Cefepime/tazobactam compared with other tazobactam combinations against problem Gram-negative bacteria

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Running head. Cefepime/tazobactam versus referred isolates

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Abstract

Piperacillin/tazobactam has long been a broad-spectrum 'workhorse' antibiotic but is compromised by resistance. One response is to re-partner tazobactam with cefepime, which is easier to protect, being less β-lactamase labile and to use a high dose and prolonged infusion. On this basis, Wockhardt are developing cefepime/tazobactam (WCK 4282) as a 2+2g q8h combination with 90 min infusion. We assessed the activity of this combination, with other tazobactam combinations as comparators, against 1632 Enterobacterales, 745 *Pseudomonas aeruginosa* and 450 other non-fermenters, as submitted to the UK national reference laboratory. These were categorised by carbapenemase-gene detection and interpretive reading of phenotypes, with MICs determined by BSAC agar dilution. Although higher values may be justifiable, based on the pharmacodynamics, we reviewed results against current cefepime breakpoints. On this basis, cefepime/tazobactam was broadly active against Enterobacterales with AmpC enzymes and ESBLs, even when these had ertapenem resistance suggesting porin loss. At 8+8 mg/L, activity extended to >90% of Enterobacterales with OXA-48 and KPC carbapenemases, although MICs for KPC producers belonging to the international *Klebsiella pneumoniae* ST258 lineage were higher; MBL producers remained resistant. Cefepime/tazobactam was less active than ceftolozane/tazobactam against *Pseudomonas aeruginosa* with AmpC derepression or high-level efflux but achieved wider antipseudomonal coverage than piperacillin/tazobactam. Activity against other non-fermenters was species-specific. Overall, cefepime/tazobactam has a spectrum exceeding those of piperacillin/tazobactam and ceftolozane/tazobactam and resembling or exceeding that of carbapenems. Used as a 'new-combination of old-agents' it has genuine potential to be 'carbapenem-sparing'.

1.0 Introduction

The antimicrobial spectrum of β–lactamase inhibitor combinations depends not only on the range of β–lactamases inactivated but also, and critically, on the partner β–lactam.[1,2] Weak-substrate β–lactams are easier to protect than those that are tightly bound and rapidly hydrolysed. If the partner β–lactam evades some β–lactamases, then the inhibitor does not need to inactivate these.

Unfortunately, the β–lactamase inhibitors developed in the last century were largely combined with very labile, difficult-to-protect, penicillins.[3,4] At the time these were seen as the drugs needing protection, particularly against classical TEM-1 enzyme, whereas oxyimino-cephalosporins were promoted as 'β–lactamase stable'. Within a few years it became apparent that oxyimino-cephalosporins were threatened by both AmpC enzymes and by emerging ESBLs, and that they too therefore might benefit from partnering with β-lactamase inhibitors. By then, however, both the cephalosporins' patent lives and those of the inhibitors were eroding, disincentivising development of new combinations.

Only the Indian market defied these generalisations, with numerous cephalosporin/inhibitor combinations commercialised, though often with dosages that are not pharmacodynamically optimised.[5,6] Cefepime/tazobactam illustrates this issue. The combination has considerable merit in principle: cefepime is a rapid permeant of Gram-negative bacteria and is relatively stable to AmpC enzymes,[7] meaning that it needs only to be protected from ESBLs and, ideally, carbapenemases, whilst tazobactam is a non-toxic, easy-to-manufacture inhibitor of most Class A β–lactamases. However, the many cefepime/tazobactam formulations available in India all have an 8:1 ratio, as in piperacillin/tazobactam, delivering only 250-750 mg tazobactam/day, based on a 2-6g daily cefepime regimen. This compares with 1.5-2g tazobactam daily for piperacillin/tazobactam

and 1.5-3g for ceftolozane/tazobactam. Such under-dosage must, inevitably, reduce coverage. To address this limitation, Wockhardt are reformulating cefepime/tazobactam (WCK 4282) as a proprietary 2+2g q8h combination, delivering 6g/tazobactam/day, with a 90-minute infusion time to maximise time above MIC.[8] The approach fits well within the concept of 'repurposing' old antibiotics to overcome resistance [9] and the high-dose combination is now entering Phase III trials. We sought to compare its activity in vitro with that of available tazobactam combinations against problem Gram-negative bacteria sent to the UK national reference laboratory.

2.0 Materials and Methods

2.1 Bacteria

The bacteria tested comprised approximately half of those submitted, to the PHE Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit from July 2015 to July-2016, as previously used for assessments of ceftolozane/tazobactam [10] and ceftazidime/avibactam.[11] The original intention was to test all the isolates received during the second half of this period but, because substantial numbers proved non-viable, this was extended to 8 months from November 2015 to early July 2016. The species distribution is shown in Table 1. Almost all the isolates had originally been collected from patient specimens tested at UK clinical diagnostic laboratories and were submitted to AMRHAI, as the UK national reference laboratory for antibiotic resistance, to the basis of unusual resistance. They should be seen as a snapshot of the types of resistance causing concern in the UK and it should be noted that there is a heavy bias to referring isolates suspected of carbapenem resistance.

Isolates were identified by MALDI-ToF (Bruker Biotyper, Bremen, Germany); resistance mechanisms were inferred based on a combination of genotype data, predominantly for carbapenemases, and interpretive reading of resistance phenotypes, as determined with agar dilution methodology using broad antibiotic panels, as described in detail previously. [10,11]

2.2 Antibiotics and susceptibility testing

Ceftolozane and ertapenem were from Wockhardt (Aurangabad, India); cefepime was purchased from Alpha Aesar (Heysham, UK), ceftazidime, piperacillin and tazobactam from Merck KGaA (Gillingham, UK). MICs were determined by British Society for Antimicrobial Chemotherapy (BSAC) methodology [12] on IsoSensitest agar (Oxoid, Basingstoke, UK). Controls included those specified in the BSAC method and an in-house panel with known resistance mechanisms. Tazobactam was used at 4 mg/L in combination with ceftolozane and piperacillin, in accordance with EUCAST guidance; the concentration for combination with cefepime was increased to 8 mg/L, reflecting the high dosage under trial.

AMRHAI is part of the Public Health England Bacteriology Department, which is UKAS ISO accredited; it participates in the National External Quality Assurance Scheme, including in respect of antimicrobial susceptibility testing.

3.0 Results

3.1 MIC distributions of tazobactam combinations

MIC distributions for cefepime/tazobactam, ceftolozane/tazobactam and piperacillin/ tazobactam are shown in Table 1 whilst Table 2 shows the fold-reductions in cefepime MIC achieved by tazobactam for the various resistance groups of Enterobacterales. Tables 3-5

then illustrate how the MIC distributions for different tazobactam combinations relate to one another and to those of carbapenems. Current EUCAST breakpoints for unprotected cefepime are S ≤1, R >4 mg/L for Enterobacterales and S ≤0.001, R >8 mg/L for *P. aeruginosa* (meaning that high dosage is always advocated for *P. aeruginosa* infections); EUCAST has no breakpoints for *Acinetobacter*. CLSI breakpoints are ≤2, >8 mg/L for Enterobacterales, with MICs of 4 and 8 mg/L defined as 'Dose-dependent susceptible, along with breakpoints of ≤8, >16 mg/L for *P. aeruginosa* and other non-fermenters. These were taken as reference points for analysis of cefepime/tazobactam results, although the extended infusion may justify a higher breakpoint (Discussion).

3.2 Enterobacterales

Against Enterobacterales 'wild-types', meaning isolates susceptible to oxyimino-cephalosporins and primarily referred for confirmation of resistance to non-β–lactams, cefepime/tazobactam was the most active combination, with a modal MIC of 0.03 mg/L, compared with 0.25 mg/L for ceftolozane/tazobactam and 2 mg/L for piperacillin/tazobactam. As would be expected, MICs of cefepime/tazobactam for these broadly susceptible wildtypes were only minimally below those of cefepime alone (Table 2).

The modal MIC of cefepime/tazobactam rose to 0.5 mg/L for AmpC producers, with 408/418 values ≤4+8 mg/L and 416/418 ≤8+8 mg/L; by contrast only 186/418 AmpC isolates were susceptible to ceftolozane/tazobactam at its ≤2+4 mg/L EUCAST breakpoint and 120/418 to piperacillin/tazobactam at ≤8+4 mg/L. Two points should be underscored here. First, the advantage of cefepime/tazobactam versus AmpC producers was largely due to the inherent stability of cefepime to these enzymes, not to tazobactam acting as an inhibitor; thus, the geometric mean reduction in cefepime MIC engendered by tazobactam was just 1.8-

fold. Secondly, many of these isolates were sent to AMRHAI because they had some degree of reduced susceptibility to carbapenems and were suspected (incorrectly) of being carbapenemase-producers; thus, for 192 of the 418 isolates the meropenem MIC exceeded the EUCAST 'screening concentration' of 0.12 mg/L and 267, including 176 *Enterobacter* spp. were resistant to ertapenem at its 0.5 mg/L EUCAST breakpoint.

Among ESBL producers, 273/306 were susceptible to cefepime/tazobactam at ≤4+8 mg/L and 283/306 at ≤8+8 mg/L (Table 1). The proportion susceptible to ceftolozane/tazobactam ≤2+4 mg/L was 198/306; whilst 126/306 were susceptible to piperacillin/tazobactam at ≤8+4 mg/L. Strong potentiation of cefepime by tazobactam was widespread (Table 2). As for the AmpC hyperproducers, ESBL producers referred to AMRHAI are mostly those (wrongly) suspected of carbapenemase production: 131 were resistant to ertapenem at 0.5 mg/L and 93 were resistant to meropenem at the 0.12 mg/L screening concentration. These are much higher proportions than among ESBL producers in general, and previous analysis indicates that such isolates have porin loss, leading to relative impermeability.[13]

MICs of cefepime/tazobactam, ceftolozane/tazobactam and meropenem for the ESBL producers correlated with those of ertapenem, which were taken as a proxy for the degree of this impermeability[9] (Table 3). Cefepime/tazobactam 4+8 mg/L remained active against 90% of these ESBL producers up to an ertapenem MIC of 0.5 mg/L; at 8+8 mg/L it remained active against 90% up to an ertapenem MIC of 2 mg/L. By contrast, ceftolozane/tazobactam 2+4 mg/L only remained active against 90% of ESBL producers up to an ertapenem MIC of 0.12 mg/L. Cefepime/tazobactam and ceftolozane/tazobactam MICs for ESBL producers correlated closely across the MIC spectrum, but with the cefepime values lower in 277/306

cases. Piperacillin/tazobactam was not included in these comparisons because its MICs additionally reflect other factors, notably the co-carriage or not of OXA-1 penicillinases.[14]

Turning to carbapenemase-producing Enterobacterales, we saw three types of behaviour (i) generalisable resistance, (ii) widespread susceptibility to both cephalosporin combinations and (iii) carbapenemase where cefepime/tazobactam and ceftolozane/tazobactam were differentiated. Generalisable resistance included the behaviours (i) that almost all carbapenemase producers, irrespective of enzyme type, were resistant to piperacillin/tazobactam 8+4 mg/L, which is therefore omitted from the detailed comparisons that follow and (ii) that almost all of the isolates with MBLs were resistant to all three tazobactam combination at any reasonable breakpoint. Widespread susceptibility to both cephalosporin combinations was seen for isolates with OXA-48 enzymes and ceftazidime MICs $\leq$4 mg/L, implying the lack of substantial ESBL co-production: among these 113/114 were inhibited by cefepime/tazobactam at $\leq$4+8 or $\leq$8+8 mg/L whilst 103/114 were inhibited by ceftolozane/tazobactam at 2+4 mg/L. The few Enterobacterales with non-KPC/GES Class A carbapenemases, comprising SME, IMI and FRI types also were widely susceptible to both cefepime/tazobactam and ceftolozane/tazobactam at these concentrations; in all these cases cefepime was not significantly potentiated by tazobactam (Table 2). This behaviour reflects the fact that cefepime approaches stability to OXA-48 and non-GES/KPC Class A carbapenemases.[15]

Cefepime/tazobactam and ceftolozane/tazobactam were substantially differentiated against carbapenemase producers with KPC or GES carbapenemases and ceftazidime-resistant isolates with OXA-48 carbapenemases, always with cefepime/tazobactam as the more active combination. Specifically, 88 of the 116 isolates with KPC carbapenemases were inhibited by cefepime/tazobactam at 4+8 mg/L and 105/116 at 8+8 mg/L, compared with

8/116 susceptible to ceftolozane tazobactam 2+4 mg/L and 3/116 to piperacillin/tazobactam. Apparent activity against isolates with KPC carbapenemases was almost entirely due to cefepime itself; there was no significant potentiation by tazobactam (Table 2). These isolates with KPC carbapenemases were mostly collected around Manchester where the 'KPC problem' largely reflects the diffusion of pKpQIL-type plasmids;[16] few belonged to the global *Klebsiella pneumoniae* ST258/512 lineages, which is generally more resistant to β–lactams.[17,18] We have variable tandem-number repeat typing data for 37 of the 71 KPC enzyme-producing *K. pneumoniae* included, and just four of these had the 3,2,2,13,2,1,3,3,1 profile typical of ST258: MICs of cefepime/tazobactam for these were 8, 8, 16 and 64 mg/L, thus falling at the high end of the present range; corresponding meropenem MICs were 16, 32, 16 and 128 mg/L, respectively. MICs of cefepime/tazobactam (or cefepime itself) correlated with those of meropenem for isolates with KPC carbapenemases across the MIC range (Table 4). In respect of the isolates with GES carbapenemases 6/10 were susceptible to cefepime/tazobactam at ≤4+8 mg/L and all were inhibited at 8+8 mg/L; by contrast all were resistant to ceftolozane/tazobactam, with MICs 8+4 to 64+4 mg/L. The extent of potentiation of cefepime by tazobactam was very variable; it is unclear whether this reflected variation in the GES enzyme type, or in respect of ESBL coproduction. It should be added that the GES family is diverse, including both carbapenemases and ESBLs and that there is a need for studies with larger collections. Among the 136 ceftazidime-resistant (i.e. putatively ESBL co-producing) isolates with OXA-48 carbapenemase, 77 were inhibited by cefepime/tazobactam at 4+8 mg/L and 90 at 8+8 mg/L, compared with just 9/136 by ceftolozane/tazobactam at 2+4 mg/L. Potentiation of cefepime by tazobactam was widely apparent for these ceftazidime-resistant isolates with OXA-48 carbapenemases (Table 2), whereas it was largely absent

(above) for ceftazidime non-resistant isolates with OXA-48 enzymes, again supporting the view that they have secondary tazobactam-inhibited β-lactamases.

Among minor groups, *Klebsiella oxytoca* with high-level K1 enzyme activity and Enterobacterales inferred to have impermeability-associated resistance were almost all inhibited by both cefepime/tazobactam 8+8 mg/L and ceftolozane/tazobactam 2+4 mg/L); tazobactam had minimal effects on the cefepime MICs for these groups.

MICs of all tazobactam combinations rose with those of ceftazidime for Enterobacterales with uncertain - and doubtless diverse - mechanisms, but cefepime/tazobactam was consistently the most active of the three combinations; potentiation of cefepime by tazobactam was seen for around half of the most ceftazidime resistant (MIC >64 mg/L) isolates, not other groups. Synergy between cefepime and tazobactam was not seen for a small further group of *K. pneumoniae* isolates, described previously, that are notable for a profile that includes raised MICs for ceftazidime and cefepime, but much less so for cefotaxime. Although no relevant β–lactamase has been demonstrated, these organisms do show strong ceftazidime/avibactam synergy.[11]

3.3 P. aeruginosa

In contrast to Enterobacterales, where cefepime/tazobactam was consistently the most active tazobactam combination, ceftolozane/tazobactam was the most active against *P. aeruginosa* on a gravimetric basis. Based on the EUCAST breakpoint of 4+4 mg/L, ceftolozane/tazobactam was active against 69/71 *P. aeruginosa* isolates inferred to have derepressed AmpC compared with 47/71 inhibited by cefepime/tazobactam at 8+8 mg/L. Corresponding proportions among isolates with raised efflux were 182/188 versus 154/188 and among those with highly raised efflux, 68/85 versus 27/85. Table 5 provides a cross plot

of cefepime/tazobactam and ceftolozane/tazobactam MICs for *P. aeruginosa* isolates deemed to have low, normal, raised and highly raised efflux, showing broad correlation across the spectrum, always with ceftolozane/tazobactam consistently the more active combination. Cefepime/tazobactam 8+8 mg/L remained more widely active than piperacillin/tazobactam 8+4 mg/L against all AmpC and efflux groups (Table 1).

P. aeruginosa isolates with MBLs and ESBLs (mostly VEB enzymes) were almost all resistant to all the tazobactam combinations whereas isolates with GES enzymes, specifically GES-5 carbapenemase, mostly were inhibited by cefepime/tazobactam at ≤8+8 mg/L whilst being resistant to ceftolozane/tazobactam at its 4+4 mg/L breakpoint. Finally, MICs of cefepime/tazobactam and ceftolozane/tazobactam rose with those of ceftazidime for *P. aeruginosa* isolates with unassigned mechanisms, mostly from cystic fibrosis patients, with ceftolozane/tazobactam again the most active combination on a gravimetric basis, followed by cefepime/tazobactam.

It should be added that tazobactam plays little useful role in the case of *P. aeruginosa*, regardless of the partner β-lactam: just 21/745 isolates showed ≥4-fold reductions in cefepime MIC in the presence of tazobactam; 15 of these were ESBL producers, 13 of which remained resistant to cefepime/tazobactam 8+8 mg/L.

3.4 Other non-fermenters

The other non-fermenters were a diverse group. The largest subgroup comprised 216 *A. baumannii*, of which at least 183 had OXA-23, -24 or 58 carbapenemases and a further 19 had metallo carbapenemases alone or together with OXA carbapenemases, giving a total of 202/216 carbapenemase positive. Only 26 of these 216 were susceptible to cefepime/tazobactam at 8+8 mg/L and eight of these owed their susceptibility to being

inhibited by tazobactam alone at 8 mg/L. Just 10/216 were inhibited by piperacillin/tazobactam 8+4 mg/L, again largely owing to susceptibility to tazobactam itself. Ceftolozane/tazobactam has no breakpoints for *Acinetobacter* spp.; its MICs were similar to those of cefepime/tazobactam.

On a simple gravimetric basis, cefepime/tazobactam was more active than ceftolozane/tazobactam against *Elizabethkingia* spp. and *Chryseobacterium* spp. and similarly active against *Burkholderia cepacia* complex, non-*aeruginosa Pseudomonas* and *S. maltophilia*. *Achromobacter* spp., and *Pandorea* spp. were largely resistant to both cephalosporin combinations at any reasonable potential breakpoint but were more often susceptible to piperacillin/tazobactam. For all these species, the effect of tazobactam on the activity of cefepime was slight, with MICs rarely reduced more than two-fold (not shown).

Wide activity of tazobactam combinations against non-*baumannii Acinetobacter* spp. reflected susceptibility to tazobactam itself: 20/38 isolates were inhibited by tazobactam at 4 mg/L and 26/38 at 8 mg/L, as in the cefepime combination.

4.0 Discussion

Piperacillin/tazobactam has been a mainstay broad-spectrum antibiotic since the 1990s, used empirically and effectively in many types of infection. Nonetheless it has limitations, and these have grown with time. Its spectrum always excluded most AmpC-hyperproducing Enterobacterales,[19] and most recent surveys find that $\geq$30% of ESBL producers are resistant.[20] Given that extracted ESBLs are inhibited by tazobactam in the assay cuvette, this behaviour may reflect the strong expression of ESBLs by currently-circulating Enterobacterales,[21] the impermeability of producer strains and/or the frequent co-production of OXA-1, an inhibitor-resistant penicillinase.[14] Even where ESBL producers

appearred susceptible to piperacillin/ tazobactam *in vitro*, outcomes were worse than with a carbapenem, at least in bacteraemia.[22] Underlying these observations is the fact that piperacillin is highly labile to many β–lactamases, making it a difficult drug to protect. Moreover, piperacillin/tazobactam MICs are notoriously difficult to measure accurately[23] and the zone diameter : MIC correlation has a shallow gradient, increasing the hazard that isolates are miscategorised.[24]

Cefepime is an attractive replacement partner for tazobactam, being both a rapid permeant of Gram-negative bacteria and near-stable to AmpC and OXA-48 β–lactamases,[3,25] both of which attack piperacillin. It is also a weaker substrate for some ESBLs, though these are diverse in relative activity, making generalisation difficult. Moreover, although cefepime is not completely stable to OXA-1 enzyme it is less vulnerable than piperacillin, meaning that this enzyme is less likely to present a problem.[26] The principle of combining tazobactam with a cephalosporin has been well-illustrated with ceftolozane/tazobactam, which showed efficacy against ESBL-producing Enterobacterales in Phase III/IV cUTI, cIAI and VAP trials.[27,28] Moreover, whereas ceftolozane/tazobactam was developed for 1+0.5g and, latterly, 2+1g q8h regimens, cefepime/tazobactam is being developed as a 2+2g regimen with a 90-min infusion time to maximise coverage. Pharmacodynamic modelling suggests that this, coupled with q8h regimen, may justify a 16+8 mg/L breakpoint, exceeding the current EUCAST and CLSI values for unprotected cefepime, which were used as reference points here.[29]

Even, however, when reviewed against the current cefepime breakpoints, cefepime/tazobactam was widely active against AmpC- and ESBL- producing Enterobacterales, including those with ertapenem resistance and reduced meropenem susceptibility, which imply porin loss. Its likely spectrum in context exceeds that of

ceftolozane/tazobactam, which is constrained by: (i) a lack of activity against AmpC-hyperproducers, (ii) weaker inherent activity, as reflected in higher MICs for 'wildtype' Enterobacterales (Table 1), (iii) greater compromise against ESBL producers with raised carbapenem MICs (Table 3) and (iv) a low assigned breakpoints (2+4 mg/L).

Harder to assess is the apparent activity of cefepime/tazobactam at 4+8 or 8+8 mg/L against large proportions of Enterobacterales with KPC carbapenemases. These results are in keeping with earlier data using panels of selected isolates,[4,30] particularly in media supplemented with human serum. They reflect the activity of cefepime itself, with no significant potentiation by tazobactam (Table 2), which is inactivated by KPC enzymes,[31] and we are unaware of any evidence that cefepime is effective in infections due to pathogens with KPC carbapenemases. Most of the isolates were clonally diverse organisms referred from hospitals around Manchester, which has a long-standing 'plasmid outbreak'.[16] Isolates from this region of the UK typically are less resistant to carbapenems and cephalosporins, including cefepime, than are representatives of the international ST258 KPC-carbapenemase-producing *K. pneumoniae* clone, which dominates in many countries.[18] Accordingly, we are cautious of claiming any widespread activity against isolates with KPC enzymes, though the aspect does need to be explored further.

Activity was also seen, at cefepime breakpoints, against the great majority of isolates with OXA-48 carbapenemases. For ceftazidime-non-resistant isolates this is unsurprising: OXA-48-like enzymes lack substantial activity against oxyimino-cephalosporins, including cefepime, which remains effective against producers in animal models.[32] What was more striking was the activity against *bla*$_{\text{OXA-48}}$-positive isolates that were resistant to ceftazidime, putatively owing to co-production of ESBLs or other secondary β–lactamases. Encouragingly, tazobactam potentiated cefepime against these isolates. In many respects, the surprise is not

that it did so, but rather that – as found here and previously[10] – tazobactam fails to similarly protect ceftolozane. The reasons for this difference remain uncertain but the present potentiation of cefepime refutes an earlier hypothesis that OXA-48-like enzymes might inactivate tazobactam.[10]

Whereas cefepime/tazobactam was the most active combination against important Enterobacterales groups, the position reversed for *P. aeruginosa*. Here, ceftolozane/tazobactam consistently was the more active combination for isolates with the two prevalent modes of cephalosporin resistance - upregulated efflux and derepressed AmpC, despite the handicap of a lower (4+4 mg/L) breakpoint. Nonetheless, cefepime remains one of only two 'old' cephalosporins with broad anti-*P. aeruginosa* activity, the other being ceftazidime. Based on EUCAST cefepime breakpoints, cefepime/tazobactam was more widely active than piperacillin/tazobactam against the raised efflux group in particular. Except against the most difficult strains, it should achieve good coverage against *P. aeruginosa*.

Carbapenemase-producing *A. baumannii* isolates were widely resistant to all tazobactam combinations whereas other *Acinetobacter* spp. were often inhibited by tazobactam itself irrespective of its partner β-lactam. MICs for other non-fermenters largely mirrored the activity of cefepime, with tazobactam giving little potentiation;, for the *B. cepacia* complex, neither EUCAST nor CLSI attaches confidence to β-lactam MICs as a guide for treatment.

All these data, for difficult isolates, support the view that cefepime/tazobactam has potential as a 'workhorse' replacement for piperacillin/tazobactam, with scope to be 'carbapenem-sparing'. This term is much abused, often being tagged to innovative antibiotics better suited to directed therapy against bacteria with critical resistances. Nonetheless, the concept is intellectually defensible in three situations. The first is for narrow-spectrum drugs

(e.g. temocillin) used as directed therapy against key resistance types where a carbapenem otherwise would be prescribed. The second is if an agent with unlinked resistance were to be used empirically to mitigate selection pressure. A plausible, if hypothetical, example would be routinely to use tigecycline in intra-abdominal sepsis, relieving pressure on β-lactams, albeit by using an agent with a more chequered side-effect profile. The third case, pertinent here, is where established agents are repurposed or combined in a manner to achieve coverage equalling a carbapenem, giving scope to spare empirical and directed carbapenem use. Although clinical experience is needed, its in-vitro coverage, based on likely breakpoints, includes difficult AmpC and ESBL producers with ertapenem resistance, as well as most Enterobacterales with OXA-48 carbapenemases. Some less-meropenem-resistant isolates with KPC carbapenemases may be within spectrum, but this needs further investigation. Although ceftolozane/tazobactam is more active against difficult *P. aeruginosa* resistance phenotypes, cefepime/tazobactam should achieve wider antipseudomonal coverage than piperacillin/tazobactam.

Only a few bacteria, not studied here, are widely susceptible to carbapenems and piperacillin/tazobactam, but would be expected to be resistant to cefepime/tazobactam, Notable examples are *Enterococcus faecalis* and *Bacteroides* spp. Coverage of these, if thought prudent for a particular setting, could easily be achieved by adding amoxicillin or metronidazole, respectively.

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Declarations

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Competing interests.

DML has undertaken Advisory Boards or ad-hoc consultancy for Accelerate, Allecra, Antabio, Centauri, Entasis, GlaxoSmithKline, J&J, Meiji, Melinta, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, T.A.Z., Tetraphase, VenatoRx, Wockhardt, Zambon. He has present paid lectures for Astellas, bioMérieux, Beckman Coulter, Cardiome, Cepheid, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. He has direct relevant shareholdings or options in Dechra, GSK, Merck, Perkin Elmer, Pfizer, and T.A.Z, amounting to <10% of portfolio value. He also has nominated holdings in Avacta, Byotrol, Destiny, Diaceutics, Evgen, Fusion Antibodies, Genedrive, Hardide, Renalytics, Scancell and Synairgen (all of which have research/products pertinent to COVID-19) through Enterprise Investment Schemes, but has no authority to trade these shares directly. **SM, PG, AV** and **NW** are members of PHE's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, which has received financial support for conference attendance, lectures, research projects, or contracted evaluations from numerous sources, including Accelerate Diagnostics, Achaogen Inc., Allecra Therapeutics, Amplex, AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp, Meiji Seika Pharma Co. Ltd, Mobidiag, Momentum Biosciences

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542 **Table 1.** MIC distribution of tazobactam combinations

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	Number of isolates with indicated MIC (mg/L)													
Row Labels	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**
Enterobacterales: Wild-types (n=71)[a]														
Cefepime/tazobactam 8 mg/L	33	20	10	2	3	1		1	1					
Ceftolozane/tazobactam 4 mg/L			8	29	18	13	1			1		1		
Piperacillin/tazobactam 4 mg/L				4	7	9	28	10	8	2			2	1
Enterobacterales: AmpC (n=418)[b]														
Cefepime/tazobactam 8 mg/L	32	45	42	70	78	70	43	28	8	2				
Ceftolozane/tazobactam 4 mg/L		1	5	24	51	48	57	49	72	56	32	18	4	1
Piperacillin/tazobactam 4 mg/L		1		2	3	5	18	53	38	54	56	70	64	54
Enterobacterales: ESBL (n=306)[c]														
Cefepime/tazobactam 8 mg/L	43	42	44	33	29	40	24	18	10	14	6			3
Ceftolozane/tazobactam 4 mg/L			10	29	55	48	56	25	20	20	19	8	7	9
Piperacillin/tazobactam 4 mg/L					2	5	34	39	46	37	30	30	21	62
Enterobacterales: AmpC + ESBL (n=27)[d]														
Cefepime/tazobactam 8 mg/L	1	2	1	2	3	4	7	5	1	1				
Ceftolozane/tazobactam 4 mg/L				1	1	1	2	4	4	2	6	2	1	3
Piperacillin/tazobactam 4 mg/L							1	1	2	1	4	3	5	10
Enterobacterales: impermeable (n=31)[e]														
Cefepime/tazobactam 8 mg/L	1		3	6	4	9	4	4						
Ceftolozane/tazobactam 4 mg/L			1	3	9	10	5	2				1		

Piperacillin/tazobactam 4 mg/L					1		2	4	8	9	2	2	1	2
Enterobacterales: *K. oxytoca*, K1 (n=4)														
Cefepime/tazobactam 8 mg/L			1		1		1				1			
Ceftolozane/tazobactam 4 mg/L						2	1		1					
Piperacillin/tazobactam 4 mg/L													1	3
Enterobacterales: KPC carbapenemases (n=116)[f]														
Cefepime/tazobactam 8 mg/L	1	2			3	11	36	35	17	3	4	4		
Ceftolozane/tazobactam 4 mg/L				1	2	1	4	23	31	27	17	4	5	1
Piperacillin/tazobactam 4 mg/L							1	2			1	5	21	86
Enterobacterales: GES carbapenemases (n=10) [g]														
Cefepime/tazobactam 8 mg/L			1	2	3		1		3					
Ceftolozane/tazobactam 4 mg/L									2		6	2		
Piperacillin/tazobactam 4 mg/L										1	2	1	3	3
Enterobacterales: Other class A carbapenemases (n=9) [h]														
Cefepime/tazobactam 8 mg/L	2	4	1	2										
Ceftolozane/tazobactam 4 mg/L				3	3	1	2							
Piperacillin/tazobactam 4 mg/L						1	1	5	1				1	
Enterobacterales: OXA-48 carbapenemases CAZ <u>≤</u>4 mg/L (n=114) [i]														
Cefepime/tazobactam 8 mg/L	1	13	34	25	20	14	4	2					1	
Ceftolozane/tazobactam 4 mg/L				10	44	33	16	10						1
Piperacillin/tazobactam 4 mg/L								1		4	1	26	41	41

Enterobacterales: OXA-48 carbapenemases CAZ >4 mg/L (n=136) [j]														
Cefepime/tazobactam 8 mg/L			3	5	20	13	17	19	13	12	11	14	5	4
Ceftolozane/tazobactam 4 mg/L						2	7	10	15	15	13	25	16	33
Piperacillin/tazobactam 4 mg/L											1	7	12	116
Enterobacterales: MBL carbapenemases (n=210) [k]														
Cefepime/tazobactam 8 mg/L	1	1		1	3	4	3	3	12	35	48	23	11	65
Ceftolozane/tazobactam 4 mg/L			1	1		1						3	1	203
Piperacillin/tazobactam 4 mg/L							1	2	3	4	6	14	21	159
Enterobacterales: MBL + OXA-48 carbapenemases (n=24) [l]														
Cefepime/tazobactam 8 mg/L										1	1		1	21
Ceftolozane/tazobactam 4 mg/L											1			23
Piperacillin/tazobactam 4 mg/L														24
Enterobacterales: unassigned, CAZ MIC ≤4 mg/L (n=58) [m]														
Cefepime/tazobactam 8 mg/L	13	9	3	6	7	8	4	2	3	3				
Ceftolozane/tazobactam 4 mg/L			6	14	16	12	6	3			1			
Piperacillin/tazobactam 4 mg/L						3	9	11	5	8	2	1	4	15
Enterobacterales: unassigned, CAZ MIC 8-32 mg/L (n=20) [n]														
Cefepime/tazobactam 8 mg/L			1	3	4		4	2	2	2		1	1	
Ceftolozane/tazobactam 4 mg/L					3		7	5	2	2	1			
Piperacillin/tazobactam 4 mg/L							3		3	1	1		1	11

Enterobacterales: unassigned, CAZ MIC ≥64 mg/L (n=64) [o]														
Cefepime/tazobactam 8 mg/L	2	3	4	4	3	7	7	1	1	11	4		4	13
Ceftolozane/tazobactam 4 mg/L			1	1	6	2	6	7	1	4	3	6	5	22
Piperacillin/tazobactam 4 mg/L				1			1	5	3	6	1	5	2	40
Enterobacterales: unassigned, type I unknowns (n=14) [p]														
Cefepime/tazobactam 8 mg/L			2			2	6	2		1	1			
Ceftolozane/tazobactam 4 mg/L				1	1	2	4	3	2	1				
Piperacillin/tazobactam 4 mg/L									2			1	1	10
***P. aeruginosa:* normal efflux/wild-type (carbenicillin MIC 32-128 mg/L) (n=96)**														
Cefepime/tazobactam 8 mg/L					1	9	37	23	22	2	1			1
Ceftolozane/tazobactam 4 mg/L			2	3	57	26	5	1	1					1
Piperacillin/tazobactam 4 mg/L					1	1	2	22	32	21	11	3		3
***P. aeruginosa:* raised efflux (carbenicillin MIC 256-512 mg/L) (n=188)**														
Cefepime/tazobactam 8 mg/L	1		1	1		1	6	37	107	22	9	2	1	
Ceftolozane/tazobactam 4 mg/L		1		2	41	97	35	6	5	1				
Piperacillin/tazobactam 4 mg/L		1		2			2	7	10	46	83	14	15	8
***P. aeruginosa:* highly raised (carbenicillin MIC 32-128 mg/L)(n=85)**														
Cefepime/tazobactam 8 mg/L							1	5	21	29	14	9	5	1
Ceftolozane/tazobactam 4 mg/L				1	6	27	25	9	11	1	3		1	1

Piperacillin/tazobactam 4 mg/L						1		2	3	17	23	22	9	8
P. aeruginosa: **low efflux (carbenicillin ≤16 mg/L) (n=44)**														
Cefepime/tazobactam 8 mg/L			2	1	2	5	6	12	10	4	1		1	
Ceftolozane/tazobactam 4 mg/L			5	6	13	17	1	1	1					
Piperacillin/tazobactam 4 mg/L			1	3	5	12	10	6	2	4		1		
P. aeruginosa: **derepressed AmpC (n=71)**														
Cefepime/tazobactam 8 mg/L							4	16	27	15	7	1	1	
Ceftolozane/tazobactam 4 mg/L					11	25	23	10		1	1			
Piperacillin/tazobactam 4 mg/L					1		2	2	1	6	8	16	18	17
P. aeruginosa: **ESBL (n=22)**														
Cefepime/tazobactam 8 mg/L								1	2		15	4		
Ceftolozane/tazobactam 4 mg/L											2	1	1	18
Piperacillin/tazobactam 4 mg/L										1	2	8	7	4
P. aeruginosa: **GES carbapenemase (n=15)**														
Cefepime/tazobactam 8 mg/L							5	4	6					
Ceftolozane/tazobactam 4 mg/L								3	9	2			1	
Piperacillin/tazobactam 4 mg/L									1	2	10	1	1	
P. aeruginosa: **MBL carbapenemase (n=81)**														
Cefepime/tazobactam 8 mg/L						1		1	3	18	16	23	8	11
Ceftolozane/tazobactam 4 mg/L							1	1				5	15	59
Piperacillin/tazobactam 4 mg/L									1	1	19	16	19	25

***P. aeruginosa:* unassigned, CAZ MIC ≤8 mg/L (n=87)**														
Cefepime/tazobactam 8 mg/L					2	20	30	22	12	1				
Ceftolozane/tazobactam 4 mg/L				2	41	32	8	3	1					
Piperacillin/tazobactam 4 mg/L						1	2	35	20	10	11	6	1	1
***P. aeruginosa:* unassigned, CAZ MIC 16-128 mg/L (n=39)**														
Cefepime/tazobactam 8 mg/L						1	3	2	11	10	7	1	4	
Ceftolozane/tazobactam 4 mg/L					2	5	15	10	3		3		1	
Piperacillin/tazobactam 4 mg/L							1	1	7	6	2	7	4	11
***P. aeruginosa:* unassigned, CAZ MIC ≥256 mg/L (n=17)**														
Cefepime/tazobactam 8 mg/L									1	2	2	4	2	6
Ceftolozane/tazobactam 4 mg/L							1	1	3	4	1	2	1	4
Piperacillin/tazobactam 4 mg/L									1	1		4	2	9
***A. baumannii:* 92.6% *with carbapenemases* (n=216)**														
Cefepime/tazobactam 8 mg/L	8	2		1		1		3	11	28	60	72	18	12
Ceftolozane/tazobactam 4 mg/L		4	1	2	1	1	7	12	27	20	66	32	7	36
Piperacillin/tazobactam 4 mg/L		3	1		3			2	1	2	1	4	5	194
Non-*baumannii Acinetobacter* spp. (n=38)														
Cefepime/tazobactam 8 mg/L	27	1		1		3	2			3	1			
Ceftolozane/tazobactam 4 mg/L		20	1	1	3	2	3	3	1	1	1	1	1	
Piperacillin/tazobactam 4 mg/L		20						4	5	3	1	1	2	2

***Burkholderia cepacia* complex. (n=48)**														
Cefepime/tazobactam 8 mg/L	3			2	3	17	7	3	2	5	2	1	1	2
Ceftolozane/tazobactam 4 mg/L		3		1	7	10	13	7	3	1	1	1		1
Piperacillin/tazobactam 4 mg/L		3		4	13	11	6	2			5		2	2
Non-*aeruginosa Pseudomonas* (n=36)														
Cefepime/tazobactam 8 mg/L			2	2	4	11	7	2	5	1	1		1	
Ceftolozane/tazobactam 4 mg/L		3	4	2	11	6	2	2		1	2			3
Piperacillin/tazobactam 4 mg/L		1		1	3		7	4	11	5	2	2		
***Achromobacter* spp. (n=33)**														
Cefepime/tazobactam 8 mg/L								2	6	5	6	7	5	2
Ceftolozane/tazobactam 4 mg/L									2	1	9	4	8	9
Piperacillin/tazobactam 4 mg/L				1	12	4	4	5	3		1	2	1	
***S. maltophilia* (n=32)**														
Cefepime/tazobactam 8 mg/L				1	4	4	4	10	4	1	2	1	1	
Ceftolozane/tazobactam 4 mg/L			1	1	4	4	5	3	5	3		3	1	2
Piperacillin/tazobactam 4 mg/L						1		1	7	1	7	5	4	6
***Pandoraea* spp. (n=20)**														
Cefepime/tazobactam 8 mg/L	3					1		2	1	4		1	6	2
Ceftolozane/tazobactam 4 mg/L								1			1	3	3	12
Piperacillin/tazobactam 4 mg/L				1		2	3	3	3	3	1		4	
***Elizabethkingia* spp. (n=8)**														
Cefepime/tazobactam 8 mg/L	2			2	1	3								
Ceftolozane/tazobactam 4 mg/L								3	2	3				

Piperacillin/tazobactam 4 mg/L								3	2	1		2		
***Chryseobacterium* spp. (n=4)**														
Cefepime/tazobactam 8 mg/L		1	2				1							
Ceftolozane/tazobactam 4 mg/L				1				1		1		1		
Piperacillin/tazobactam 4 mg/L			1						1	1			1	
Rare non-fermenters (n=15)														
Cefepime/tazobactam 8 mg/L	2	1		1		3		2	3	1				2
Ceftolozane/tazobactam 4 mg/L		1		1		1	2		2	2	1	1	1	3
Piperacillin/tazobactam 4 mg/L		1			4	1	2	1		1		1	1	3

[a] *E. coli* (n=15), *Klebsiella* spp. (n=15), *Enterobacter* spp. (n=17), Others (n=24)
[b] *E. coli* (n=47), *Klebsiella* spp. (n=98), *Enterobacter* spp. (n=230), Others (n=43)
[c] *E. coli* (n=145), *Klebsiella* spp. (n=140), *Enterobacter* spp. (n=20), Others (n=1)
[d] *E. coli* (n=11), *Klebsiella* spp. (n=3), *Enterobacter* spp. (n=12), Others (n=1)
[e] *E. coli* (n=12), *Klebsiella* spp. (n=16), *Enterobacter* spp. (n=3), Others (n=0)
[f] *E. coli* (n=20), *Klebsiella* spp. (n=74), *Enterobacter* spp. (n=19), Others (n=3)
[g] *E. coli* (n=4), *Klebsiella* spp. (n=4), *Enterobacter* spp. (n=0), Others (n=2)
[h] *E. coli* (n=0), *Klebsiella* spp. (n=0), *Enterobacter* spp. (n=6), Others (n=3)
[i] *E. coli* (n=60), *Klebsiella* spp. (n=34), *Enterobacter* spp. (n=14), Others (n=6)
[j] *E. coli* (n=36), *Klebsiella* spp. (n=77), *Enterobacter* spp. (n=15), Others (n=8)
[k] *E. coli* (n=68), *Klebsiella* spp. (n=108), *Enterobacter* spp. (n=22), Others (n=12)
[l] *E. coli* (n=1), *Klebsiella* spp. (n=23), *Enterobacter* spp. (n=0), Others (n=0)
[m] *E. coli* (n=29), *Klebsiella* spp. (n=21), *Enterobacter* spp. (n=3), Others (n=5)
[n] *E. coli* (n=2), *Klebsiella* spp. (n=16), *Enterobacter* spp. (n=2), Others (n=0)
[o] *E. coli* (n=11), *Klebsiella* spp. (n=53), *Enterobacter* spp. (n=0), Others (n=0)
[p] *E. coli* (n=0), *Klebsiella* spp. (n=14), *Enterobacter* spp. (n=0), Others (n=0)

563 **Table 2**. Distribution of fold reductions in cefepime MIC in the presence of 8 mg/L tazobactam for Enterobacterales groups.
564

Row Labels	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4192	Total
Wild-type for β-lactamase		3	29	35	1	2	1									71
AmpC	2	19	144	164	68	17	2		1	1						418
ESBL + AmpC		1	1	2	2	5	2	4	5	3	2					27
ESBL		3	25	18	13	13	21	23	41	39	41	39	19	8	3	306
K1 hyperproducer			2		2											4
Impermeability	1	2	17	10	1											31
KPC		2	35	61	12	3		1	1				1			116
GES			2			4			2	2						10
Other class A carbapenemase			4	5												9
MBL		4	120	55	21	3	5		1	1						210
MBL (NDM) + OXA-48			22	1		1										24
OXA-48 CAZ-S/I		4	53	37	3	7	6	2	1	1						114
OXA-48-CAZ-R		3	22	22	19	18	18	17	14	2	1					136
Unassigned ceftazidime MIC ≤4			19	30	7	2										58
Unassigned ceftazidime MIC 8-32		1	9	7	2					1						20
Unassigned ceftazidime MIC >64			20	7	4	9	1		8	7	3	4	1			64
K. pneumoniae CAZ/CPM-R CTX-S/I			10	3		1										14
Grand Total	**3**	**42**	**534**	**457**	**155**	**85**	**56**	**47**	**74**	**57**	**47**	**43**	**21**	**8**	**3**	**1632**

565

566 **Table 3.** Cefepime/tazobactam and ceftolozane/tazobactam MICs for ESBL producers in relation to those of ertapenem, taken as a

567 proxy for impermeability.

Ertapenem MIC (mg/L)			Distribution of MICs of cefepime/tazobactam ESBL producers with the ertapenem MIC indicated in the left-hand column (mg/L)												
	0.03	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**Total**
≤0.016	12	3	1												16
0.03	10	15	4	1	2	1									33
0.06	9	4	11	3		2	1								30
0.125	7	9	5	1	1	1	3								27
0.25	1	5	6	5	4	1				1					23
0.5	3	4	7	10	4	2	6	6	3	1					46
1	1	1	6	5	4	7	2	6	3	1					36
2		1	2	6	7	10	4	2	2		1				35
4			1	1	4	5	2	1		4	1				19
8			1	1	2	4	1	2	1	5	1			2	20
16					1	3	4				2				10

32						4	1	1	1	2	1				10
64														1	1
Total	**43**	**42**	**44**	**33**	**29**	**40**	**24**	**18**	**10**	**14**	**6**	**0**	**0**	**3**	**306**
			Distribution of MICs of ceftolozane/tazobactam for ESBL producers with the ertapenem MIC indicated in the left-hand column (mg/L)												
≤0.016			8	6	1	1									16
0.03			2	11	14	4	2								33
0.06				7	14	7	2								30
0.125				1	10	9	5			2					27
0.25					4	5	9	2		2	1				23
0.5				1	6	5	15	6	4	5	4				46
1				1	2	6	8	4	4	4	3	3	1		36
2				2	2	7	9	3	3	1	4	1	2	1	35
4						2	2	3	5	3	1	1	1	1	19
8					2	1	3	2	1	1	3	2	1	4	20
16							1	3	2		1			3	10

32						1		2	1	2	2	1	1		10
64													1		1
Total			**10**	**29**	**55**	**48**	**56**	**25**	**20**	**20**	**19**	**8**	**7**	**9**	**306**
	Distribution of MICs of meropenem for ESBL producers with the ertapenem MIC indicated in the left-hand column (mg/L)														
Row Labels	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**					**Total**
≤0.016	16														16
0.03	33														33
0.06	26	3	1												30
0.125	17	9	1												27
0.25	4	15	4												23
0.5	4	25	16	1											46
1	1	12	14	6	3										36
2		3	9	19	4										35
4				4	8	5	2								19
8				1	3	12	4								20

16						3	3	3	1						10
32						1	1	7	1						10
64										1					1
Grand Total	**101**	**67**	**45**	**31**	**18**	**21**	**10**	**10**	**2**	**1**					**306**

568

569

Table 4. Cross relation of cefepime/tazobactam and meropenem MICs for Enterobacterales with KPC carbapenemases

	No. isolates with indicated combination of MIC values												
	Cefepime/tazobactam MIC (mg/L)												
MIC meropenem (mg/L)	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**Total**
0.03	1	1											2
0.06		1											1
0.12													
0.25													
0.5					1	2	1	1					5
1							1						1
2						4	5	3					12
4					1	3	4	6	1				15
8					1	1	18	12	2				34
16						1	6	10	6	2			25
32							1	3	4		2	1	11
64									3	1	1		5
128									1		1	3	5
Grand Total	**1**	**2**	**0**	**0**	**3**	**11**	**36**	**35**	**17**	**3**	**4**	**4**	**116**

573 **Table 5.** Cross relation of cefepime/tazobactam and ceftolozane/tazobactam MICs for *P. aeruginosa* with low, normal, raise or highly

574 raised efflux function

Cefepime/tazobactam MIC (mg/L)	**No. isolates with indicated combination of MIC values**													
	Ceftolozane/tazobactam MIC (mg/L)													
	0.06	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**>128**	**Total**
0.03	1													1
0.06														
0.125		1	1		1									3
0.25		1	1											2
0.5		2	1											3
1		2	4	8	1									15
2		1	5	35	9									50
4				40	33	2	1	1						77
8				29	101	27	2	1						160
16				4	19	26	7	1						57
32					2	7	6	8	1				1	25
64				1	1	4		4		1				11
128							1	2	1	2		1		7
>128								1					1	2
Grand Total	**1**	**7**	**12**	**117**	**167**	**66**	**17**	**18**	**2**	**3**		**1**	**2**	**413**

575