203) and *E. coli* (n=124) (Table 1). The major common feature, along with some degree of cephalosporin resistance, was the absence of synergy between cephalosporins and clavulanate or cloxacillin, and between imipenem and EDTA. The lack of ceftazidime/clavulanate synergy is illustrated in fig 1a. Prior to adding ceftazidime/avibactam to the test panel, we believed that these isolates mostly had β-lactamase-independent, modes of resistance but subsequently were surprised by the large proportion with potentiation was seen. Thus, among all 379 isolates, 199 were resistant to ceftazidime 8 mg/L and 195 to ceftazidime/clavulanate 8+2 mg/L but only 26 to ceftazidime/avibactam 8+4 mg/L (fig 1b).

Two regularly-seen *K. pneumoniae* phenotypes ('Type I' and 'Type II') accounted for many of these isolates, and MIC data are illustrated in Table 5. Type I isolates were resistant to cefepime and ceftazidime, with MICs 8-64 mg/L, but remained borderline susceptible to cefotaxime, with MICs 1-4 mg/L. Type II isolates were resistant to all three oxyimino-cephalosporins, with MICs 32->256 mg/L. Both types were resistant to cefoxitin, piperacillin/tazobactam, and amoxicillin/clavulanate. Temocillin MICs were raised above the 4-8 mg/L values typical for *K. pneumoniae*, but mostly remained $\leq$64 mg/L. Carbapenem MICs were raised, with almost all non-susceptible to ertapenem at EUCAST's 0.5 mg/L breakpoint; many, particularly among Type II isolates, were highly resistant, with MICs >16 mg/L. Both types have been referred from multiple hospitals over the past 3-4 years and are non-clonal, based on Variable Number Tandem Repeat typing.[22] They varied in fluoroquinolone and aminoglycoside susceptibility. Crucially, while cephalosporin MICs were not reduced by clavulanate or cloxacillin, those of ceftazidime were reduced by avibactam, mostly falling to 1-4 mg/L.

Whole genome sequencing of 10 Type I representatives, mostly pre-dating the present series, confirmed clonal diversity and found seven to have only the SHV-1 β-lactamase typical of *K. pneumoniae*, without mutations to the coding or promoter sequences; single representatives had SHV-27 (an ESBL), SHV-36 (unknown spectrum) or SHV-1 plus TEM-10 (an ESBL). Increased read depth, relative to *gyrA* and *parC*, suggested that bla_{SHV} was amplified in most cases whilst *ompK35* was inactivated by an identical frame shift mutation in all isolates and *ompK36* was inactivated in most by various mutations or insertions. The genes encoding the essential PBPs (1, 2 and 3) were conserved, without mutations. Sequencing of four Type II isolates variously revealed CTX-M-15 plus OXA-1, CMY-42 plus OXA-1, CTX-M-15, OXA-1 plus SHV-53 and CTX-M-33, OXA-1, SHV-11 and TEM-1.

None of the genetic changes seen for Type I isolates adequately explains their phenotypes (see Discussion). Further bioinformatic analysis failed to find motifs suggesting additional β–lactamase genes, and clover leaf (Hodge) tests were negative for both carbapenems and oxyimino-cephalosporins.

Pseudomonas aeruginosa

Data were obtained for 1384 *P. aeruginosa.* Analysis must be cautious because, unlike for Enterobacteriaceae, we used ceftazidime/avibactam in categorising these isolates,[7] distinguishing those with derepressed AmpC (carbenicillin MIC ≤128 mg/L, cefotaxime MIC > carbenicillin MIC and ceftazidime MIC > 4x ceftazidime/avibactam MIC) from those with up-regulated efflux (carbenicillin, piperacillin/tazobactam and ceftazidime MICs raised in approximate proportion, without ceftazidime/avibactam potentiation).

Among 147 putative AmpC-derepressed *P. aeruginosa*, 94.6% were susceptible to ceftazidime/avibactam 8+4 mg/L versus 21.0% to ceftazidime 8 mg/L and 96.6% to ceftolozane/tazobactam 4+4 mg/L. Among 388 with moderately raised efflux (carbenicillin

MICs 256-512 mg/L), 86.1% were susceptible to ceftazidime/avibactam, 65.7% to ceftazidime and 99.7% to ceftolozane/tazobactam. Among 149 with highly raised efflux (carbenicillin MICs >512 mg/L), 41.6% were susceptible to ceftazidime/avibactam, 27.5% to ceftazidime and 95.3% to ceftolozane/tazobactam. The gain versus AmpC-derepressed isolates doubtless reflects β-lactamase inhibition of; that versus 'efflux isolates' was largely a thresholding effect, with the ceftazidime MIC reduced from 16 to 8 mg/L thus crossing the breakpoint but remaining within one doubling dilution of the ceftazidime value. Four hundred and ten *P. aeruginosa* isolates were non-susceptible to *all* of carbenicillin, piperacillin/tazobactam, ceftazidime, imipenem and meropenem at EUCAST breakpoints. Of these, 28.7% were susceptible to ceftazidime/avibactam 8+4 mg/L and 52.6% to ceftolozane/tazobactam 4+4 mg/L, rising to 43.3% and 81.6%, respectively, if isolates with metallo-carbapenemases (n = 118, mostly VIM types), ESBLs (n = 31 mostly VEB) or GES enzymes (n = 4) were excluded.

Discussion

These data are for 'problem' isolates sent to PHE's reference laboratory, and therefore with a heavy bias to resistance. They show ceftazidime/avibactam broadly active against: (i) Enterobacteriaceae with KPC, GES and other class A carbapenemases, (ii) Enterobacteriaceae with OXA-48-like enzymes, irrespective of susceptibility to ceftazidime alone, and (iii) Enterobacteriaceae with ESBLs or AmpC enzymes, irrespective of the impermeability traits that confer resistance to ertapenem. Lastly, ceftazidime/avibactam 8+4 mg/L remained active against 87% (fig. 1b) of the 199 Enterobacteriaceae with unassigned mechanisms, but which were resistant to ceftazidime alone at 8 mg/L, including members of the widely encountered Type I and II phenotypes of *K. pneumoniae* illustrated in Table 5.

Activity against KPC-, ESBL- and AmpC- producers is in keeping with the known ability of avibactam to inhibit these enzymes.[2,3] Ceftazidime itself remains active against a sizeable minority of Enterobacteriaceae with OXA-48-like enzymes, whereas others are highly

resistant, as illustrated by the bi-modal MIC distribution in Table 2. The explanation is that OXA-48-like enzymes do not, themselves,[4] attack ceftazidime, but that many producers also have further mechanisms - most often ESBLs[23] - that confer resistance. Avibactam gave weak potentiation of ceftazidime against ceftazidime-susceptible isolates with OXA-48-like enzymes, but strongly potentiated ceftazidime against those with high-level resistance, presumably via inhibition of these secondary β-lactamases.

The only major gaps in ceftazidime/avibactam's spectrum, as is well recognised,[2,3] were metallo-carbapenemase producers. These accounted for a little over one-third of carbapenemase-producing Enterobacteriaceae referred to AMRHAI (302/873 = 34.6% in the period reviewed). Their actual proportion may be lower since: (i) isolates with KPC carbapenemases are concentrated in a few hospitals in Northwest England, which no longer refer all producers, and (ii) isolates with metallo-carbapenemases, particularly NDM, are highly resistant and unlikely to be missed, whereas many with OXA-48-like enzymes have marginal carbapenem resistance, likely leading to under-detection. Proportions of non-metallo- versus metallo-carbapenemases vary globally, with KPC types predominating in the Americas, Italy, Greece and China; OXA-48 in Turkey, Romania and Spain, and NDM in South Asia; strains with both OXA-48 and NDM appear prevalent in the Middle East.[24,25]

A few isolates with KPC and OXA-48 enzymes were resistant to ceftazidime/avibactam on primary testing, but resistance was only confirmed for one *K. pneumoniae* with a KPC carbapenemase. It is impossible to ascertain whether initial results for the others were in error or whether unstable resistance had been lost. Sequencing revealed that the stably-resistant *K. pneumoniae* isolate produced KPC-2 carbapenemase and its behaviour possibly reflected the activity of this enzyme together with impermeability. It lacked the bla_{KPC} mutations associated with emerging ceftazidime/avibactam resistance during therapy, and found also in mutants generated *in vitro*; these cluster around the Ω-loop and increase affinity for ceftazidime, protecting against binding of avibactam.[20,21,26] Emerging resistance to ceftazidime/avibactam in an isolate with an OXA-48 enzyme was associated with Pro170Ser

and Thr264Ile substitutions to a co-produced CTX-M-14 ESBL, without changes to OXA-48 itself.[27]

Retained activity against isolates with combinations of ESBL or AmpC and impermeability was striking. Although such strains rarely cause outbreaks and often are unstable, they are not infrequent and can be selected during carbapenem therapy, complicating treatment .[28,29]

The broad activity of ceftazidime/avibactam against ceftazidime-resistant isolates with unassigned mechanisms is intriguing, especially as these were almost all resistant to ceftazidime/clavulanate (fig. 1). The obvious explanation is that these isolates have unsuspected β–lactamases, inhibited by avibactam, but not by clavulanate, cloxacillin or tazobactam. However, for the two largest groups, i.e. the Type I and II *K. pneumoniae* in Table 5 – we have been unable to find any such enzyme: the Type I isolates largely have an increased copy number classical bla_{SHV-1}, which is chromosomal and ubiquitous in *K. pneumoniae*,[30] along with inactivation of *ompK35* and *ompK36,* whilst the Type II unknowns had various ESBL or AmpC enzymes. Further analysis has concentrated on the Type I isolates as the simpler case. A quarter century ago, Petit *et al.*[31] cautiously associated increased expression of SHV-1 enzyme with resistance to ceftazidime but not cefotaxime in *K. pneumoniae*, as in our Type I isolates. However, (i) their strains, unlike ours, had ceftazidime/clavulanate synergy, as would be expected, and (ii) they did not seek non-β-lactamase-mediated mechanisms. It may be that the porin mutations in our isolates excluded clavulanate more effectively that avibactam, reconciling this discrepancy. But, if so, the distinction was remarkably clear cut, whereas significant cephalosporin/clavulanate synergy typically is retained for impermeable, ertapenem-resistant, ESBL producers of the type detailed in the bottom rows of Table 3 (see also ref. 19). An alternative hypothesis, speculative but plausible, is that these organisms have some perturbation (in the broadest sense) of cell wall synthesis that simultaneously confers (a) reduced susceptibility to multiple β-lactams and (b) vulnerability ceftazidime/avibactam synergy by a mechanism other than β-

lactamase inhibition. Potentiation of cephalosporins independently of β-lactamase inhibition is a common feature of other DBOs, notably nacubactam (RG6080/OP0595) or zidebactam and seems to depend on the DBO interacting with PBP2 whilst the partner β-lactam attacks PBP3.[32] The absence of PBP gene changes in *K. pneumoniae* with the Type I and II phenotypes does not refute these speculations, for it is established that the consequence of DBO- and mecillinam- mediated inhibition of PBP2 are modulated by mutations to genes involved in the stringent response rather than directly in peptidoglycan biogenesis. The threat posed by these phenotypes is debatable: on the one hand they are widely scattered and regularly referred, moreover the Type II isolates are very broadly resistant to β-lactams other than ceftazidime/avibactam; on the other hand we have not seen outbreaks, and susceptibility rates to non-β-lactams are high, particularly for Type I isolates, meaning that treatment options remain (Table 5).

We have only included a limited analysis for *P. aeruginosa* because we used ceftazidime/avibactam MICs to help categorise resistance mechanisms.[7] Nevertheless the findings are entirely compatible with the view, inherently plausible and supported by previous work, that avibactam substantially overcomes AmpC-mediated ceftazidime resistance,[6] but not that due to efflux. Ceftolozane/tazobactam, by contrast, retains activity against >95% of isolates with either of these mechanisms.[7] Neither inhibitor combination overcomes metallo-carbapenemases nor VEB-type ESBL-mediated resistance in the species, but these mechanisms are uncommon in the UK.

In summary, these data show that ceftazidime/avibactam has activity against most problem Enterobacteriaceae groups seen in the UK, as referred to the national reference laboratory. Its activity extends to two frequently-referred *K. pneumoniae* phenotypes where ceftazidime resistance is not obviously β–lactamase-mediated; these remain under active investigation. The isolates studied here pre-date clinical use of ceftazidime/avibactam in the UK and, as the drug enters use, attention will need to be paid to any emergence of resistance.

Shields and colleagues, in Pittsburgh, saw emerging resistance in 3/31 cases where ceftazidime/avibactam was used to treat severe infections due to *K. pneumoniae* ST258 with KPC carbapenemases.[21] These mutations –and similar ones selected by ourselves *in vitro*- make KPC enzymes into 'better' ceftazidimases,[20,26] but also reduce carbapenemase activity. An interesting possibility is that co-administration of meropenem might block this route to resistance, counter-selecting against any mutation that degraded carbapenemase activity and thus 'forcing' the KPC enzyme to remain vulnerable to avibactam.

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Transparency declaration

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Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Trius Therapeutics, VenatoRx Pharmaceuticals and Wockhardt Ltd.

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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	

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

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

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

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

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*			

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

* MIC > indicated value

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
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	>16	
≤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

	No. isolates with indicated ceftazidime/avibactam MIC (mg/L)											Grand Total
Ertapenem MIC (mg/L)	≤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

505 **Table 5.** MICs of ceftazidime/avibactam and comparators against *K. pneumonia*e 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 ≥ indicated value

507 ** MIC ≤ 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

Figure legends

MIC distributions of (a) ceftazidime/clavulanate and (b) ceftazidime/avibactam in relation to those of unprotected ceftazidime for Enterobacteriaceae (n=379) with unassigned resistance mechanisms

Figure 1.

Panel a)

	MIC ceftazidime (mg/L)													
Ceftazidime/ clavulanate MIC (mg/L)	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	Grand Total
≤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

Panel b)

Ceftazidime/ avibactam MIC (mg/L)	MIC ceftazidime (mg/L)													
	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	Grand Total
≤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