Activity of cefepime/zidebactam (WCK 5222) against 'problem' antibiotic-resistant Gram-negative bacteria sent to a national reference laboratory

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Running head. Cefepime/zidebactam versus referred isolates

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Abstract

Introduction. Triple-action diazabicyclooctanes, e.g., zidebactam, combine β-lactamase inhibition, antibacterial activity, and 'enhancement' of PBP3-targetted β-lactams. We examined the activity of cefepime/zidebactam against consecutive 'problem' Gram-negative bacteria referred to the UK national reference laboratory. **Materials and Methods.** MICs were determined by BSAC agar dilution for 1632 Enterobacterales, 745 *Pseudomonas aeruginosa* and 450 other non-fermenters, categorised by carbapenemase detection and interpretive reading. **Results**. Universal susceptibility to cefepime/zidebactam 8+8 mg/L was seen for otherwise multidrug-resistant Enterobacterales with AmpC, extended-spectrum, K1, KPC and OXA-48-like β-lactamases, or with impermeability and 'unassigned' mechanisms. Unlike ceftazidime/avibactam and all other comparators, cefepime/zidebactam 8+8 mg/L also inhibited most (190/210, 90.5%) Enterobacterales with MBLs. Resistance in the remaining minority of MBL producers, and in 13/24 with NDM MBLs plus OXA-48-like enzymes, was associated with *Klebsiella pneumoniae* ST14. For *Pseudomonas aeruginosa*, MICs of cefepime/zidebactam with efflux grade, but exceeded 8+8 mg/L for only 11/85 isolates even in the highly-raised efflux group. Among 103 *P. aeruginosa* with ESBLs or MBLs 97 (94.5%) were inhibited by cefepime/zidebactam 8+8 mg/L whereas fewer than 15% were susceptible to any comparator. MICs for *Acinetobacter baumannii* with acquired OXA carbapenemases clustered around 8+8 to 32+32 mg/L, with higher values for MBL producers. A strong enhancer effect augmented activity against many isolates that were highly resistant to cefepime and zidebactam alone and which had mechanisms not inhibited by zidebactam. **Conclusion.** Assuming successful clinical trials, cefepime/zidebactam has scope to widely overcome critical resistances in both Enterobacterales and non-fermenters.

Introduction

Diazabicyclooctanes (DBOs) are one of two new β-lactamase inhibitor classes to enter clinical use, boronates being the other.[1] Two DBOs are now licenced: avibactam, combined with ceftazidime, and relebactam, combined with imipenem/cilastatin. Both are 'pure' inhibitors, targeting Class A and C β-lactamases, also some class D types in the avibactam's case.[2] Avibactam inhibits the growth of a few Enterobacterales isolates, principally *Escherichia coli,* at 4-16 mg/L but, otherwise, has little direct antibiotic activity. Relebactam MICs consistently exceed 32 mg/L.[3,4]

DBOs can, however, be modified to target PBP2 of Gram-negative bacteria, as with nacubactam, zidebactam and durlobactam, which all have MICs within likely clinical ranges for Enterobacterales and, in the case of zidebactam, also for *Pseudomonas aeruginosa*.[3-6] This activity is vulnerable to high-frequency mutations, precluding development as standalone antibiotics, but three factors should mitigate this hazard in combination use. First, and obviously, β-lactamase inhibitory activity remains. Secondly, the resistance-conferring mutations are compensatory and, under DBO challenge, 'resistant' mutants grow as round forms of questionable virulence.[7] Third, continued attack on PBP2 by the DBO, combined with attack on PBP3 by its partner β-lactam engenders a β–lactamase-inhibition-independent synergy dubbed the 'enhancer effect,'[3,6,8] meaning that MICs of β-lactam/DBO combinations often remain low even when an isolate is unequivocally resistant to both components alone and has a β-lactamase that is not inhibited by the DBO.

DBOs that combine β-lactamase inhibition, PBP2 attack and enhancer effect are dubbed 'triple action' molecules, and their combinations with PBP3-targetted β-lactams achieve extremely wide spectra. In the present study we examined the activity of

cefepime/zidebactam (WCK 5222) against consecutive reference laboratory submissions of Gram-negative bacteria, taken as a sample of problem strains circulating in the UK.

Materials and Methods

Bacteria

The test collection has been described previously: it comprised around half of the Gram-negative bacteria submitted to the PHE Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit (AMRHAI) from July 2015 to July-2016, as previously used for assessments of ceftolozane/tazobactam ceftazidime/avibactam against 'problem' organisms.[9,10] The same subset was recently used for cefepime/tazobactam.[11] Most isolates were originally referred owing to unusual resistance to carbapenems or other β-lactams, but some are referred owing to suspected resistance to e.g., polymyxins, and remain fully susceptible to β-lactams. Identification was by MALDI-ToF (Bruker Biotyper, Bremen, Germany). Resistance mechanisms were the categorised based on genotype data, predominantly for carbapenemases, and interpretive reading of phenotypes, as detailed previously.[9-11] Upregulated efflux in *P. aeruginosa* was inferred based on raised and inter-related MICs for carbenicillin, cefotaxime, piperacillin/tazobactam and ceftazidime, along with the absence of ceftazidime/avibactam synergy; it was graded as 'raised' if the carbenicillin MIC was 256-512 mg/L and 'highly raised in the carbenicillin MIC was >512 mg/L.[10] Sequence types were based on previous WGS or variable number tandem repeat data.[12]

Antibiotics and susceptibility testing

Zidebactam, ertapenem, ceftolozane and avibactam were from Wockhardt (Aurangabad, India); other antibiotics were from Alpha Aesar (Heysham, UK) or Merck KGaA (Gillingham,

UK). MICs were determined by BSAC agar dilution on IsoSensitest agar (Oxoid, Basingstoke, UK)[13] and interpreted versus current (v.10.0) EUCAST guidance.[14] Certificates of Analysis data were provided for each antibiotic, irrespective of source, and were used to derive potencies (as per CLSI guidance), with appropriate correction factors then used when preparing antibiotic dilutions. Cefepime/zidebactam was tested as a 1:1 ratio; tazobactam was used at a fixed 4 mg/L in combinations with ceftolozane and piperacillin; avibactam at a fixed 4 mg/L combined with ceftazidime. Across 33 sets of MIC testing plates for eight antibiotics there were just nine instances where values for one of the four control strains (*Escherichia coli* ATCC 25922 and ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603) fell outside the accepted control limits.

Results.

Resistance profile of the test collection

Susceptibility data for comparator antibiotics are summarised in Table 1, also showing the species distributions. Most reference laboratory submissions are broadly resistant (Table 1), and are referred for precisely that reason. Small groups of susceptible organisms (i.e., 'wild-type' Enterobacterales and 'low' and 'normal' efflux *P. aeruginosa*) largely were submitted owing to other resistances, e.g., to polymyxins not represented here. Ceftazidime/avibactam 8+4 mg/L was the most active comparator against Enterobacterales, achieving >90% coverage against all groups except for those with MBLs, which were almost universally resistant. A high prevalence of resistance to ertapenem and ceftolozane/tazobactam among ESBL and AmpC producers reflects the fact that most such isolates are referred on suspicion of carbapenemase production, but then transpire to have impermeability together with a non-carbapenemase enzyme.[9,10] High rates of meropenem 'susceptibility' among isolates with

OXA-48-like and KPC carbapenemases is typical producers circulating in the UK. Such 'susceptibility' among carbapenemase producers is not, in our view, an indicator of likely response, given presence of the carbapenemase.[15]

For *Pseudomonas aeruginosa*, all comparators showed diminishing activity as the grade of efflux (or of ceftazidime resistance among isolates with unassigned mechanisms) increased, with ceftolozane/tazobactam the least compromised agent (Table 1). Ceftazidime/avibactam as well as ceftolozane/tazobactam retained activity against over 90% of isolates with derepressed AmpC. No comparator was active against even 15% *P. aeruginosa* isolates with ESBLs and MBLs, although ceftazidime/avibactam was widely active against those with GES carbapenemases. Other non-fermenters are omitted from Table 1, since EUCAST largely lacks specific breakpoints. Among 216 *A. baumannii*, 183 had acquired bla_{OXA} carbapenemase genes alone, 19 had MBL genes alone or together with acquired bla_{OXA} whilst 14 had neither, though they retained intrinsic $bla_{OXA\text{-}51}$. Susceptibility rates to meropenem 8 mg/L among these three groups were 3.3%, 0% and 86%, respectively. Further agents were examined during prior reference testing, again by BSAC methodology. Among all 1632 Enterobacterales, 1356 (83.1%) were resistant to cefotaxime, 2 mg/L; 923 (56.6%) to ciprofloxacin, 0.5 mg/L and 643 (39.4%) to gentamicin, 2 mg/L, corresponding to EUCAST 'high' breakpoints; among the 745 *P. aeruginosa*, 351 (47.1%) were resistant to ceftazidime, 8 mg/L, 483 (64.8%) to ciprofloxacin, 0.5 mg/L and 217 (29.1%) to tobramycin 2 mg/L. It should be cautioned that some groups – particularly *K. oxytoca* with high level K1 enzyme were small. MICs were determined once but, in 80% of cases were in essential (i.e. +/- 1 doubling dilution) agreement with previous reference testing. [9,10]

MICs of zidebactam, cefepime, and their combination: Enterobacterales

Zidebactam differed from all comparators in that its MICs (fig. 1) strongly related to species among Enterobacterales. Values ≤2 mg/L were recorded for 90% of *E. coli* and 75% of *Enterobacter* spp. whereas MICs >128 mg/L were seen for all Proteeae and 90% of *Serratia* spp. Values for *Klebsiella* spp. straggled widely, being >128 mg/L for 65.7% of isolates but with a long tail of lower values. MICs were difficult to read, particularly for *Klebsiella* spp.: we took the lowest concentration at which confluent growth was substantially diminished; persistent thin confluent films, trailing colonies and resurgence after skipped tubes were disregarded.

Whilst high MICs of cefepime and zidebactam alone were frequent in many groups (Table 2), high MICs for cefepime/zidebactam 1:1 were extremely rare. Also, and in contrast to standalone zidebactam, MIC endpoints were discrete and readily readable. Thus, based on a tentative 8+8 mg/L breakpoint, cefepime/zidebactam was universally active against Enterobacterales with Class A (KPC, GES and other) and D (OXA-48-like) carbapenemases, AmpC β-lactamases, ESBLs, K1 β-lactamases, impermeability or other unassigned modes of resistance. For all these groups the new combination thus achieved a spectrum qualitatively equal to ceftazidime/avibactam, taken as the most-active comparator; quantitatively, activity was stronger, with MICs of cefepime /zidebactam mostly two- or four-fold lower than those of ceftazidime/avibactam (Table 3). Lastly, the activity of cefepime/zidebactam 8+8 mg/L encompassed most (190/210, 90.5%) MBL producers along with 11/24 (49%) that had both MBLs (always NDM) together with OXA-48-like enzymes. Very few members of these groups (9/220 and 1/24, respectively) were susceptible to ceftazidime/avibactam, with resistance to other comparators almost ubiquitous (Table 1).

Resistance to cefepime/zidebactam 8+8 mg/L among Enterobacterales was confined to just 33/1632 isolates, comprising 20/210 with MBLs alone and 13/24 with both MBLs

(always NDM) and OXA-48-like enzymes (Table 2). The high proportion of 'resistance' among the latter group was notable and appeared to be substantially clonal. All 13 were *K. pneumoniae* and 10, from six hospitals, were ST14 variants with OXA-232 as their 'OXA-48-like' enzyme. Among the 11/24 'NDM+OXA-48-like isolates' that were inhibited by cefepime/zidebactam at 8+8 mg/L, 10 were *K. pneumoniae,* eight of which had been typed. Four of the eight, all with cefepime/zidebactam MICs of 2-8 mg/L, belonged to ST14; three had OXA-232. The remaining four 'susceptible' isolates belonged to other lineages: three were inhibited by cefepime/zidebactam at 0.5-2 mg/L, with one MIC of 4 mg/L; only one had OXA-232 whereas the other three had classical OXA-48 Among the 20 'cefepime/zidebactam-resistant' MBL-producing Enterobacterales isolates lacking OXA-48-like enzymes, 19 were *K. pneumoniae* (from a total of 104 MBL-producing *K. pneumoniae*). These 19 were from 14 laboratories; 10 had typing data indicating ST14, with seven untyped. Among the 85 MBL-positive/OXA-48-negative *K. pneumoniae* that were inhibited by cefepime/zidebactam at $\leq$8+8 mg/L, 66 had been typed and only 3 had VNTR profiles suggesting ST14.

MICs of zidebactam, cefepime, and their combination: non-fermenters

For *P. aeruginosa*, 76% of all zidebactam MICs fell across the range 4-16 mg/L, with a trend for the modes and ranges to rise as one progressed upwards (i) through grades of efflux, from 'low' to 'highly raised', and (ii) from low to high ceftazidime MICs among isolates with unassigned mechanisms (which are likely to have and efflux component). In the case of the raised and highly raised efflux groups, also the 'unassigned ceftazidime MIC >256 mg/L' group, a few cefepime/zidebactam MICs extended to 32+32 mg/L. Nonetheless, the proportions of isolates with cefepime/zidebactam MICs >8+8 mg/L only exceeded 10% for the groups with highly raised efflux (12.9%, versus 20.0% resistant to ceftolozane/tazobactam 4+4 mg/L as

194 the most active comparator) or with unassigned mechanisms and ceftazidime MICs 16-128
195 mg/L (12.8%, versus 17.9% for ceftolozane/tazobactam, again the most active comparator)
196 or ≥256 mg/L (23.5%); versus 88.2% for ceftolozane/tazobactam and ceftazidime/avibactam
197 as the most active comparators). Among *P. aeruginosa* with ESBLs or MBLs, 97/103 were
198 inhibited by cefepime/zidebactam at 8+8 mg/L, whereas fewer than 15% of these isolates
199 were susceptible to any comparator (Table 1). MICs of cefepime/zidebactam are cross-
200 plotted against those of ceftolozane/tazobactam, as the most active antipseudomonal
201 comparator, in Table 4. Ceftolozane/tazobactam remained the more active agent up to its
202 4 mg/L breakpoint but this pattern reversed at higher ceftolozane/tazobactam MICs where
203 the population was dominated by isolates with ESBLs or MBLs.
204 Most other non-fermenter groups besides *P. aeruginosa* had high-level inherent
205 resistance to zidebactam alone, with 90 to 100% of MICs >128 mg/L for *Acinetobacter*
206 *baumannii*, other *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Chryseobacterium* spp.,
207 *Elizabethkingia* spp. and *Achromobacter* spp. For all groups, however, addition of zidebactam
208 caused a downward shift in the cefepime MIC distribution, most marked for the
209 *Chryseobacterium* / *Elizabethkingia* group and least for *Pandoraea* spp.
210 *A. baumannii* deserves particular comment as the most important of these non-
211 fermenters. Among 216 *A. baumannii* isolates included, 183 (84.7%) had acquired OXA
212 carbapenemase, with a positive PCR test for one or more of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$,or $bla_{\text{OXA-58}}$.
213 Cefepime/zidebactam MICs for these clustered around 8-16 mg/L, compared with 64 mg/L
214 for cefepime alone and >128 mg/L for zidebactam alone; MICs of cefepime/zidebactam for
215 MBL producing *A. baumannii* were higher, with all values ≥32 mg/L.
216
217 *Extent and nature of cefepime/zidebactam interactions*

Ordinarily, for a β-lactamase inhibitor, it is useful to review the distribution of fold MIC reductions achieved, defining the resistance groups where potentiation is strongest. This approach fails for triple action DBOs because, for many isolates, the combination MICs predominantly reflect the antibacterial activity of the DBO. It is also inappropriate when, as here, this situation necessitates using a fixed partner : inhibitor ratio, rather than a fixed inhibitor concentration. Calculating ΣFIC indices proved equally unsatisfactory, partly owing to the lack of complete chequerboards, but primarily because the scope for synergy was limited whenever an isolate was highly susceptible to zidebactam.

Straightforward analysis was, however, possible for those organisms that were highly resistant to both cefepime and zidebactam, with MICs >32 mg/L (Table 5). Among the 1632 Enterobacterales, 225 met these criteria: 108 with enzymes expected to be inhibited by zidebactam (i.e., ESBLs, KPC) or with little activity against cefepime (AmpC and OXA-48-like) All these isolates were strongly inhibited by cefepime/zidebactam, with MICs $\leq$2+2 mg/L. A further 42/225 had unassigned mechanisms and, among these, 35 were inhibited by cefepime/zidebactam 4+4 mg/L and all at 8+8 mg/L. Last, 75/225 had MBLs alone or in combination with OXA-48 and, among these, 45 were inhibited by cefepime/zidebactam at 8+8 mg/L, indicating a maximal ΣFIC of $\leq$0.25 [calculated as ($\leq$8/$\geq$64) + ($\leq$8/$\geq$64), with the possibility that a lower value might arise somewhere on a complete isobologram.

Only 18 *P. aeruginosa* met the dual criteria of cefepime and zidebactam MICs $\geq$64 mg/L, with 13 of these belonging to the raised/highly raised efflux categories or to 'unassigned' groups with raised or highly raised ceftazidime MICs. Ten of the 18 were susceptible to cefepime/zidebactam at $\leq$8+8 mg/L, indicating an ΣFIC of $\leq$0.25 [($\leq$8/$\geq$64) + ($\leq$8/$\geq$64)]; seven of the eight exceptions were organisms with highly raised efflux, or which belonged to unassigned groups with raised or highly raised ceftazidime MICs.

The criteria of cefepime and zidebactam MICs >32 mg/L mg/L were met by large majorities (118/183 and 19/19, respectively) of the *A. baumannii* isolates with acquired OXA or metallo carbapenemases. Unlike for other groups with high-level resistance to cefepime and zidebactam individually, most continued to need relatively high concentrations of cefepime/zidebactam for inhibition, with MICs clustered around 8+8 to 32+32 mg/L for isolates with acquired OXA carbapenemase and ≥128 mg/L for those with MBLs.

Discussion

Cefepime/zidebactam has significant potential against extremely resistant Gram-negative bacteria: zidebactam has lower MICs than other triple-action DBOs, notably nacubactam[16] whilst cefepime is an easy-to-protect partner, rapidly penetrating Gram-negative bacteria[17] and being relatively stable to AmpC and OXA-48-like enzymes.[18,19] Moreover, cefepime attacks PBP3, promoting a strong enhancer effect.[16]

Several MIC studies have been published already for cefepime/zidebactam.[4,5,6,20-22] The present investigation extends knowledge by using a fixed cefepime : zidebactam ratio, not a fixed zidebactam concentration, and in testing all isolates referred to AMRHAI, not solely those with pre-defined mechanisms. Using a fixed ratio avoids the problem that many isolates are otherwise inhibited by low fixed concentrations of zidebactam, with the partner β-lactam playing no role, whereas this cannot reasonably be the situation in the patient with dynamically changing drug concentrations. Testing all referred isolates ensured inclusion of often excluded groups: this is important for *P. aeruginosa*, where complex mixed mechanisms are prevalent in isolates from chronic respiratory disease.[22-24] Other strongly represented groups, not widely tested previously, include *P. aeruginosa* with upregulated efflux and Enterobacterales with impermeability as well as ESBL or AmpC activity.

Zidebactam MICs were ≤2 mg/L for most *E. coli* and *Enterobacter*, with values of 4 to 16 mg/L for 75% of *P. aeruginosa* isolates. These results agree with previous data, as do high MICs for almost all Proteeae, *Serratia* spp and non-fermenters besides *P. aeruginosa*.[5,6,21] The behaviour of *Klebsiella* spp. is more uncertain: we found MICs >32 mg/L for 496/700 (71%) isolates of the genus, with only 129/700 (18%) inhibited at 2 mg/L. By contrast, others have reported an MIC_{90} of 2 mg/L[21] and, for an earlier collection, we found 69% of *Klebsiella* spp. inhibited at ≤2 mg/L.[5] Impermeability contributes to zidebactam resistance in *Klebsiella*, since this trait is almost universal among ertapenem-resistant ESBL and AmpC producers (not shown), however, this cannot explain study-to-study differences, which may relate e.g. to the use of IsoSensitest agar here but Mueller-Minton media previously, perhaps by modulating the stringent response, which affects susceptibility to PBP2-targeted DBOs.[7]

MICs of cefepime/zidebactam were reviewed here against a tentative breakpoint of 8+8 mg/L, matching the upper edge of CLSI's 'Dose-dependent Susceptibility' for Enterobacterales and the CLSI and EUCAST (high dose) breakpoints for *P. aeruginosa*. EUCAST's cefepime breakpoints for Enterobacterales are lower, at S ≤1/R >4 mg/L, but were reduced to minimise categorising ESBL producers as susceptible. This issue should not apply for cefepime/zidebactam, which inhibits ESBLs and is intended for high dosage (2+2g q8h, with prolonged infusion). By analogy, ceftazidime/avibactam has an S ≤8/R >8 mg/L EUCAST breakpoint for Enterobacterales, compared with S ≤1/R >4 mg/L for ceftazidime alone. Higher breakpoints may be justifiable: a human-simulated cefepime/zidebactam regimen gave bactericidal levels in the murine lung up to MICs of 16 mg/L for *P. aeruginosa*[25] whilst (below) an unusually brief *T >MIC* may be adequate for *A. baumannii.*

Among 1632 Enterobacterales, cefepime/zidebactam MICs >8+8 mg/L were seen for just 20/210 isolates with MBLs and 13/24 with both an MBL (NDM) and OXA-48-like

carbapenemase. These proportions agree with a recent American study of 275 isolates with NDM carbapenemases.[26] The present 33 'resistant' Enterobacterales comprised 32 *K. pneumoniae* and one *E. coli*. Although we lack comprehensive typing data, it is clear that the *K. pneumoniae* disproportionately included members of ST14 and its variants and that, where these also had an OXA-48-like enzyme, it was generally OXA-232. There are reports of an ST14 *K. pneumoniae* lineage with NDM-1 and the (otherwise uncommon) OXA-232 variant of OXA-48, including from the Arabian Peninsula [27-29] and the USA.[30] It may be that an international clone with these enzymes is circulating and is resistant to cefepime/zidebactam. We previously noted, with a overlapping strain series, an association between ST14 *K. pneumoniae* and raised cefepime/taniborbactam MICs.[12] Caution is, however warranted because ST14 is frequent, and is known to acquire MBLs repeatedly and independently.[31]

For *P. aeruginosa*, MICs of zidebactam and cefepime/zidebactam rose with the extent of efflux activity, as seen for all β-lactams except imipenem.[32] Nonetheless, these MIC rises were less pronounced than for unprotected cefepime, suggesting that zidebactam is not effluxed strongly. Also of note: almost all (97/103) *P. aeruginosa* with MBLs or ESBLs were inhibited by cefepime/zidebactam at 8+8 mg/L whereas isolates with these enzymes are almost universally resistant to available β-lactams and combinations except, possibly, cefiderocol.[33] These data point towards a role for in the treatment of difficult *P. aeruginosa* infections, regardless of whether these have complex mutational resistance or potent acquired β-lactamases, as are common in e.g., the Middle East,[34] Latin America[35] and Russia.[36]

The behaviour of non-fermenters besides *P. aeruginosa* was species specific, but only *Achromobacter* spp. and *Pandoraea* spp. consistently required MICs >8+8 mg/L. In the case of *A. baumannii*, the MIC distribution for isolates with acquired OXA-23, -24 or -58 β-lactamases – the principal carbapenemases of Acinetobacter – clustered from 8+8 to 32+32

mg/L, whereas MICs for isolates with MBLs mostly exceeded 32 mg/L. These results resemble those reported previously.[5,37] Although MICs for *A. baumannii* are high compared with current cefepime breakpoints, higher breakpoints of cefepime/zidebactam may be warranted for *Acinetobacter* spp., based on an unusually brief (15% of dosage interval) *T >MIC* being required for efficacy in animal models.[38] Human simulated regimens achieved eradication of *A. baumannii* from the murine lung up to an MIC of 64 mg/L.[39]

In summary, the present data, coupled with previous experience, underscore the broad potential of cefepime/zidebactam, particularly against Enterobacterales and *P. aeruginosa* with MBLs and other carbapenemases. Although higher MICs are seen for *A. baumannii* with OXA carbapenemases, these may be partially offset by a relatively shorter *T >MIC* being needed for efficacy.

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482 **Table 1.** Susceptibility of the collection to established antibiotics (%)

	Cefepime		CAZ/AVI	TOL/TAZ	PIP/TAZ	Ertapenem	Meropenem	
Enterobacterales Breakpoints mg/L (EUCAST S and S+I)	≤1	≤4	≤8+4	≤2+4	≤8+4	≤0.5	≤2	≤8
KPC carbapenemases (n=116) [a]	3.5%	52.6%	99.1%	6.9%	2.6%	2.6%	18.1%	60.3%
GES carbapenemases (n=10) [b]	20.0%	30.0%	100%	0%	0%	0%	40.0%	60.0%
Other class A carbapenemase (n=9) [c]	100%	100%	100%	100%	88.9%	0%	11.1%	44.4%
MBL carbapenemases (n=210) [d]	1.4%	3.8%	4.3%	1.4%	2.0%	5.24%	10.5%	28.6%
MBL (NDM) + OXA-48 (n=24) [e]	0%	0%	4.2%	0%	0%	0%	0%	4.2%
OXA-48 ceftazidime-S/I (n=114) [f]	79.0%	95.6%	100%	90.4%	0.9%	14.9%	84.8%	(96.5%)
OXA-48-ceftazidime-R (n=136) [g]	9.6%	19.1%	98.5%	6.6%	0%	0.7%	62.5%	(74.3%)
AmpC (n=418) [h]	66.8%	91.1%	99.8%	44.5%	28.7%	36.1%	94.0%	98.6%
ESBL (n=307) [i]	12.8%	22.2%	100%	64.7%	41.2%	57.2%	95.8%	99.7%
ESBL + AmpC (n=27) [j]	14.8%	25.9%	100%	18.5%	14.8%	33.3%	100%	100%
K1 hyperproducing *K. oxytoca* (n=4)	25.0%	75.0%	100%	75.0%	0%	75.0%	100%	100%
Impermeability (n=31) [k]	74.2%	93.6%	100%	90.3%	48.4%	41.9%	96.8%	100%
K. pneumoniae 'Type 1 unknown' (n=14)	28.6%	78.6%	100%	57.1%	14.3%	35.7%	85.7%	100%

Unassigned ceftazidime MIC ≤4 mg/L (n=58) [l]	70.7%	86.2%	100%	93.1%	48.3%	70.7%	98.3%	100%
Unassigned ceftazidime MIC 8-32 mg/L (n=20) [m]	40.0%	60.0%	100%	50.0%	30.0%	45.0%	75.0%	90.0%
Unassigned ceftazidime MIC >32 mg/L (n=64) [n]	9.4%	12.5%	96.88%	25.0%	15.6%	18.8%	56.3%	89.1%
Wildtype for β-lactamase (n=69) [o]	98.6%	100%	100%	98.6%	94.9%	84.3% [p]	100%	100%
P. aeruginosa Breakpoints mg/L (EUCAST S and S+I)		≤8	≤8+4	≤4+4	≤16+4	Not active	≤2	≤8
MBL carbapenemase (n=81)		4.9%	2.5%	2.5%	2.5%	-	0%	12.4%
GES carbapenemase (n=15)		93.3%	93.3%	20.0%	20.0%	-	0%	6.7%
AmpC carbapenemase (n=71)		49.3%	93.0%	97.2%	16.9%	-	23.95	71.8%
ESBL (n=22)		4.6%	9.1%	0%	4.5%	-	0%	4.6%
Efflux low (n=44)		84.1% [q]	100%	97.7%	97.8%	-	63.6% [r]	97.7% [r]
Efflux normal (n=96)		92.7%	96.9%	97.9%	82.3%	-	28.1% [r]	82.3% [r]
Efflux raised (n=188)		77.7%	86.7%	96.8%	36.2%	-	10.1% [r]	37.8% [r]
Efflux highly raised (n=85)		30.6%	49.4%	80.0%	27.1%	-	9.4% [r]	24.7% [r]
Unassigned ceftazidime MIC ≤8 mg/L (n=87)		97.7%	100%	98.9%	78.2%	-	34.5%	94.3%
Unassigned, ceftazidime 16-128 mg/L (n=39)		35.9%	69.2%	82.1%	38.5%	-	10.3%	59.0%
Unassigned, ceftazidime ≥256 mg/L (n=17)		5.9%	11.8%	11.8%	11.8%	-	0%%	11.8%

Table 1 - notes

[a] *E. coli* (n=20), *Klebsiella* spp. (n=74), *Enterobacter* spp. (n=19), Others (n=3)
[b] *E. coli* (n=4), *Klebsiella* spp. (n=4), *Enterobacter* spp. (n=0), Others (n=2)
[c] *E. coli* (n=0), *Klebsiella* spp. (n=0), *Enterobacter* spp. (n=6), Others (n=3)
[d] *E. coli* (n=68), *Klebsiella* spp. (n=108), *Enterobacter* spp. (n=22), Others (n=12)
[e] *E. coli* (n=1), *Klebsiella* spp. (n=23), *Enterobacter* spp. (n=0), Others (n=0)
[f] *E. coli* (n=60), *Klebsiella* spp. (n=34), *Enterobacter* spp. (n=14), Others (n=6)
[g] *E. coli* (n=36), *Klebsiella* spp. (n=77), *Enterobacter* spp. (n=15), Others (n=8)
[h] *E. coli* (n=47), *Klebsiella* spp. (n=98), *Enterobacter* spp. (n=230), Others (n=43)
[i] *E. coli* (n=145), *Klebsiella* spp. (n=140), *Enterobacter* spp. (n=20), Others (n=1)
[j] *E. coli* (n=11), *Klebsiella* spp. (n=3), *Enterobacter* spp. (n=12), Others (n=1)
[k] *E. coli* (n=12), *Klebsiella* spp. (n=17), *Enterobacter* spp. (n=3), Others (n=0)
[l] *E. coli* (n=29), *Klebsiella* spp. (n=21), *Enterobacter* spp. (n=3), Others (n=5)
[m] *E. coli* (n=2), *Klebsiella* spp. (n=16), *Enterobacter* spp. (n=2), Others (n=0)
[n] *E. coli* (n=11), *Klebsiella* spp. (n=53), *Enterobacter* spp. (n=0), Others (n=0)
[o] *E. coli* (n=15), *Klebsiella* spp. (n=14), *Enterobacter* spp. (n=17), Others (n=24)
[p] 10/11 resistant isolates were *Enterobacter* spp. / *K. aerogenes* with MICs 1 mg/L, reduced permeability is possible but not categorised as such because no other result supported doing so.
[q] Lower susceptibility rate than among 'efflux normal' because minor pumps, some of which specifically recognise cefepime may be upregulated when minor pumps are down-regulated.[16]
[r] Rates elevated by propensity of some laboratories that specifically refer *P. aeruginosa* isolates based on meropenem resistance; these rates are not representative of *P. aeruginosa* that solely have upregulated efflux

Abbreviations: S, susceptible; I, EUCAST increased-dose susceptible; R resistant; CAZ/AVI, ceftazidime/avibactam; TOL/TAZ, ceftolozane/tazobactam, PIP/TAZ, piperacillin/tazobactam.

Susceptibility rates less than 90% are shaded.

513 **Table 2.** MIC distributions of cefepime, zidebactam and cefepime/zidebactam for principal groups
514

Row Labels		No. isolates with indicated MIC (mg/L)													
Enterobacterales [a]		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
KPC carbapenemase (n=116)	FEP		2				2	22	35	30	8	5	5	5	2
	ZID			14	13	17	7	2	4		4			1	54
	FPZ	1	1	18	63	21	11	1							
GES carbapenemase (n=10)	FEP					1	1	1				4	3		
	ZID					1		1	3			1			4
	FPZ			1		6		1	2						
Other class A carbapenemase (n=9)	FEP		3	4	2										
	ZID			2	2	1							1		3
	FPZ		5	3	1										
MBL carbapenemase (n=210)	FEP		2			1		2	3	9	25	22	42	26	78
	ZID		1	44	30	19	7	4	3	2	4	4	2	2	88
	FPZ	2	2	38	37	21	22	27	25	16	14	2	2	2	
MBL (NDM) + OXA-48 carbapenemase (n=24)	FEP												1	1	22
	ZID				1	1	2			1	2				17
	FPZ				1	2	1	2	3	2	3	10			
OXA-48 carbapenemase Ceftazidime MIC $\leq$4 mg/L (n=114)	FEP		5	23	22	25	15	11	8	2		1	1		1
	ZID		4	41	23	6	5	2		1				3	29
	FPZ	1	34	65	12	1		1							
OXA-48-carbapenemase	FEP				2	6	5	8	5	5	12	13	17	12	51

Ceftazidime >4 mg/L (n=136)	ZID		2	15	26	6	5	9	5	4	2		3	5	54
	FPZ		1	26	47	26	21	15							
AmpC β-lactamase (n=418)	FEP		44	38	58	72	67	54	48	26	9	2			
	ZID		4	31	56	**68**	45	29	23	12	7	3	8	5	127
	FPZ	33	76	103	**106**	61	26	13							
ESBL (n=307)	FEP		8	7	8	9	7	12	17	18	28	30	40	29	94
	ZID		3	56	55	35	16	15	3	4	6	4	5	4	101
	FPZ	4	24	81	84	61	35	13	4	1					
ESBL + AmpC β-lactamase (n=27)	FEP		1	1		1	1	2	1	1	3	3	1	4	8
	ZID		1	2	6	6	7								5
	FPZ	1	1	7	7	6	5								
K1 hyperproducing *K. oxytoca* (n=4)	FEP					1		2				1			
	ZID							1							3
	FPZ			2	1		1								
Impermeability (n=32)	FEP		2	1	3	9	9	3	3	2					
	ZID			5	7	2	1	1	1		1	1			13
	FPZ	1	3	16	8	2	1	1							
Unassigned Ceftazidime MIC ≤4 mg/L (n=58)	FEP		16	5	4	7	9	7	2	1	5	2			
	ZID		1	16	9	1	1	3	2	1	1	4	2	1	16
	FPZ	15	7	16	10	6	3	1							
Unassigned Ceftazidime	FEP				3	1	4	2	2	3		1	2		2
	ZID				1	2		1			1		2	2	11

MIC 8-32 mg/L (n=20)	FPZ			6	4	4	1	3	1	1					
Unassigned Ceftazidime MIC >32 mg/L (n=64)	FEP		1	3	1	1		2		1	1	1	6	6	41
	ZID		2	1	3	1	2	3	2	2	4		3		41
	FPZ		4	5	2	6	14	14	12	7					
Type 1 unknown (n=14)	FEP				1		3	5	2	1	1	1			
	ZID			2				1			1				10
	FPZ			3	4	5		1	1						
Wildtype for β-lactamase (n=69)	FEP		51	9	3	4	1		1						
	ZID			23	15	2	1		1				2		26
	FPZ	34	27	7	2	1	1								34
P. aeruginosa															
MBL carbapenemase (n=81)	FEP						1		1	2	19	15	22	11	10
	ZID						2	2	19	44	11			1	2
	FPZ						2	7	29	38	5				
GES carbapenemase (n=15)	FEP							4	5	5	1				
	ZID								6	8	1				
	FPZ						3	7	5						
AmpC β-lactamase (n=71)	FEP							3	9	23	22	9	3	1	1
	ZID					1	3	4	20	17	13	4	3	2	4
	FPZ					1	6	14	39	10	1				
ESBL (n=22)	FEP									1			2	6	13
	ZID							1	1	9	10		1		
	FPZ							2	1	18	1				

Efflux low (n=44)	FEP				1	3	6	4	10	13	4	2		1	
	ZID				1	2	6	12	7	4	1	4	1	2	4
	FPZ	1	2		1	7	13	13	6	1					
Efflux normal (n=96)	FEP				1		7	32	31	18	5	1			1
	ZID				1	1	4	11	40	28	6		2		3
	FPZ			1		5	14	35	37	4					
Efflux raised (n=188)	FEP		1	1	1		1	4	35	103	31	8	2	1	
	ZID	1				1		4	14	73	73	11	2	3	6
	FPZ	1		1		2	2	14	73	81	13	1			
Efflux highly raised (n=85)	FEP							1	3	22	25	11	16	5	2
	ZID				1	1	2	5	7	25	21	9	3	6	5
	FPZ				1	1	3	5	17	47	10	1			
Unassigned Ceftazidime MIC ≤8 mg/L (n=87)	FEP						17	27	28	13	2				
	ZID					1	2	8	50	23	1			2	
	FPZ					2	24	42	17	2					
Unassigned Ceftazidime MIC 16-128 mg/L (n=39)	FEP							4	2	8	8	12	1	4	
	ZID					1		2	7	13	7	2			
	FPZ				1	1	2	11	10	9	5				
Unassigned Ceftazidime MIC ≥256 mg/L (n=17)	FEP									1	1	4	1	2	8
	ZID							1	1	6			3	1	5
	FPZ							2	4	7	3	1			

A. baumannii															
Acquired OXA carbapenemases (n=183)	FEP								3	3	8	50	85	30	3
	ZID														183
	FPZ		1			2		2	22	71	55	28	1	1	
MBL +/- OXA carbapenemase (n=19)	FEP												1	2	16
	ZID														19
	FPZ											2	4	7	4
No acquired carbapenemase (n=14)	FEP		1		1			2	4	2	1	2			1
	ZID														14
	FPZ	1		1			1	4	2	1	3		1		
Acinetobacter non-*baumannii* (n=38)	FEP		1	3	1	5	3	7	4	9	1	2	1	1	
	ZID										1	1			36
	FPZ	3	1	3	3	3	2	6	10	3	4				
Burkholderia spp. (n=48)	FEP					1	8	13	10	1	7	3	1	1	3
	ZID										4	9	8	3	24
	FPZ					3	10	12	12	6	4				1
Pseudomonas non-*aeruginosa* (n=36)	FEP		2	3	2	2	8	9	2	5	1	1	1		
	ZID							1	1	4	7	4	4	3	11
	FPZ		1	3	1	3	12	7	6	1		2			
Achromobacter spp. (n=33)	FEP								2	5	4	9	6	6	1
	ZID														33
	FPZ								2	7	6	9	7	2	
S. maltophilia (n=32)	FEP					4	2	3	8	4	5	3	2	1	

	ZID														32
	FPZ			1		4	7	7	11		1	1			
Pandoraea spp. (n=20)	FEP									1	3	1	6	4	5
	ZID												1		19
	FPZ									1	3	1	5	6	4
Elizabethkingia/ Chryseobacterium spp. (n=12)	FEP				1				2		6	2	1		
	ZID														12
	FPZ					1	2	2	3	2		2			
Rare non-fermenters (n=15)	FEP		1		1		1	3		4	2	1			2
	ZID				1								1	1	12
	FPZ	1			2	1	2	2	2	2	1		1		1

[a] For species split, see footnotes to Table 1

Abbreviations: FEP, cefepime; ZID, zidebactam; FPZ, cefepime/zidebactam 1:1

Table 3. Interrelation of cefepime/zidebactam and ceftazidime/avibactam MICs for Enterobacterales (n=1632)

Ceftazidime/ avibactam MIC (mg/L)	No isolates with indicated cefepime/zidebactam MIC (mg/L)													Grand Total
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	
≤0.06	36	25	18	11	4	1								95
0.12	34	51	53	17	3	1	1							160
0.25	18	67	144	69	20	13	1							332
0.5	4	36	98	120	69	21	6	2						356
1		3	33	95	73	55	25	5	1					290
2		1	11	29	27	17	25	7	3					120
4				9	6	8	4	4	3					34
8				4	4	2	2	2	1					15
16				3	1				1					5
32				2	2									4
64			2	1				1						4
128			1	1	2		1	1						6
>128		2	35	28	17	24	28	26	18	17	12	2	2	211
Grand Total	92	185	395	389	228	142	93	48	27	17	12	2	2	1632

The dark grey cells indicate equal MICs for both agents: isolates in cells below this line were more susceptible to cefepime/zidebactam, those in cells above the line were more susceptible to ceftazidime/avibactam.

The light grey area encompasses isolates (n=230) resistant to ceftazidime/avibactam (MIC >8+8 mg/L). Of these, 224/230 had MBLs alone or in combination with OXA-48-like enzymes

Table 4. Interrelation of cefepime/zidebactam and ceftolozane/tazobactam MICs for *P. aeruginosa* (n=745)

Ceftolozane/tazobactam MIC (mg/L)	No isolates with indicated cefepime/zidebactam MIC (mg/L)											Grand Total
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	
≤0.06	1											1
0.125	1			1	3	1	1					7
0.25		2			4	4	3	1				14
0.5			1		8	41	56	50	15			171
1			1		4	12	50	87	66	9		229
2				2		4	12	42	42	11	1	114
4						1	11	11	18	3	1	45
8						5	8	7	11	3		34
16							1	4	3	1		9
32							1	3	2	4		10
64							2	2	3		1	8
128							5	3	11	1		20
>128						1	2	28	46	6		83
Grand Total	2	2	2	3	19	69	152	238	217	38	3	745

The dark grey yellow cells indicate equal MICs for both agents: isolates in cells below this line were more susceptible to cefepime/zidebactam, those in cells above the line were more susceptible to ceftolozane/tazobactam.

The light grey area encompasses isolates (n=164) resistant to ceftolozane/tazobactam. Of these, 113/164 had carbapenemases or ESBLs

Table 5. MIC distributions of cefepime/zidebactam for Enterobacterales with MICs 64 mg/L for both cefepime and zidebactam, by mechanism

	MIC (mg/L)										
Enterobacterales	**≤0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**≥128**	**Total**
KPC carbapenemase [a]			4	1							5
MBL carbapenemase [b]	1	2	2	10	15	11	11	2	2	2	58
MBL (NDM) + OXA-48 carbapenemase [c]		1		1	1	1	3	10			17
OXA-48 carbapenemase ceftazidime S/I [d]				1							1
OXA-48- carbapenemase ceftazidime R [e]	2	13	13	9							37
ESBL [f]	7	28	21	6	2	1					65
Unassigned ceftazidime MIC 8-32 mg/L [g]				1	1	1					3
Unassigned ceftazidime MIC >32 mg/L [h]		4	11	10	8	6					39
P. aeruginosa											
MBL carbapenemase						1					1
AmpC β-lactamase					1	1					2
ESBL							1				1
Efflux low				1							1
Efflux very raised					1	4	2				7
Unassigned ceftazidime MIC 16-128 mg/L						1	1				2
Unassigned ceftazidime MIC >256 mg/L							3	1			4
A. baumannii											
OXA-23/24/58 carbapenemase					1	36	51	28	1	1	118
MBL +/- OXA-23/58 carbapenemase								2	4	13	19
No acquired carbapenemase										1	1

[a] *Klebsiella* spp. (n=4); others (n=1)

[b] *Klebsiella* spp. (n=55); *Enterobacter* spp. (n=2); others (n=1)

[c] *Klebsiella* spp. (n=17)

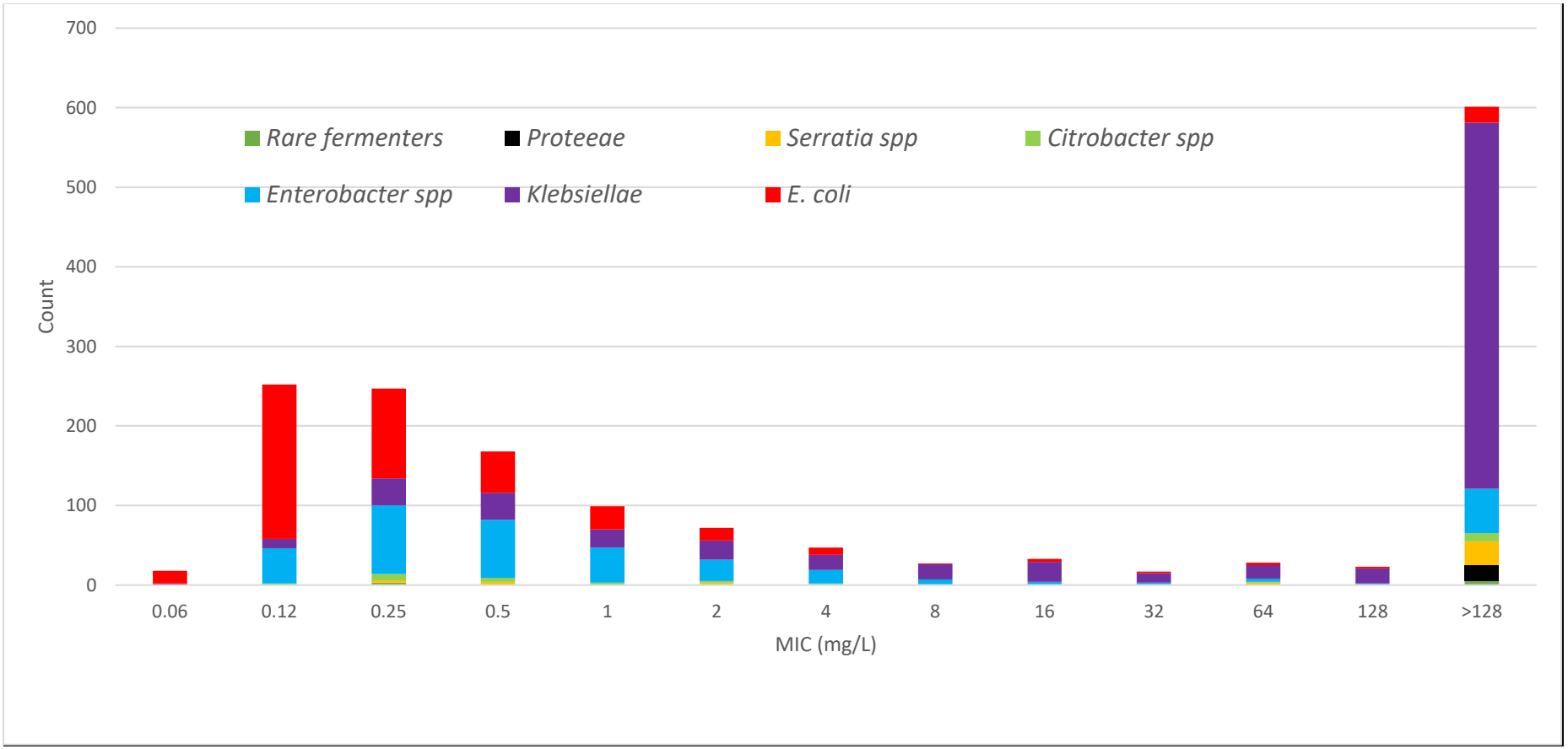
[d] *Klebsiella* spp. (n=1)

[e] *Klebsiella* spp. (n=36); others (n=1)

[f] *Klebsiella* spp. (n=56); *E. coli* (n=9)

[g] *Klebsiella* spp. (n=3)

[h] *Klebsiella* spp. (n=36); *E. coli* (n=3)



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555 **Figure 1**. MIC distributions of zidebactam for Enterobacterales