In vitro activity of cefepime/zidebactam (WCK 5222) against Gram-negative bacteria

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Running head: cefepime/zidebactam *versus* gram-negatives

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Background. Diazabicyclooctanes (DBOs) inhibit Class A, C and some Class D β-lactamases. A few also bind PBP2, conferring direct antibacterial activity and a β-lactamase-independent 'enhancer' effect, potentiating β-lactams targeting PBP3. We tested a novel DBO, zidebactam, combined with cefepime. **Methods**. CLSI agar dilution MICs were determined with cefepime/zidebactam in a chequerboard format. Bactericidal activity was also measured. **Results**. Zidebactam MICs were ≤2 mg/L (mostly 0.12-0.5 mg/L) for most *Escherichia coli, Klebsiella, Citrobacter* and *Enterobacter* spp., but were >32 mg/L for Proteeae, most *Serratia* and a few *E. coli, Klebsiella* and *Enterobacter/Citrobacter*. The antibacterial activity of zidebactam dominated chequerboard studies for Enterobacteriaceae, but potentiation of cefepime was apparent for zidebactam-resistant isolates with class A and C enzymes, illustrating β-lactamase inhibition. Overall, cefepime/zidebactam inhibited almost all Enterobacteriaceae with AmpC, ESBL, K1, KPC and OXA-48-like β-lactamases at 1+1 mg/L and also 29/35 isolates with metallo-carbapenemases, including several resistant to zidebactam alone. Zidebactam MICs for 36/50 *Pseudomonas aeruginosa* were 4-16 mg/L, and majorities of AmpC, metallo-β-lactamase-producing and cystic fibrosis isolates were susceptible to cefepime/zidebactam 8+8 mg/L. Zidebactam MICs for *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* were >32 mg/L; potentiation of cefepime was frequent for *S. maltophilia*, but minimal for *A. baumannii*. Kill curve results largely supported MICs. **Conclusion**. Zidebactam represents a second triple action DBO following RG6080, with lower MICs for Enterobacteriaceae and *P. aeruginosa*. Clinical evaluation of cefepime/zidebactam must critically evaluate the reliance that can be placed on this direct antibacterial activity and on the enhancer effect as well as β-lactamase inhibition.

Introduction

Diazabicyclooctanes (DBOs) are among the most promising new β-lactamase inhibitors.[1] The first member of the class, avibactam, is already marketed in combination with ceftazidime and is under investigation combined with aztreonam[1,2] whilst a second analogue, relebactam, has undergone is now in phase III development combined with imipenem-cilastatin.[3] Avibactam and relebactam act solely as inhibitors of class A, C and some class D β–lactamases at clinical concentrations, though avibactam does directly inhibit the growth of many *Escherichia coli* strains at concentrations a little above the 4 mg/L routinely used in MIC tests. Avibactam MICs for other species are higher.

Some developmental DBOs have greater direct antibacterial activity. RG6080/OP0595 (Meiji, Fedora, Roche) not only has similar β-lactamase inhibitory activity to avibactam, but also has MICs of around 1-4 mg/L for most *E. coli, Klebsiella, Enterobacter* and *Citrobacter* spp., contingent on attacking penicillin-binding protein (PBP)2.[4,5] Proteeae and non-fermenters are resistant, with MICs >32 mg/L. Like mecillinam[6] – another PBP2-targeting agent – RG6080 also synergises or 'enhances' the activity of PBP3-targeted β-lactams against many *E. coli*, *Klebsiella* spp. and *Enterobacter* spp., regardless of whether these produce β-lactamases. The enhancer effect is retained against some strains and mutants with resistance to the antibacterial action of OP0595 and these additional activities allow β-lactam-RG6080 combinations to achieve in-vitro activity against many Enterobacteriaceae with metallo-β-lactamases (MBLs), even though these evade inhibition by DBOs.[4,5,7]

In the present study we characterised the activity of a second DBO with direct antibacterial activity, zidebactam (Wockhardt, WCK 5107, figure 1), tested in combination with cefepime, which is its intended clinical partner β–lactam (see e.g. https://clinicaltrials.gov/ct2/show/NCT02707107). The cefepime/zidebactam combination is also known by the code number WCK 5222.

Materials and methods

Isolates

Isolates (n=269) were reference submissions to Public Health England from UK diagnostic laboratories, or were collected in during resistance surveys. The distribution of resistance

mechanisms by species is shown in Table 1. Isolates were identified using API20E or API20NE strips (bioMerieux, Marcy l'Etoile, France) or by MALDI-ToF mass spectroscopy (Maldi-Biotyper, Bruker, Bremen, Germany), with the exception that *Acinetobacter baumannii* were identified by PCR detection of $bla_{\text{OXA-51-like}}$.[8] Carbapenemase genes were identified by PCR or sequencing;[9] other mechanisms were inferred from phenotype and (where available) genotype data.

Susceptibility testing

MICs of cefepime (US Pharmacopoeial Convention, Rockville, USA) were determined by CLSI agar dilution[10] in a chequerboard format with zidebactam (Wockhardt, Aurangabad, India) included at 0.06-8 mg/L. Comparator antibiotics were tested in parallel and comprised: piperacillin (Sigma-Aldrich, Poole, UK) with 4 mg/L tazobactam (Wockhardt), ceftazidime (Sigma-Aldrich) alone and with 4 mg/L avibactam (Wockhardt), also meropenem (Sequoia Research Products, Pangbourne, UK).

Killing curves

Bacteria were grown overnight, with shaking, in Mueller-Hinton Broth at 37^{o}C then diluted 1000-fold into 100 ml of fresh warm broth. Incubation was continued, with shaking, for 90 min to bring the cells into early log phase. The cultures were then divided into 10-ml volumes and antibiotics or combinations were added, with incubation continued as before. This point was defined as T_0, and a single count was performed, representing the starting point for all curves with that strain. Further counts were performed on all cultures at T+1h, T+2h T+4h, T+6h, T+8h (non-fermenters only) and T+24h. Counts were by the Miles and Misra method and 'bactericidal' is used in the classical sense, as meaning 'causing some initial reduction in bacterial counts', irrespective of the extent or duration these reductions.

Results

Antibacterial activity of zidebactam

The great majority (92/102) of isolates of *E. coli*, *Enterobacter* spp. and *Citrobacter* spp. were susceptible to zidebactam at $\leq$1 mg/L, with 86/102 MICs clustered from 0.12 to 0.5 mg/L (Table 2). MICs for *Klebsiella* spp. were more bimodally distributed, with 40/58 values from 0.12-2 mg/L and 16/58 at $\geq$32 mg/L. Trailing end points and surviving colonies made reading difficult, especially with

Klebsiella spp. Zidebactam MICs also were bimodal for *Serratia* spp., but with most (7/10) values ≥32 mg/L. All Proteeae (n=19) were resistant, with MICs ≥32 mg/L. No relationship was apparent between zidebactam MICs and the β-lactamase phenotypes and genotypes for which the Enterobacteriaceae were selected for inclusion in the study.

In the case of *P. aeruginosa*, MICs for 36/50 isolates were in the range 4-16 mg/L, but the median values for AmpC- and MBL-producing isolates (8 mg/L) were one doubling dilution higher than for the susceptible controls (4 mg/L) and the median for the increased-efflux isolates was a further two-fold higher, at 16 mg/L. Zidebactam MICs for *A. baumannii* and *Stenotrophomonas maltophilia* universally exceeded 32 mg/L.

Combination activity of cefepime/zidebactam: Enterobacteriaceae

At 1 mg/L (EUCAST's susceptible breakpoint, http://www.eucast.org) unprotected cefepime inhibited only 6/33 ESBL producers, 26/35 AmpC hyperproducers, 4/5 K1 hyperproducers, 7/15 with OXA-48-like enzymes, and none of those with KPC (n=30) or metallo-β-lactamases (MBLs, n=35) (Table 3). Addition of zidebactam increased these proportions markedly, so that cefepime/zidebactam 1+1 mg/L was active against all 33 Enterobacteriaceae with ESBLs, all 35 with hyper-produced AmpC enzymes, all five with hyper-produced K1 enzyme (n=5), all 15 with OXA-48-like carbapenemases, 29/30 with KPC enzymes and 29/35 with MBLs. The sole KPC isolate that was resistant to 1+1 mg/L was an *Enterobacter cloacae* that was inhibited by zidebactam alone at 4 mg/L and by cefepime/zidebactam at 8+2 or 4+4 mg/L. Much of this gain in spectrum reflected the direct antibacterial activity of zidebactam, which inhibited many *E. coli, Klebsiella, Enterobacter* and *Citrobacter* spp. isolates at 1 mg/L (above, Table 2).

The β–lactamase inhibitory activity and enhancer effects of zidebactam became evident for the minority of Enterobacteriaceae with high MICs for the DBO, taken here as MIC ≥16 mg/L, which are line-listed in Table 4. Strong, dose-dependent synergy was seen for all zidebactam-resistant Enterobacteriaceae isolates with class A β–lactamases, including ESBLs (which were mostly CTX-M types based on higher MICs for cefotaxime than ceftazidime) and KPC types, with cefepime MICs of 2 to >256 mg/L reduced below 1 mg/L even by zidebactam at 1 mg/L or less. The sole 'zidebactam-resistant' (MIC >32 mg/L) representative with an AmpC enzyme (*S. marcescens* SE01046) had only intermediate resistance to cefepime, with an MIC of 2 mg/L reduced to ≤0.03 mg/L by zidebactam at

1 mg/L. Good cefepime/zidebactam synergy was seen for two zidebactam-resistant isolates with OXA-48 carbapenemase, but this oxacillinase has little activity against cefepime[11] and it is most likely that the synergy reflected inhibition of co-produced ESBLs, which were not identified in this study. Potentiation of cefepime by zidebactam was variable for the zidebactam-resistant metallo-carbapenemase producers, being at least eight-fold for two *K. pneumoniae*, (H113980340 and H112240413) one *Morganella morganii* (H092540314) and one *Providencia stuartii* (H124880510), all of which were susceptible to cefepime/zidebactam at 2+1 mg/L, but weak or absent for all three *P. rettgeri* (H123140552, H123560843 and H124880511) and the one *E. coli* (H130680324) where the cefepime/zidebactam MIC remained >64+8 mg/L.

Ceftazidime-avibactam, tested as a comparator, was active against all ESBL, K1, AmpC, OXA-48 and KPC strains at its 8+4 mg/L EUCAST and FDA breakpoint. Its MICs were higher than for cefepime/zidebactam, largely owing to the lack of direct antibacterial activity by avibactam; more critically, almost all (33/25) MBL producers were resistant to ceftazidime/avibactam, even at 8+4 mg/L. The other comparators had very limited activity against this highly resistant strain collection. Unprotected ceftazidime was only active against control strains, K1-enzyme-hyperproducing *K. oxytoca* and those isolates that had OXA-48-like enzymes but lacked ESBLs. Non-susceptibility rates to piperacillin/tazobactam (8+4 mg/L) exceeded 90% among isolates with AmpC, K1, OXA-48-like, KPC enzymes of MBLs; meropenem resistance was near universal among the MBL- and KPC-producing isolates, though MICs for many with OXA-48-like enzymes remained around the CLSI and EUCAST susceptible breakpoints of 1 and 2 mg/L.

Combination activity of cefepime/zidebactam: Non-fermenters

At 8 mg/L, the antibacterial activity of zidebactam dominated combination results for *P. aeruginosa*, with 33/50 isolates inhibited by the DBO alone (Table 2). Largely owing to this, 9/10 isolates with derepressed AmpC, 8/10 with MBLs, 8/10 with up-regulated efflux and 9/10 cystic fibrosis isolates were susceptible to cefepime/zidebactam at 8+8 mg/L. Even at 4 mg/L, zidebactam increased the proportion of strains counting as susceptible to cefepime (MIC $\leq$8 mg/L) from 2/10 to 8/10 for AmpC hyperproducers, 2/10 to 6/10 for efflux strains and from 0/10 to 4/10 for cystic fibrosis isolates, although 9/10 MBL producers remained resistant.

Cefepime MICs for *A. baumannii* isolates with OXA carbapenemases were mostly reduced by one doubling dilution by zidebactam at 4 or 8 mg/L, with modal values falling from 32 to 16 mg/L (Table 2); MICs for susceptible controls or those with NDM MBLs were not reduced. MICs for *S. maltophilia* isolates were reduced by zidebactam: without the DBO only 2/10 isolates were susceptible to cefepime at 8 mg/L but this proportion rose to 7/10 with zidebactam present at 4 or 8 mg/L.

Killing curves

Killing curves were determined with two isolates each of *K. pneumoniae*, *E. coli* and *P. aeruginosa*, all producing NDM metallo-carbapenemases. In each case, the test strains per species were chosen to include one susceptible to zidebactam and one resistant, though the differential was much greater in the Enterobacteriaceae pairs than for the *P. aeruginosa* (see figure 2 and its legend). A single *A. baumannii* strain with OXA-23 carbapenemase was also tested; this, like all members of its species, was highly resistant to zidebactam. Cefepime MICs were $\geq$256 mg/L for all these organisms.

Both the zidebactam-susceptible (H113840625 MIC 0.25 mg/L, panel 2a) and –more surprisingly- the zidebactam-resistant (H113980340, MIC >32 mg/L, panel 2b) NDM *K. pneumoniae* were killed by zidebactam at 4 mg/L, though the extent of killing was reduced for the resistant organism (l.5 log maximum after 4 h exposure *versus* 3 log). The cefepime/zidebactam combinations (1+4 and 8+4 mg/L) combinations achieved 3-4 log kills for both organisms and it is notable that the zidebactam-resistant *K. pneumoniae* H113980340 was likewise susceptible to cefepime/zidebactam combinations in MIC tests (Table 4). For the two NDM-positive *E. coli* (H131020913, zidebactam MIC 0.25, panel 2c and H130680324 MIC 16 mg/L panel 2d), killing simply tracked MICs. Thus, for the zidebactam-susceptible organism, zidebactam and its cefepime combinations achieved extensive killing whereas, for the resistant strain, neither zidebactam nor its combinations achieved significant kill at the concentrations studied. Corresponding with this result, and unlike for *K. pneumoniae* H113840625, there was no hint of an enhancer effect for cefepime/zidebactam in MIC combination studies for this *E. coli* strain (Table 4).

Zidebactam MICs were 8 and 32 mg/L for the two NDM-positive *P. aeruginosa* strains (H130680310, panel 2e/2g and H131800691 panel 2f/2h, respectively). There was some suppression

of growth for the more susceptible strain with zidebactam alone at 8 mg/L or cefepime/zidebactam 16+8 mg/L, whereas the more resistant strain was unaffected. A 2 - 4 log bactericidal effect was achieved within 8h for both strains with cefepime/zidebactam at higher concentrations (panels g and h), though only once the zidebactam was present at MIC (32 mg/L). The *A. baumannii* strain, H104940508, with OXA-23 enzyme, was highly resistant to zidebactam (MIC >32 mg/L); cefepime/zidebactam 8+4 mg/L had little effect, but cefepime/zidebactam 16+8 did achieve bacteriostasis, a result in keeping with the MIC of 32+8 mg/L.

In most cases where cefepime/zidebactam achieved substantial killing there was overnight re-growth. Nevertheless, where examined, the organisms remained susceptible in repeat MIC tests with cefepime/zidebactam and did not represent resistant mutants.

Discussion

Zidebactam represents a second DBO with multiple activities, acting not only as a β–lactamase inhibitor but also as a direct antibacterial and exerting an enhancer effect with PBP3-targeting β-lactams. Key differences from RG6080 are (i) that the MICs of zidebactam for susceptible Enterobacteriaceae are lower, typically falling into the 0.12-0.5 mg/L range rather than 1-4 mg/L and (ii) that zidebactam alone inhibited many *P. aeruginosa* at 4-8 mg/L whereas MICs of OP0595/RG6080 consistently exceed 32 mg/L for this species. Proteeae, most *Serratia*, *A. baumannii* and *S. maltophilia* remained resistant, exactly as with RG6080. The antibacterial activity of zidebactam is believed to depend on binding to PBP2, as with RG6080;[12] it is uncertain if the lower MICs of zidebactam reflect increased target affinity, a more favourable balance of permeation and efflux, or combination of all the three or other factors. Raised zidebactam MICs (typically 16-32 mg/L *versus* 4-8 mg/L) for *P. aeruginosa* were associated with strains known to have up-regulated efflux, indicating that the molecule does not entirely evade this mechanism. Otherwise, however, no association was seen between the MICs of zidebactam and the resistance mechanisms for which the isolates were selected. This is in keeping with experience that raised MICs of OP0595/RG6080 were associated primarily not with 'conventional' β-lactam resistance mechanisms, but with mutations that activate the stringent response, thereby compensating for inactivation of PBP2.[13] Similar types of mutation can confer resistance to mecillinam, which also targets PBP2.[14] The fact that PBP2 itself

remains unaltered means that the enhancer effect can remain even when the antibacterial activity has been lost.[15]

Despite its low MICs, zidebactam is better suited for development in combination than as a single agent, owing (again like OP0595/RG6080) to a high frequency of mutational resistance (Wockhardt, data on file). Cefepime has been chosen as a partner agent, based (i) on its broad spectrum and good safety record, (ii) wide range of licensed indication, (iii) relative stability to AmpC enzymes – whi

ch can mutate to resist to DBO inhibition[16] and (iv) on an enhancer effect being most likely with agents, such as cefepime, that target PBP3.[4] Even at 1+1 mg/L (i.e. below any likely breakpoint for a high dosage formulation) cefepime/zidebactam was active against almost all Enterobacteriaceae with AmpC, ESBL, K1, OXA-48 and KPC β–lactamases and the great majority (29/35) of those with MBLs. Even when zidebactam itself lacked activity, the combination retained activity against Enterobacteriaceae with class A and C β–lactamases, which is in keeping with kinetic data showing that zidebactam inhibits these enzymes.[17] Activity was also retained against both zidebactam-resistant klebsiellas with OXA-48 carbapenemase, though – given cefepime's stability to OXA-48[18] – it is most likely that this result reflected inhibition of co-produced ESBLs rather than of OXA-48 itself. Combination activity was more variable against the small number of zidebactam-resistant Enterobacteriaceae with MBLs, but the observation of strong synergy between cefepime and zidebactam for several of these organisms, notably *K. pneumoniae* H113980340, *P. stuartii* H124880510 and *M. morganii* H092540314 supports the view of an enhancer effect, and or the inhibition of co-produced ESBLs. Potentiation against *S. maltophilia* was widespread and may reflect either an enhancer effect or, more probably, inhibition of the L-2 cephalosporinase, which confers resistance to cefepime.[19]

The killing curves, done with pairs of NDM-carbapenemase-positive zidebactam-susceptible and -resistant *E. coli, K. pneumoniae* and *P. aeruginosa* largely supported the MIC data with the notable exceptions that zidebactam achieved some killing of the 'zidebactam-resistant' *K. pneumoniae* strain H113980340. Moreover cefepime/zidebactam achieved equally extensive killing of this strain as of its zidebactam-susceptible counterpart (H113840625), whereas there was minimal

killing of the NDM-positive zidebactam-resistant *E. coli* H130480324 by cefepime/zidebactam This variability recapitulates that seen in MIC studies here and previously with OP0595-resistant strains and mutants;[5,7] though it should be added that zidebactam-resistance (Table 2) and the lack of an enhancer effect (Wockhardt, data on file) seem exceptional in *E. coli*. Such variation may reflect the diversity of different mutations that can underlie resistance to PBP2-targeted DBOs, though precise relationships remain uncertain. In summary, these finding further illustrate the expanding potential of the DBO class. The first member of the class to enter clinical use, avibactam, has been successfully used, combined with ceftazidime, for infections due to Gram-negative bacteria with KPC carbapenemases,[20] though these were poorly represented in Phase III trials. Zidebactam and RG6080 extend this potential by adding direct antibacterial activity and an enhancer effect, contingent on binding to PBP2, with zidebactam having lower MICs for Enterobacteriaceae and *P. aeruginosa* than RG6080. The result is that β-lactam combinations based on these DBOs have an in-vitro spectrum that includes many MBL-producing Enterobacteriaceae – with 80% of these organisms susceptible at 1+1 mg/L in the case of cefepime/zidebactam. Even MBL-producing *P. aeruginosa* were mostly susceptible to cefepime/zidebactam at 8+8 mg/L, though MICs for *A. baumannii* with OXA carbapenemases were higher. Only clinical trials and experience will reveal the extent to which these additional potentials are realised and, until then, some uncertainty will remain about the risk for selection of resistance to the antibacterial effect of these DBOs and strain-to-strain variability in the enhancer effect.

Acknowledgements. We also are grateful to Drs Mahesh Patel and Sachin Bhagwat of Wockhardt for many helpful discussions and debates. These data were presented, in part, at Microbe 2016, Boston, Ma., USA; Abstract Sun-439.

Funding. We are grateful to Wockhardt Ltd. for financial support of these studies.

Transparency declarations.

DML: Advisory Boards or ad-hoc consultancy Accelerate, Achaogen, Adenium, Allecra, AstraZeneca, Auspherix, Basilea, BioVersys, Centauri, Discuva, Meiji, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx, Wockhardt, Zambon, Zealand, Paid lectures – AstraZeneca, Cepheid, Merck and Nordic. Relevant shareholdings in– Dechra, GSK, Merck, Perkin Elmer, Pfizer amounting to <10% of portfolio

value. **All others:** No personal interests to declare. However, PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Achaogen Inc, Allecra Antiinfectives GmbH, Amplex, AstraZeneca UK Ltd, Becton Dickinson Diagnostics, The BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline Services Ltd, Henry Stewart Talks, IHMA Ltd, Merck Sharpe & Dohme Corp, Meiji Seika Kiasya Ltd, Momentum Biosciences Ltd, Nordic Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Rokitan Ltd, Smith & Nephew UK Ltd, Trius Therapeutics, VenatoRx and Wockhardt Ltd.

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343 **Table 1**: Species and genera represented in Enterobacteriaceae groups

Species	Resistance mechanisms					
	ESBL	AmpC	KPC	OXA-48-like	MBL [a]	Susceptible controls
E. coli	10	10	10	5	10	5
Klebsiella	10	5	10	10	10	5
Enterobacter/Citrobacter[b]	10	10	10	0	10	5
Serratia		5				5
Proteeae[c]	4	5			5	5

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345 [a] 20 with NDM enzymes and 15 with VIM types

346 [b] 12 *C. freundii* and 33 *Enterobacter* spp.

347 [c]13 *M. morganii*, 4 *Providencia* spp. and 2 *Proteus* spp.

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Table 2. MIC distributions of zidebactam by species and, for *P. aeruginosa*, resistance mechanism

	No isolates with indicated MIC (mg/L)										
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
E. coli (n=50)	3	28	12	5	1				1		
Klebsiella spp. (n=58)		3	17	17	2	1	1	1		13	3
Enterobacter and *Citrobacter* spp. (n=52)		11	20	10	2		1			1	7
Serratia spp. (n=10)			1	1				1		7	
Proteeae (n=6)											6
P. aeruginosa (n=50)											
β-Lactam susceptible controls (n=10)						3*	5	1			1
AmpC derepressed (n=10)							2	5	3		
MBL producers (n=10)								6	2	1	1
Up-regulated efflux (n=10)								3	2	5	
Cystic fibrosis, mixed mechanisms (n=10)						1*	2	2	3	1	1
A. baumannii (n=30)											30
S. maltophilia (n=10)											10

353 **Table 3.** MIC distributions for cefepime/zidebactam and comparator agents in relation to resistance groups and zidebactam concentrations.

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Control Enterobacteriaceae, without cephalosporin-hydrolysing β-lactamases or carbapenemases (n=25)																
Cefepime	-	12	7	4	2											
CPM/Zid, 0.06	1	16	3	3	2											
CPM/Zid, 0.12	9	10	1	4	1											
CPM/Zid, 0.25	13	8	3	1												
CPM/Zid, 0.5	14	11														
CPM/Zid, 1	14	11														
CPM/Zid, 2	14	11														
CPM/Zid, 4	14	11														
CPM/Zid, 8	14	11														
PIP/TAZ, 4	-				4	1		11	6	3						
Ceftazidime	-		1	9	10	3	2									
CAZ/AVI, 4	-	5	4	6	7	2	1									
Meropenem	-	14	8	3												
Extended-spectrum β-lactamase-producing Enterobacteriaceae (n=33)																
Cefepime						2	4	8	3	1	4	1	3		1	6
CPM/Zid, 0.06			1	6	4	4	7	3	2	2	2	2				
CPM/Zid, 0.12	16	4	2	1	3	2	1	2		1	1					
CPM/Zid, 0.25	24	5	1	1				1		1						
CPM/Zid, 0.5	27	3	1	1			1									

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
CPM/Zid, 1	27	5				1										
CPM/Zid, 2	27	5			1											
CPM/Zid, 4	27	5		1												
CPM/Zid, 8	27	5	1													
PIP/TAZ, 4	-						1	6	11	6	3	1	1		1	3
Ceftazidime	-					1	3	3	2		2	3	6	9		4
CAZ/AVI, 4	-	1	2	7	15	6	1	1								
Meropenem	-	20	11	2												
		K. oxytoca, hyper-produced K1 β-lactamase (n=5)														
Cefepime						1	3		1							
CPM/Zid, 0.06				2	1	2										
CPM/Zid, 0.12			4	1												
CPM/Zid, 0.25		5														
CPM/Zid, 0.5	3	2														
CPM/Zid, 1	3	2														
CPM/Zid, 2	3	2														
CPM/Zid, 4	3	2														
CPM/Zid, 8	3	2														
PIP/TAZ, 4															1	4
Ceftazidime						4	1									
CAZ/AVI, 4				3	2											
Meropenem		3	2													

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
AmpC β-lactamase-producing Enterobacteriaceae (n=																
Cefepime		2	2	5	4	7	6	6	3							
CPM/Zid, 0.06	0	5	7	11	3	2	4	3								
CPM/Zid, 0.12	4	14	7	2	1	4	3									
CPM/Zid, 0.25	15	9	5	2	4											
CPM/Zid, 0.5	23	10	2													
CPM/Zid, 1	25	8	2													
CPM/Zid, 2	25	8	2													
CPM/Zid, 4	25	8	2													
CPM/Zid, 8	27	6	2													
PIP/TAZ, 4					2			1		5	7	6	6	4	3	11
Ceftazidime							1	1	1	1	5	6	9	10	1	
CAZ/AVI, 4		1	3	3	11	14	3									
Meropenem		13	14	4	4											
KPC β-lactamase-producing Enterobacteriaceae																
Cefepime								1	1	3	5	2	3	7	2	6
CPM/Zid, 0.06	1				1			3	7	1	8	4	1	2	1	1
CPM/Zid, 0.12	6				2	4	1		3	7	1	2	2	1	1	
CPM/Zid, 0.25	17	3		1	1	1			1		2		3	1		
CPM/Zid, 0.5	24	1	1		1							2		1		

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
CPM/Zid, 1	26	1	1	1									1			
CPM/Zid, 2	27	1	1							1						
CPM/Zid, 4	28	2														
CPM/Zid, 8	28	2														
PIP/TAZ, 4													1	2	2	25
Ceftazidime									1	7	3	7	7	1	1	3
CAZ/AVI, 4		6	1	6	5	7	3	1	1							
Meropenem							1	2	5	6	4	8	4**			
						OXA-48 β-lactamase-producing Enterobacteriaceae										
Cefepime					3	1	3					1	5	1		1
CPM/Zid, 0.06	1		2	1	1	2		1	2	4		1				
CPM/Zid, 0.12	5	2	2	1		1		2	1		1					
CPM/Zid, 0.25	6	5		1	2				1							
CPM/Zid, 0.5	11	2	2													
CPM/Zid, 1	11	4														
CPM/Zid, 2	11	4														
CPM/Zid, 4	12	3														
CPM/Zid, 8	12	3														
PIP/TAZ, 4														2	6	7
Ceftazidime				1	3	2		1	3				3	2		
CAZ/AVI, 4			1	5	7	2										
Meropenem			1			3	6	2	1			2				

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
MBL-producing Enterobacteriaceae																
Cefepime					1				1	2	4	4	7	3	5	8
CPM/Zid, 0.06					1		1	1	3	7	1	2	8	4	5	2
CPM/Zid, 0.12	5	1		1	1	1	4	1		6	2	3	3	2	5	
CPM/Zid, 0.25	20	1			1		1		3	2	1		1		4	1
CPM/Zid, 0.5	25			2	1	1	1	1					1		3	
CPM/Zid, 1	26		1		1	1	1	1					1		3	
CPM/Zid, 2	27				1	1	1	1					1		3	
CPM/Zid, 4	27				1	1	1	1					1		3	
CPM/Zid, 8	27				2		1	1					1		3	
PIP/TAZ, 4												1	1	1	7	25
Ceftazidime										1			1	4	3	26
CAZ/AVI, 4							1			1	5	3	1			24
Meropenem								1	9	5	3	9	5	3		
Control *P. aeruginosa*																
Cefepime					1		2	3	2	1	1					
CPM/Zid, 4	7						3									
CPM/Zid, 8	9					1[a]										
PIP/TAZ, 4						1		1	3	4			1			
Ceftazidime						2	2	3	2		1					
CAZ/AVI, 4						4[a]	2	4								

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Meropenem		1	1	2	5		1									
							P. aeruginosa, derepressed for AmpC β-lactamase									
Cefepime									1	1	2	5		1		
CPM/Zid, 4	1						1		2	4	1	1				
CPM/Zid, 8	7						1	1			1					
PIP/TAZ, 4												1	1	1	3	4
Ceftazidime										1	1	1	4	2	1	
CAZ/AVI, 4							1	2	3	3	1					
Meropenem						2	6	1	1							
							P. aeruginosa, with MBLs									
Cefepime											1	2		1	1	5
CPM/Zid, 4										1		1	2	1	1	4
CPM/Zid, 8	6					2[a]									1	1
PIP/TAZ, 4												1	4		2	3
Ceftazidime												2	1	1		6
CAZ/AVI, 4												2	1	1		6
Meropenem												2	1	7[b]		
							P. aeruginosa, with upregulated efflux									
Cefepime										2	6	2				
CPM/Zid, 4						1[a]				5	4					

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
CPM/Zid, 8	2						1	1	1	3	2					
PIP/TAZ, 4												4	2	1	2	1
Ceftazidime										5	1	2	2			
CAZ/AVI, 4									1	8	1					
Meropenem											1	6	3			
P. aeruginosa, cystic fibrosis isolates																
Cefepime													2	1	5	2
CPM/Zid, 4	3					1[a]						2	2	2		
CPM/Zid, 8	5					2[a]		2					1			
PIP/TAZ, 4										1					2	7
Ceftazidime															4	6
CAZ/AVI, 4										3		1	2	1	3	
Meropenem										2	4	2	2			
A. baumannii, susceptible controls																
Cefepime							2	2	1							
CPM/Zid, 4							1	3	1							
CPM/Zid, 8								5								
PIP/TAZ, 4						4[a]				1						
Ceftazidime							2	2	1							
CAZ/AVI, 4								2	2	1						
Meropenem					4	1										

Antibiotic and [inhibitor], mg/L	Inhibited by zidebactam alone (n)	Number of isolates with indicated MIC (mg/L)														
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
A. baumannii, OXA carbapenemases																
Cefepime											3	12	4			1
CPM/Zid, 4										2	10	5	3			
CPM/Zid, 8								1		2	9	6	2			
PIP/TAZ, 4												2	0	1	4	13
Ceftazidime										1	2		2	7	2	6
CAZ/AVI, 4									1	1	6	5	2	1		4
Meropenem									1	1	1	11	4	2[b]		
A. baumannii, metallo (NDM) carbapenemases																
Cefepime																5
CPM/Zid, 4															1	4
CPM/Zid, 8															1	4
PIP/TAZ, 4																5
Ceftazidime																5
CAZ/AVI, 4																5
Meropenem													1	4		
S. maltophilia																
Cefepime								1		2	2	3	2			
CPM/Zid, 4							1	1	3	2	2	1				
CPM/Zid, 8						1[a]		1	3	2	2	1				
PIP/TAZ, 4											1		3		1	5
Ceftazidime						1		1		1	1	1	1	1	2	1

CAZ/AVI, 4	1	1	1	1	1	2		3
Meropenem						1	9 [b]	

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355 * MIC ≤ indicated value; **MIC ≥indicated value; Abbreviations: CAZ+AVI, ceftazidime/avibactam, CPM-Zid, cefepime/zidebactam; PIP+TAZ Piperacillin-tazobactam

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357 **Table 4:** Combination behaviour against Enterobacteriaceae with zidebactam MICs ≥16 mg/L and cefepime ≥2 mg/L

		MIC Zidebactam	Cefepime MIC (mg/L) with zidebactam at:								
Specimen ID	Species	(mg/L)	0	0.06	0.12	0.25	0.5	1	2	4	8
SE01046	*S. marcescens,* AmpC	>32	2	1	0.25	0.25	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
H053420099	*K. pneumoniae,* CTX-M 9 gp	>32	64	32	16	8	0.125	≤0.03	≤0.03	≤0.03	≤0.03
NCTC 13465	*K. pneumoniae,* CTX-M-25	>32	16	1	0.5	0.06	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
Mei 1	*K. pneumoniae,* ESBL	>32	2	0.06	0.125	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
SE06031	*M. morganii,* CTX-M 1 group	>32	4	0.25	0.06	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
H053460141	*Proteus* spp., ESBL	>32	>256	32	8	2	1	0.5	0.25	0.125	0.06
LN09056	*P. mirabilis,* ESBL	>32	>256	1	0.25	0.125	0.06	≤0.03	≤0.03	≤0.03	≤0.03
H092260700	*Klebsiella* spp., OXA-48 + ESBL	>32	64	8	2	0.25	0.06	≤0.03	≤0.03	≤0.03	≤0.03
H112860135	*Klebsiella* spp., OXA-48 + ESBL	>32	>256	8	2	0.125	0.06	≤0.03	≤0.03	≤0.03	≤0.03
H131480242	*M. morganii,* ESBL	>32	>256	>256	>256	256	128	≤0.03	≤0.03	≤0.03	≤0.03
H124240625	*K. pneumoniae,* KPC + SHV	>32	256	128	64	64	32	0.125	0.06	≤0.03	≤0.03
H114600525	*E. aerogenes,* KPC	>32	64	16	8	4	0.06	≤0.03	≤0.03	≤0.03	≤0.03
H113980340	*K. pneumoniae,* NDM, Azt-R	>32	256	64	32	8	0.25	0.25	0.25	0.25	0.25
H112240413	*K. pneumoniae,* VIM, Azt-R	>32	4	2	2	1	0.5	0.5	0.5	0.5	0.25
H130680324	*E. coli,* NDM, Azt-R	16	>256	256	256	256	256	256	256	256	256

H092540314	*M. morganii,* NDM, Azt-I	>32	64	8	8	4	1	1	1	1	1
H123140552	*P. rettgeri,* NDM, Azt-R	>32	>256	>256	256	256	256	256	256	256	256
H123560843	*P. rettgeri,* NDM, VEB, CMY-14 Azt-R	>32	>256	256	256	256	256	256	256	256	256
H124880510	*P. stuartii,* NDM, Azt-S	>32	16	16	16	16	2	2	2	2	2
H124880511	*P. rettgeri,* NDM, Azt-S	>32	64	64	64	64	64	64	64	64	64

Azt-S/I/R: aztreonam susceptible, intermediate or resistant, based on prior testing by BSAC methodology and taken as an indicator of ESBL/AmpC presence or absence in MBL producing isolate

Figure 1. Structure of zidebactam

Figure 2. Killing curves for Gram-negative bacteria with NDM carbapenemases by cefepime, zidebactam and their combinations. Panel (a) *K. pneumoniae* H113840625 with MICs cefepime 256 mg/L, zidebactam 0.25 mg/L, meropenem 32 mg/L; (b) *K. pneumoniae* H113980340 with MICs cefepime 256 mg/L, zidebactam >32 mg/L, meropenem 32 mg/L; (c) *E. coli* H131020913 with MICs cefepime >256 mg/L, zidebactam 0.25 mg/L , meropenem 64 mg/L; (d) *E. coli* H130480324 with MICs cefepime >256 mg/L, zidebactam 16 mg/L, meropenem 32 mg/L; (e and f) *P. aeruginosa* H130680310 with MICs cefepime >256 mg/L, zidebactam 8 mg/L cefepime and meropenem >64 mg/L; (g and h) *P. aeruginosa* H131800691 with MICs cefepime >256 mg/L, zidebactam 32 mg/L cefepime and meropenem 64 mg/L and (i), *A. baumannii* H104940508 with OXA-23 carbapenemase with MICs cefepime >256 mg/L, zidebactam >32 mg/L and meropenem 32 mg/L.

374 **Figure 1**

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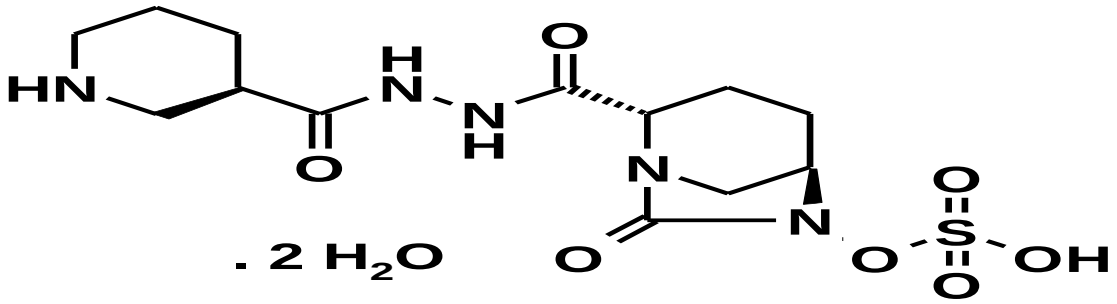

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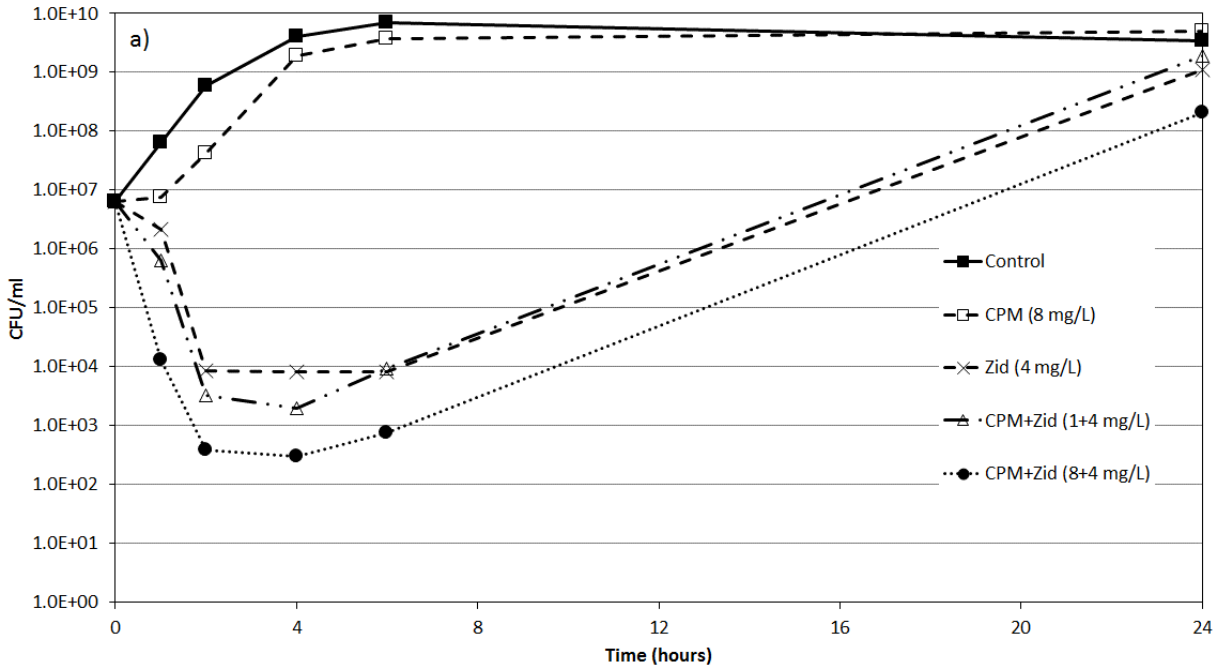
379 Figure 2



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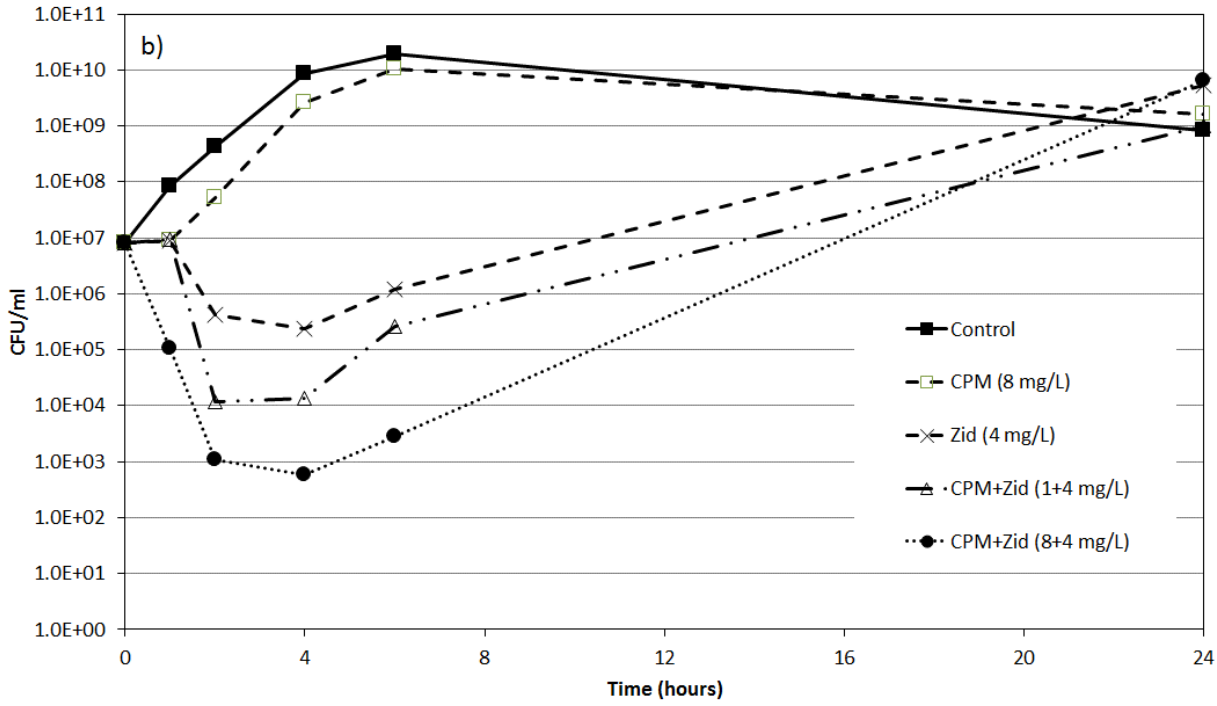

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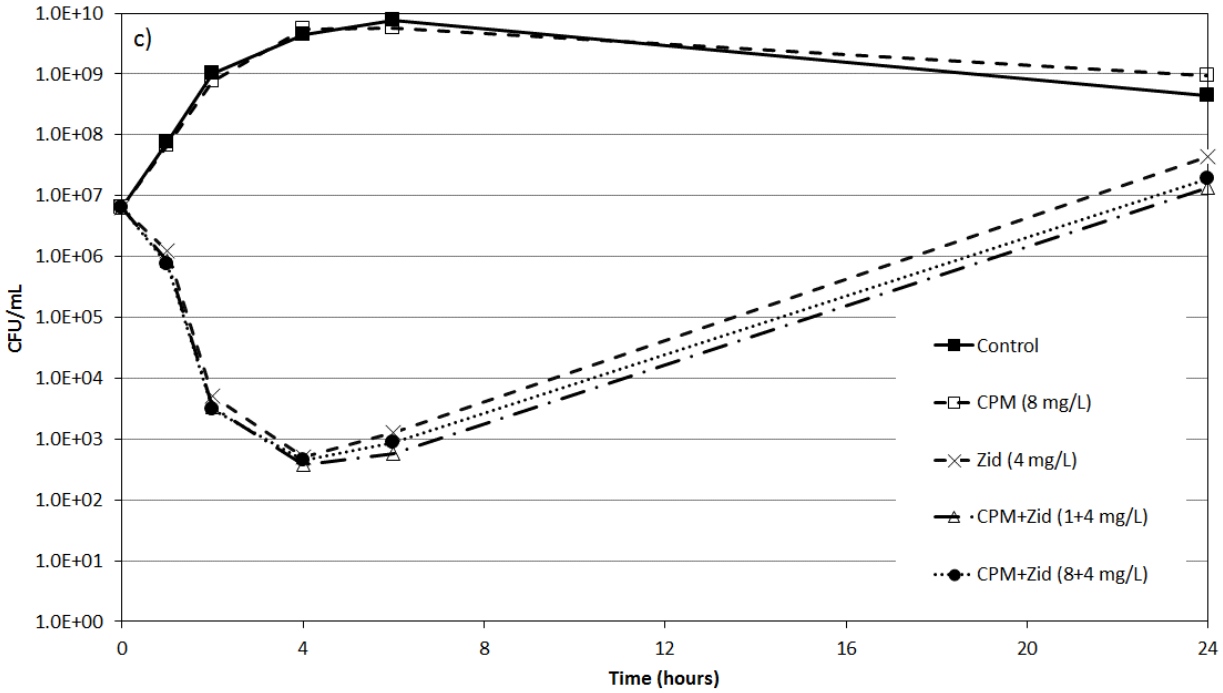

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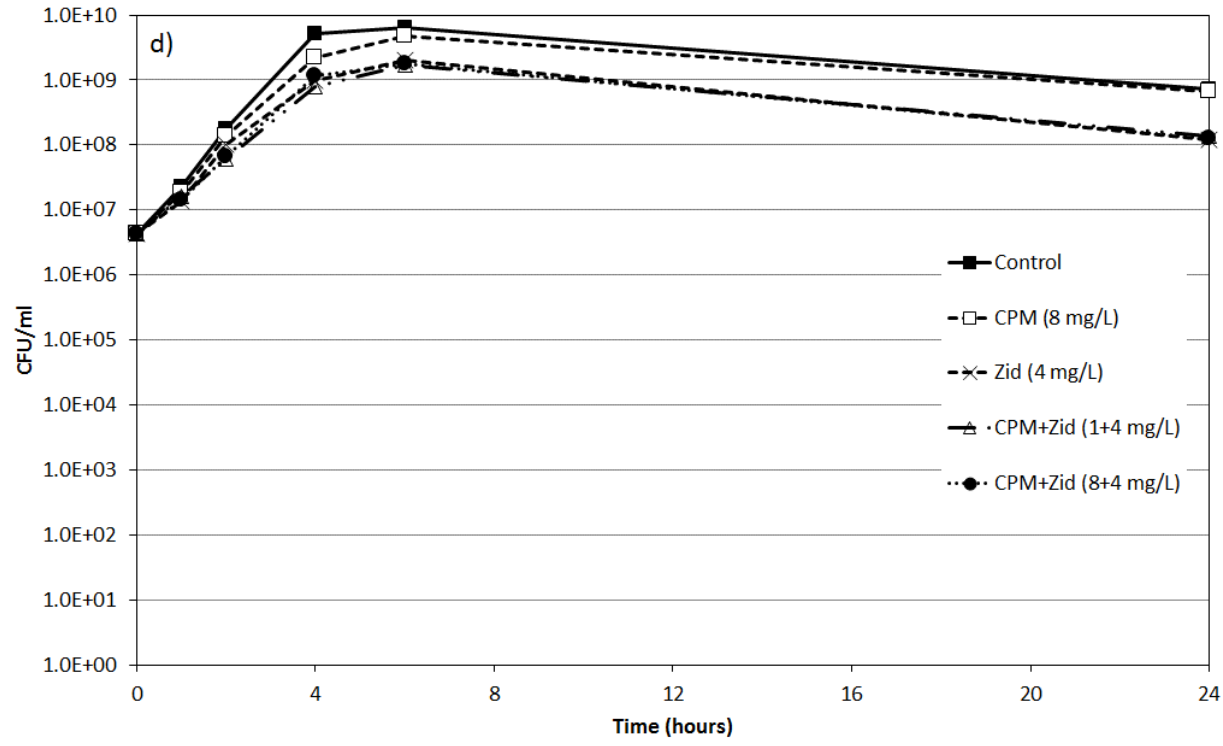

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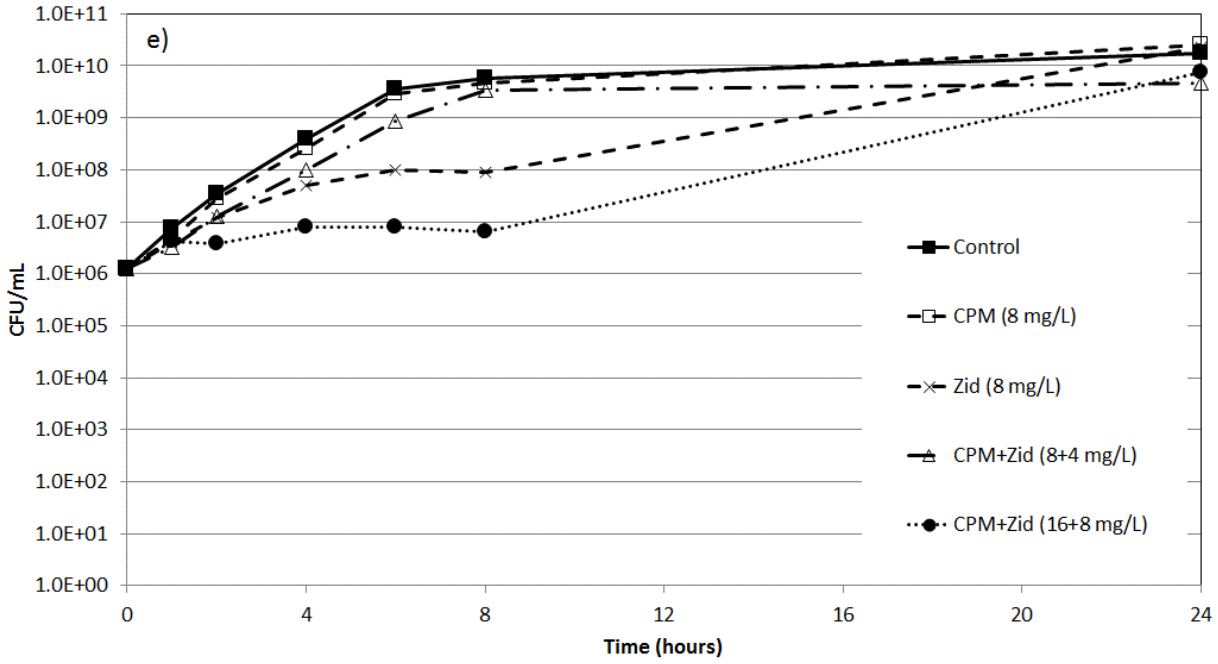
e)
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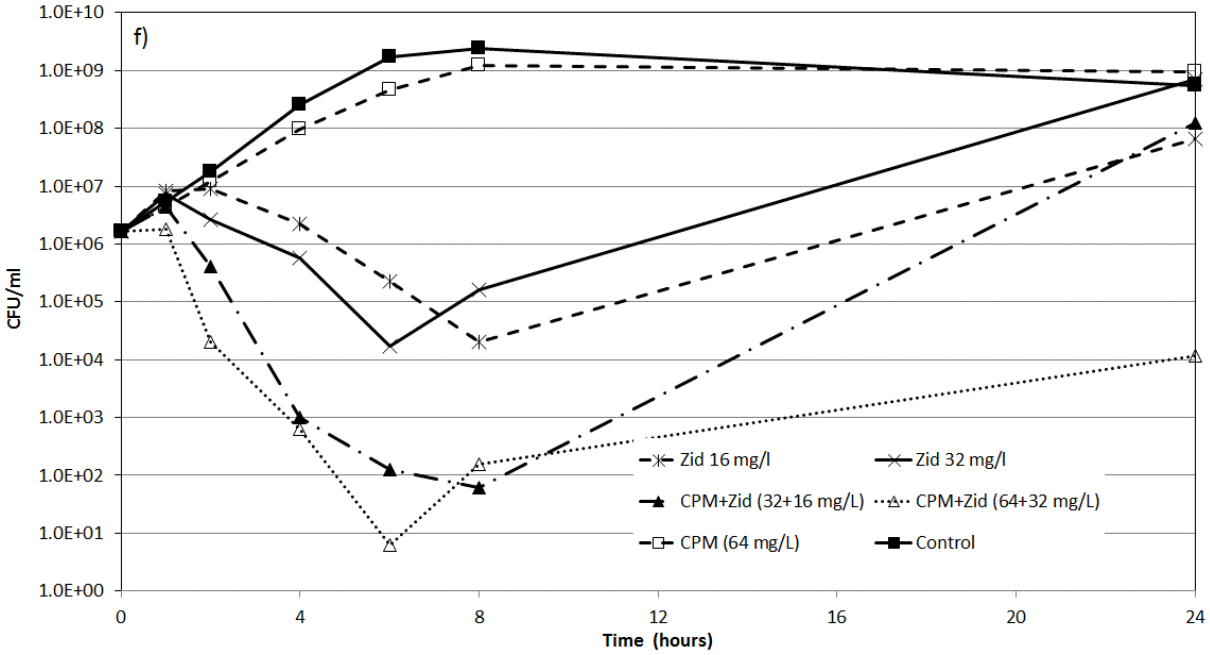
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
CFU/mL
0
4
8
12
16
20
24
Time (hours)
Control
CPM (8 mg/L)
Zid (8 mg/L)
CPM+Zid (8+4 mg/L)
CPM+Zid (16+8 mg/L)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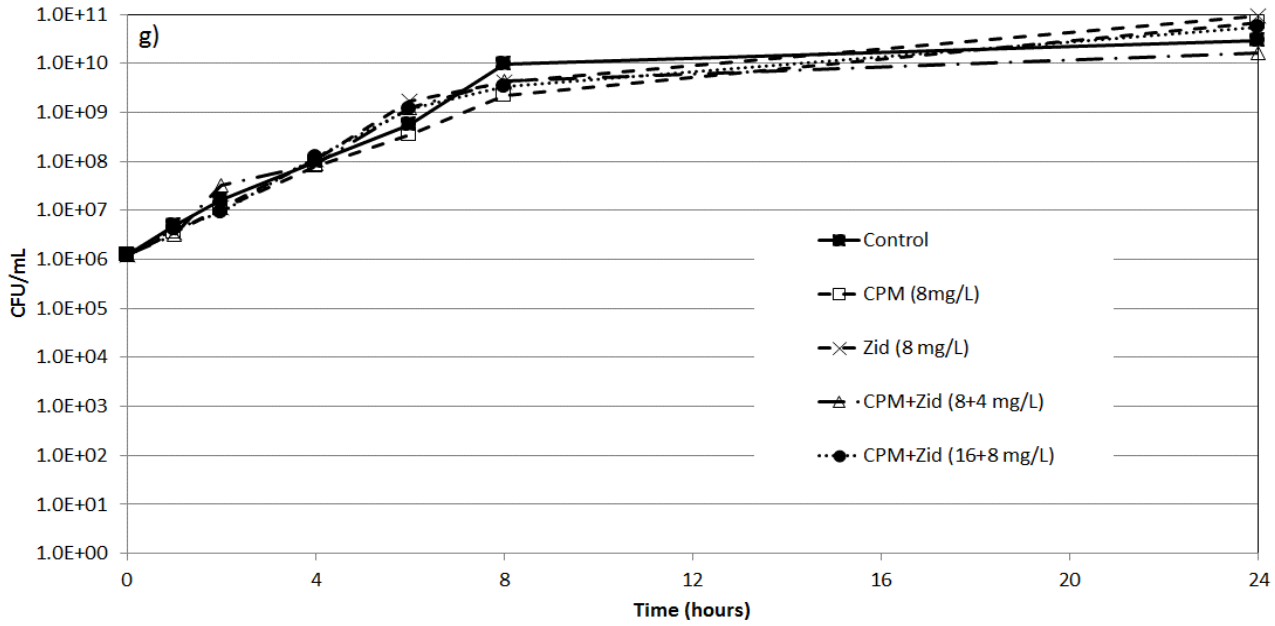
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
CFU/ml
0
4
8
12
16
20
24
Time (hours)
Zid 16 mg/l
Zid 32 mg/l
CPM+Zid (32+16 mg/L)
CPM+Zid (64+32 mg/L)
CPM (64 mg/L)
Control



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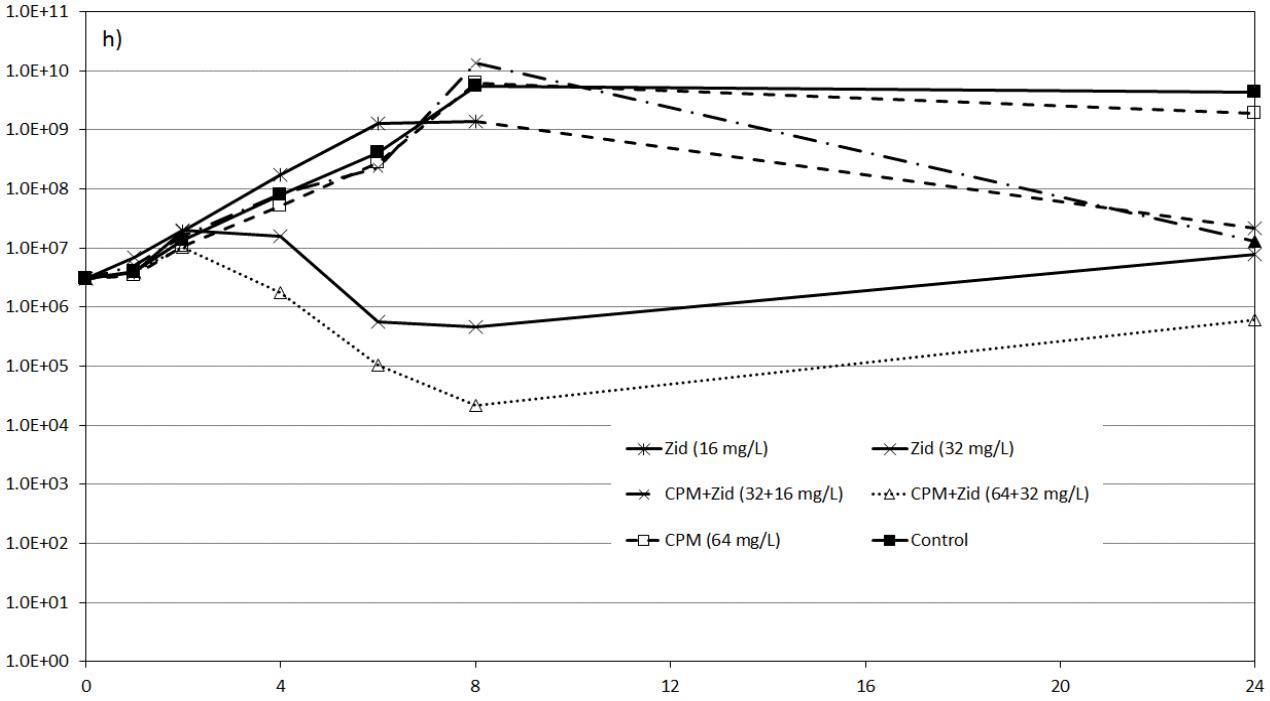

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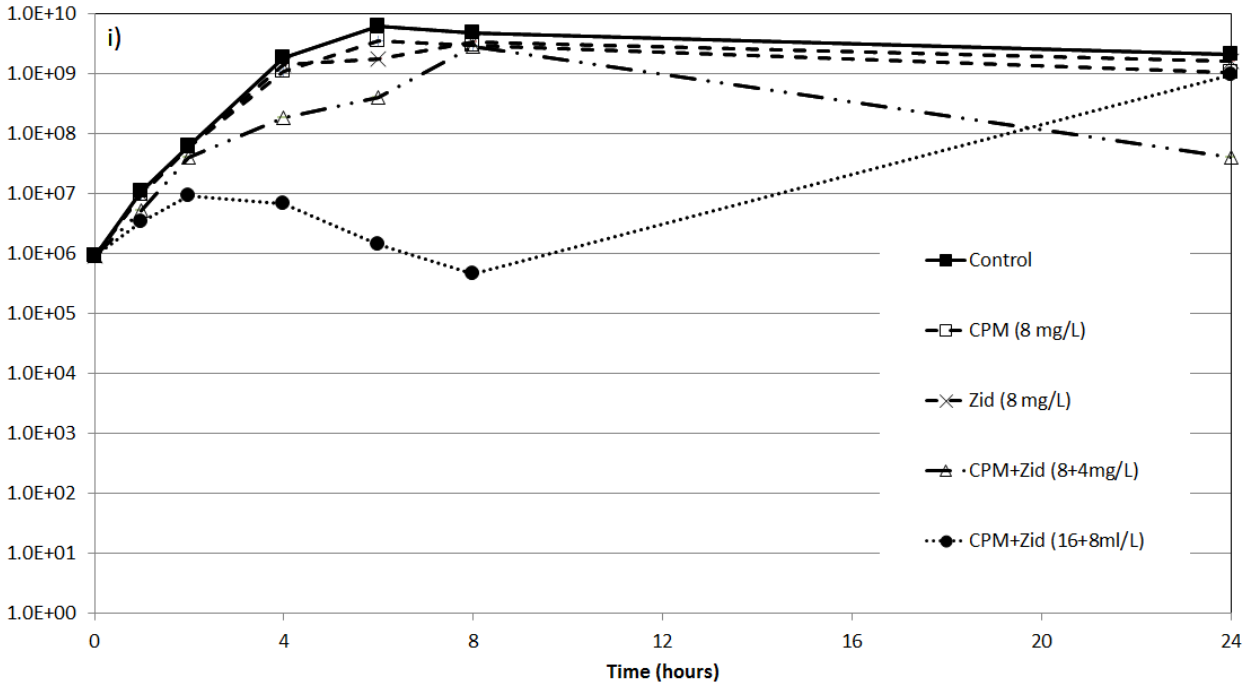
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1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
0
4
8
12
16
20
24
Time (hours)
Control
CPM (8 mg/L)
Zid (8 mg/L)
CPM+Zid (8+4mg/L)
CPM+Zid (16+8ml/L)

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