Activity of nacubactam (RG6080/OP0595) combinations against metallo-β-lactamase-producing Enterobacteriaceae

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Background. Diazabicyclooctanes (DBOs) are promising β–lactamase inhibitors. Some, including nacubactam (OP0595/RG6080), also bind PBP2, and have an enhancer effect, allowing activity against Enterobacteriaceae with MBLs, which DBOs do not inhibit. We tested the activity of nacubactam-β–lactam combinations against MBL-producing Enterobacteriaceae. **Materials/Methods.** Test panels comprised: (i) 210 consecutive Enterobacteriaceae with NDM or VIM MBLs, as referred by UK diagnostic laboratories and, (ii) 99 supplementary MBL-producing Enterobacteriaceae, representing less prevalent phenotypes, species and enzymes. MICs were determined by CLSI agar dilution. **Results.** MICs of nacubactam alone were bimodal, clustering at 1-8 mg/L or >32 mg/L: >85% of values for *Escherichia coli* and *Enterobacter* fell into the low-MIC cluster, whereas *Proteeae* were universally resistant and *Klebsiella* divided between the two groups. Depending on the prospective breakpoint (4+4 or 8+4 mg/L), and on whether all isolates were considered or solely the Consecutive panel, meropenem/nacubactam and cefepime/nacubactam inhibited 80.3 to 93.3% of MBL producers, with substantial gains over nacubactam alone. Against the most resistant isolates – comprising 57 organisms with MICs of nacubactam >32 mg/L, cefepime $\geq$128 mg/L and meropenem $\geq$128 mg/L – cefepime/nacubactam 8+4 mg/L inhibited 63.2% and meropenem/nacubactam 8+4 mg/L inhibited 43.9%. Aztreonam/nacubactam - incorporating an MBL-stable β-lactam partner - was almost universally active against the MBL producers and, unlike aztreonam/ avibactam, had an enhancer effect. **Conclusions.** Nacubactam combinations, including those using MBL-labile β–lactams, e.g. meropenem and cefepime, can overcome most MBL-mediated

resistance. This behaviour reflects nacubactam's direct antibacterial and enhancer activity.

Introduction

Diazabicyclooctanes (DBOs) are potent non-β–lactam inhibitors of β-lactamases.[1] Avibactam is the sole analogue so far licensed, partnered with ceftazidime. It is also in Phase III trials combined with aztreonam. Four further DBOs – ETX2514 (Entasis),[2] nacubactam (RG6080/OP0595, Roche, Fedora, Meiji),[3] relebactam (MK-7655, Merck),[4] and zidebactam (WCK5107, Wockhardt)[5] – have progressed into clinical development.

DBOs inhibit most or all Class A and C β-lactamases, whilst activity against Class D β-lactamases varies with the particular enzyme and inhibitor.[1-5] Although DBOs do not inhibit MBLs (Class B β-lactamases), which are an expanding problem worldwide[6] this limitation may be overcome in either of two ways. Firstly, as with aztreonam/avibactam, the DBO can be combined with a monobactam, as these are stable to MBLs and need only to be protected from any co-produced ESBL or AmpC enzyme(s).[7,8] Alternatively, several developmental DBOs – notably nacubactam, ETX2514 and zidebactam – have significant affinity for PBP2 of many Gram-negative species.[3,5,9,10] This allows them to exert both a direct antibacterial effect and, like mecillinam (which also targets PBP2), an 'enhancer' mechanism, potentiating partner β-lactams that bind to PBP3. This combination of direct and enhancer-based activity means that combinations of MBL-labile β-lactams with nacubactam, ETX2514 or zidebactam can retain activity against MBL-producing Enterobacteriaceae[3,5,9] (also *Pseudomonas aeruginosa* in the case of zidebactam[10]). Although the antibacterial activity of these DBOs is vulnerable to high-frequency mutational

resistance the enhancer effect is often retained against DBO-resistant mutants.[3,5,9,11,12]

We assessed the activity of nacubactam combinations against MBL producers by testing against isolates sent to the UK reference laboratory.

Materials and methods

Isolates

Two groups of MBL-producing Enterobacteriaceae were used: the Consecutive and Supplementary Collections. The 'Consecutive' Collection comprised 158 non-duplicate Enterobacteriaceae with NDM MBLs and 52 with VIM MBLs, as consecutively referred to PHE's AMRHAI Reference Unit from UK diagnostic labs from May 2014 to Dec 2015. The 'Supplementary' Collection comprised 99 pre-2014 Enterobacteriaceae selected to add IMP enzymes, and to augment the numbers of under-represented species and aztreonam-susceptible phenotypes. Bacterial species were identified by MALDI-ToF mass spectroscopy, whilst MBL genes were identified by PCR[13,14] or Illumina-based WGS.[12]

Antibiotics

Nacubactam was from Roche (Basel, Switzerland); avibactam from TCG Lifesciences (Pune, India); aztreonam and cefepime from Alfa Aesar (Heysham, UK); and meropenem from Sequoia Research Products (Pangbourne, UK).

Susceptibility testing

MICs were determined by CLSI agar dilution[15] using Mueller-Hinton media from Oxoid/Thermofisher (Basingstoke, UK). When end-points trailed, growth of ≥4 colonies was counted as significant. Aztreonam, cefepime and meropenem were tested, as doubling dilutions, with nacubactam at 0, 1, 2 and 4 mg/L, or with avibactam at 4 mg/L. 'Synergy' was defined as a ≥3 doubling dilution reduction in the partner β-lactam MIC in the presence of the DBO.

Results and Discussion

Behaviour of nacubactam alone

MIC distributions of nacubactam alone for the Combined Collection (i.e. Consecutive and Supplementary Collections combined, n =309) are shown in Table 1. Values for *Proteeae* were almost all >32 mg/L, whereas those for other genera were bimodal, with peaks at 1-8 and >32 mg/L. MICs for most (>88%) *E. coli* and *Enterobacter* spp. fell into the lower peak, with few high values; those for *Klebsiella* spp. were widely scattered and complicated by trailing end points, but mostly fell into the higher peak, with 84/157 values >32 mg/L. MICs of avibactam alone, which was included as a control, were ≤4 mg/L for just 3/309 isolates (1%), with values >4 mg/L for the remaining 99%.

Analysis of the behaviour of nacubactam in combination

Depicting the MIC distributions for combinations triple-action DBOs (i.e. those with direct antibacterial and enhancer effects as well as acting as β-lactamase

inhibitors) is challenging. If MICs are expressed relative to the β–lactam, as is conventional for β-lactam/β-lactamase inhibitor combinations, values can be low either (i) because the DBO potentiates the β–lactam, or (ii) because the isolate is inhibited by the DBO itself. In addition, a distinction must be drawn between the behaviour of combinations involving cefepime and meropenem, which are MBL-labile, and those involving aztreonam, which is stable to MBLs. For cefepime and meropenem combinations, a low MIC requires either antibacterial activity by the DBO or a strong enhancer effect whereas a low MIC for an aztreonam combinations may be achieved solely by inhibition of other coproduced β–lactamases. MBL-producers lacking ESBL or AmpC activity are anyway susceptible to aztreonam.

To capture these nuances, two presentations are provided. Firstly, in Table 2, conventional MIC distributions are shown for the Combined and Consecutive Collections, and for various subsets. These are compared with the MIC distributions for the unprotected β–lactam and for the corresponding combination with avibactam (4 mg/L), which lacks direct antibacterial and enhancer activities. Secondly, Table 3 illustrates the proportions of different groups of isolates susceptible to meropenem, cefepime and aztreonam at 1, 2, 4 or 8 mg/L, as determined in the presence of nacubactam at 0, 1, 2 or 4 mg/L, or with avibactam at 4 mg/L. These β–lactam concentrations were chosen to straddle the current spectrum of EUCAST and CLSI breakpoints (EUCAST, cefepime and aztreonam S $\leq$1, R >4, meropenem, S $\leq$2, R >8; CLSI cefepime and aztreonam S $\leq$2, R >8, [with 4 and 8 mg/L designated ‘Dose-Dependent Susceptible for cefepime]; meropenem, S $\leq$1, R >4 mg/L).

MICs of meropenem and cefepime combined with DBOs

As would be expected, the great majority of MBL producers were resistant to unprotected meropenem and cefepime. Most, however, became susceptible to these agents when they were combined with nacubactam, 4 mg/L (Table 2). Thus, meropenem/nacubactam at 8+4 mg/L was active against 87.1% of the 210 Consecutive isolates, which provide the best representation of currently circulating MBL producers, whilst cefepime/nacubactam 8+4 mg/L was active against 93.3% of these isolates. Corresponding proportions susceptible to meropenem/avibactam and cefepime/avibactam 8+4 mg/L were much smaller, at 24.8% and 22.4%, respectively.

The wide activity of meropenem/nacubactam and cefepime/ nacubactam 8+4 mg/L combinations against *Escherichia coli* and *Enterobacter* spp*.,* was substantially attributable to the direct antibacterial activity of nacubactam against these species (see Table 1). However meropenem/nacubactam 8+4 mg/L and cefepime/nacubactam 8+4 mg/L also were active against 127 (80.9%) and 141 (89.8%) of 157 MBL-positive *Klebsiella* spp. respectively (Table 2), whereas nacubactam 4 mg/L alone only inhibited only 40 (25.5%) of these isolates (Table 1). These gains in activity, relative to nacubactam alone, are best explained by the enhancer effect and are most clearly illustrated by data for the Combined Collection in Table 3.

Overall, addition of nacubactam at 1, 2 or 4 mg/L allowed meropenem 8 mg/L to inhibit 53.7%, 80.9% and 84.8% of all MBL producers; corresponding

proportions for equivalent cefepime combinations were 47.2%, 85.4% and 90.0%, respectively whereas the proportions inhibited by nacubactam alone at 1, 2 or 4 mg/L were only 12.6%, 35.0% and 49.2%, respectively (Table 1). Similarly-large gains in activity compared with nacubactam alone were apparent when other prospective meropenem and cefepime breakpoints were considered, when the Consecutive Collection alone was considered, or when only NDM *Klebsiella* spp. (as. the most populous group) were considered (Table 3).

In general, cefepime/nacubactam combinations inhibited a slightly larger proportion of MBL producers than the corresponding meropenem/nacubactam combinations when the nacubactam concentration was 2 or 4 mg/L whereas the position reversed, with meropenem/nacubactam more active, when the nacubactam concentration was 1 mg/L. The activity of meropenem/nacubactam and cefepime/nacubactam did not show any clear relationship to MBL type (IMP, NDM or VIM), nor to aztreonam susceptibility and resistance, which is a proxy for whether or not ESBL or AmpC enzymes are co-produced (Table 2).

Forty-seven isolates from the Combined Collection were resistant to meropenem/nacubactam 8+4 mg/L. These comprised 30 *Klebsiella* spp., 9 *Proteeae*, 4 *Citrobacter* spp., 3 *E. coli* and one *Enterobacter* spp.; 36 had NDM MBLs, 9 had VIM and two IMP. Although *Klebsiella* spp. and NDM dominated, it should be recalled that these were the most populous species (159/309, 51.5%) and MBL (200/309, 64.7%) type across the whole collection; the presence of 9/15 *Proteeae* and 4/10 *Citrobacter* spp. is more noteworthy and underscores the frequent resistance to these groups to the antibacterial action

of nacubactam (Table 1). Synergy between meropenem and 4 mg/L nacubactam was often weak or absent for *Proteeae*, with meropenem MICs reduced ≥8-fold in only 1/15 cases; synergy was greater with cefepime, where ≥8-fold MIC reductions were seen for 11/15 Proteeae.

MICs of aztreonam combined with DBOs

As noted earlier, aztreonam combinations differ from the others considered here insofar as they utilise a β–lactam that is not a substrate for MBLs, meaning that low MICs are to be anticipated so long as the inhibitor inactivates any co-produced monobactam-hydrolysing ESBL or AmpC enzyme.[7,8] Thus, aztreonam/avibactam 4+4 mg/L inhibited 96.4% of the Combined Collection and 96.7% of the Consecutive Collection, rising to 98.1% and 99.5% respectively at 8+4 mg/L. Aztreonam/nacubactam performed similarly, inhibiting 99.7% of the Combined Collection and 99.5% of the Consecutive Collection at either 4+4 or 8+4 mg/L. Six isolates were not susceptible to aztreonam/avibactam at 8+4 mg/L; these comprised four *E. coli* and two *Providencia* spp. The sole isolate resistant to aztreonam/nacubactam at 4+4 or 8+4 mg/L was an *E. coli* (MIC 32+4 mg/L) that was also highly resistant to all other nacubactam combinations, with MICs >128+4 mg/L for all cefepime and meropenem combinations.

Nacubactam combinations against nacubactam-resistant isolates

Isolates that are resistant to the antibacterial activity of both nacubactam and its MBL-labile antibiotic partners are of particular interest, because low

combination MICs here must depend upon the enhancer effect.[9] Accordingly, Table 4 shows the MIC distributions of nacubactam combinations, compared with unprotected β–lactams and avibactam combinations, against the 110 isolates for which the nacubactam MICs were >32 mg/L, and for the 57 of these that were highly resistant to meropenem and cefepime, with MICs ≥128 mg/L.

Nacubactam combinations retained activity against many of these difficult organisms. Thus, at 8+4 mg/L, meropenem/nacubactam inhibited 61.8% of all isolates resistant to nacubactam at 32 mg/L, compared with only 22.7% for meropenem/avibactam; similarly, cefepime/nacubactam 8+4 mg/L inhibited 75.5% of the Combined Collection compared with 15.5% for cefepime/avibactam. Given that avibactam should inhibit co-produced ESBLs and AmpC enzymes as efficiently as nacubactam, the gain in activity of the nacubactam combinations relative to those involving avibactam is ascribed to the enhancer effect. Against the 57 isolates that were highly resistant to cefepime and meropenem (MIC ≥128 mg/L) as well as to nacubactam (MIC >32 mg/L), 43.9% were inhibited by meropenem/nacubactam 8+4 mg/L and 63.2% by cefepime/nacubactam 8+4 mg/L. None of these 57 was susceptible to meropenem/avibactam or cefepime/avibactam 8+4 mg/L.

Based on prospective 4+4 or 8+4 mg/L breakpoints, both aztreonam/avibactam and aztreonam/nacubactam had near universal activity against the nacubactam- and β-lactam- resistant isolates. In addition, and interestingly, nacubactam, unlike avibactam, potentiated aztreonam against many nacubactam-resistant (MIC >32 mg/L) isolates that were susceptible to aztreonam on CLSI criteria, with MICs ≤2 mg/L (n=29, Table 5). Such isolates are unlikely to have significant AmpC or ESBL activity, firstly because of the

low aztreonam MICs and secondly because, if they did have such enzymes, aztreonam/avibactam synergy would be anticipated. Accordingly, aztreonam/nacubactam, synergy here is interpreted as a further manifestation of the enhancer effect.

Conclusion

Along with boronates, DBOs are among the most promising new-generation β–lactamase inhibitors.[1] A limitation is that DBOs do not directly inhibit MBLs, which are a rising global problem,[6,16] whereas some of these enzymes are inhibited by developmental boronates such as VNRX-5133[17] (VenatoRx), though not by vaborbactam, which is the sole licensed analogue. Routes around this limitation are to combine the DBO with an MBL-stable monobactam, as with aztreonam/avibactam,[7,8] or to use a triple-action DBO, such as nacubactam or zidebactam.[3,5.9,10] Although the direct antibacterial activity of triple action DBOs is vulnerable to high frequency mutations that compensate for inhibition of PBP2,[3,9,11,12] these commonly leave a functional enhancer effect; moreover, DBO-resistant mutants grow as round forms under DBO challenge,[9,12] and the ability of these to sustain infection is questionable.

Despite utilising MBL-labile β–lactams, both meropenem/nacubactam and cefepime/nacubactam achieved wide activity against MBL producers, independently of the MBL type and the isolates' aztreonam-resistance status. Activity did vary with species, with raised meropenem/nacubactam and cefepime/nacubactam MICs more frequent among *Proteeae*. These are uncommon hosts for MBLs in most countries,[16,18] though there is a scatter of

reports, notably of *Providencia* spp. with NDM enzymes in Latin America.[19,20] Meropenem/nacubactam or cefepime/nacubactam retained activity against many MBL producers that had high-level resistance to these molecules individually (Table 4). This behaviour is believed to reflect the enhancer effect, contingent on simultaneous attack on PBP2 by nacubactam and PBP3 by the partner β-lactam. Although meropenem itself has significant affinity for PBP2, it is not so primarily directed against this target as imipenem, and also has potent affinity for PBP3.[21,22]

Aztreonam/nacubactam (and aztreonam/avibactam) achieved wider activity against MBL-producing Enterobacteriaceae than meropenem/nacubactam or cefepime/nacubactam. However, their overall spectrum is narrower, owing to aztreonam having limited activity against *Pseudomonas* and none against Gram-positive genera or anaerobes.[23] Moreover, aztreonam, which targets only PBP3, is more weakly bactericidal than cephalosporins and carbapenems, which target multiple PBPs. On the other hand, some will consider a narrower spectrum to be ecologically preferable, and note that aztreonam has the advantages of limited cross-allergenicity with other β–lactams and little selectivity for *Clostridium difficile*.[24,25]

The data presented here, coupled with the near universal activity of nacubactam combinations against isolates with non-metallo carbapenemases[3,9] supports progression of nacubactam combinations into clinical development.

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Transparency declaration

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Table 1. MIC distributions of nacubactam, tested alone, by species, Combined Collection (n=309)

	No isolates with indicated MIC (mg/L)							
Genus/Group	≤1	2	4	8	16	32	>32	Total
Citrobacter spp.		1	1	2		3	3	10
Enterobacter spp.	10	24	11			1	4	50
Escherichia coli	22	29	14	3	3	1	5	77
Klebsiella spp.	7	15	18	15	12	6	84	157
Proteeae[a]				1			14	15
Grand Total	39	69	44	21	15	11	110	309

[a] Comprising 14 *Providencia* spp. and 1 *Morganella morganii*

Table 2. MIC distributions of DBO 4 mg/L combinations, by species, MBL type and aztreonam resistance

MIC (mg/L)	No isolates with indicated MIC												
	β-Lactam/nacubactam 4 mg/L; isolate subsets										Combined Collection (n=309)		
	Consecutive Collection (n=210)	Combined Collection, by species					Combined Collection, by MBL type			Combined Collection, by aztreonam MIC		β-Lactam-nacubactam, 4 mg/L	β-Lactam-avibactam, 4 mg/L	β-Lactam alone, no DBO
		Citro-bacter	*Entero-bacter*	*E. coli*	*Kleb-siella*	*Prot-eeae*	IMP	NDM	VIM	>2 mg/L	≤2 mg/L			
Meropenem combinations														
≤0.03	113	3	43	67	51		13	105	46	110	54	164	6	2
0.06	16	1	2	2	15		1	13	6	12	8	20	0	
0.125	13	2		2	12			7	9	12	4	16	2	
0.25	6		2		6			2	6	4	4	8		
0.5	4				4			3	1	4		4		
1	3			2	5	1	2	5	1	5	3	8	3	1
2	9				10	2		6	6	10	2	12	24	2
4	12		1		13	2		12	4	12	4	16	13	18
8	7		1	1	11	1	1	11	2	10	4	14	37	26
16	7	2			7	4		10	3	8	5	13	27	32
32	6	1			6	1		6	2	4	4	8	31	28

64	4	1			6		1	4	2	5	2	7	69	57
128	6		1	2	8	3	1	11	2	13	1	14	47	75
>128	4			1	3	1		5		3	2	5	50	68
Cefepime combinations														
≤0.03	125	4	45	69	61		14	114	51	119	60	179	8	1
0.06	10		2	2	9			8	5	9	4	13	1	1
0.125	13	1		1	15			6	11	14	3	17	2	
0.25	7			2	9		1	6	4	8	3	11	0	
0.5	6	1			5			3	3	5	1	6	1	
1	8				7	4		6	5	6	5	11	5	
2	10	2	1		9			11	1	12		12	22	3
4	6	1			9		1	8	1	8	2	10	14	9
8	11				17	2	1	13	5	13	6	19	15	15
16	4		1		5	4		7	3	3	7	10	14	11
32	4				5	1		6		5	1	6	12	21
64	3	1			4		1	3	1	3	2	5	25	16
128	1				1			1		1		1	51	34
>128	2		1	3	1	4	1	8		6	3	9	139	198

Aztreonam combinations														
≤0.03	188	9	48	74	128	8	17	174	76	176	91	267	17	1
0.06	14		1		18	3	1	14	7	19	3	22	45	13
0.125	5	1			9	1		5	6	10	1	11	87	20
0.25	1		1		2		1	1	1	2	1	3	73	24
0.5						1		1		1		1	44	6
1	1			1				1			1	1	19	10
2						2							7	16
4				1				3		3		3	6	7
8													5	7
16													5	10
32	1			1				1		1		1	1	17
64														28
128														35
>128														115
Total	**210**	**10**	**50**	**77**	**157**	**15**	**19**	**200**	**90**	**212**	**97**	**309**	**309**	**309**

421 **Table 3.** Susceptibility to DBO combinations compared with susceptibility to DBOs alone

	% of isolates susceptible to β-lactam at stated concentration when combined with:				
	No DBO	Nacubactam 1 mg/L	Nacubactam 2 mg/L	Nacubactam 4 mg/L	Avibactam 4 mg/L
Combined Collection (n=309)					
DBO alone	**-**	**12.6**	**35.0**	**49.2**	**1.0**
Meropenem, 1 mg/L + DBO	1.0	35.6	64.4	71.2	3.6
Meropenem, 2 mg/L + DBO	1.6	40.1	70.2	75.1	11.3
Meropenem, 4 mg/L + DBO	7.4	47.2	74.8	80.3	15.5
Meropenem, 8 mg/L + DBO	19.1	53.7	80.9	84.8	27.5
Cefepime, 1 mg/L + DBO	0.6	34.3	69.6	76.7	5.5
Cefepime, 2 mg/L + DBO	1.6	39.5	74.8	80.6	12.6
Cefepime, 4 mg/L+ DBO	4.5	41.7	79.6	83.8	17.2
Cefepime, 8 mg/L + DBO	9.4	47.2	85.4	90.0	22.0
Aztreonam, 1 mg/L + DBO	23.9	86.4	97.7	98.7	92.2
Aztreonam, 2 mg/L + DBO	29.1	91.9	98.4	98.7	94.5
Aztreonam, 4 mg/L+ DBO	31.4	95.8	99.0	99.7	96.4
Aztreonam, 8mg/L + DBO	33.7	96.8	99.0	99.7	98.1
Consecutive Collection (n=210)					
DBO alone	**-**	**14.8**	**35.7**	**50.0**	**1.4**
Meropenem, 1 mg/L + DBO	0.5	35.7	66.2	73.8	4.3
Meropenem, 2 mg/L + DBO	1.0	40.5	73.8	78.1	11.9
Meropenem, 4 mg/L + DBO	3.8	45.7	78.6	83.8	15.7
Meropenem, 8 mg/L + DBO	11.4	52.9	84.3	87.1	24.8
Cefepime, 1 mg/L + DBO	0.0	34.3	71.4	80.5	6.2

Cefepime, 2 mg/L + DBO	0.5	39.0	77.6	85.2	12.4
Cefepime, 4 mg/L + DBO	2.4	41.9	83.8	88.1	17.1
Cefepime, 8 mg/L + DBO	6.2	47.1	90.0	93.3	22.4
Aztreonam, 1 mg/L + DBO	16.7	85.7	98.6	99.5	91.4
Aztreonam, 2 mg/L + DBO	19.5	91.0	99.0	99.5	94.3
Aztreonam, 4 mg/L + DBO	22.9	95.2	99.5	99.5	96.7
Aztreonam, 8mg/L + DBO	25.2	96.2	99.5	99.5	99.5
All NDM *Klebsiella* (n=104)					
DBO alone	**-**	**3.8**	**15.4**	**26.0**	**0.0**
Meropenem, 1 mg/L + DBO	0.0	10.6	44.2	57.7	0.0
Meropenem, 2 mg/L + DBO	0.0	12.5	53.8	62.5	0.0
Meropenem, 4 mg/L + DBO	0.0	16.3	62.5	71.2	1.0
Meropenem, 8 mg/L + DBO	0.0	23.1	74.0	79.8	1.0
Cefepime, 1 mg/L + DBO	0.0	10.6	52.9	62.5	1.0
Cefepime, 2 mg/L + DBO	0.0	13.5	59.6	70.2	1.0
Cefepime, 4 mg/L + DBO	0.0	16.3	73.1	76.9	1.0
Cefepime, 8 mg/L + DBO	0.0	20.2	82.7	88.5	1.0
Aztreonam, 1 mg/L + DBO	12.5	85.6	100.0	100.0	100.0
Aztreonam, 2 mg/L + DBO	12.5	90.4	100.0	100.0	100.0
Aztreonam, 4 mg/L + DBO	12.5	96.2	100.0	100.0	100.0
Aztreonam, 8 mg/L + DBO	15.4	96.2	100.0	100.0	100.0

422

423 **Table 4.** Performance of DBO combinations against MBL producers highly resistant to nacubactam

	No. isolates with MIC of:											
	Among all isolates with nacubactam MIC >32 mg/L (n=110)									Among isolates with nacubactam MIC >32 mg/L and cefepime and meropenem MICs ≥128 mg/L (n=57)		
MIC mg/L	MEM	MEM/NAC 4 mg/L	MEM /AVI 4 mg/L	CPM	CPM/NAC 4 mg/L	CPM/AVI 4 mg/L	AZT	AZT/NAC 4 mg/L	AZT/AVI 4 mg/L	MEM/NAC 4 mg/L	CPM/NAC 4 mg/L	AZT/ NAC 4 mg/L
<=0.03		2			5		1	74	7	2	2	34
0.06		5			3		4	20	13	2	1	13
0.125		7			12		6	10	41	2	3	5
0.25		3			9		9	3	25		3	2
0.5		2			5		2	1	19	1	2	1
1		7			10	2	3		2	2	2	
2		12	5		10	9	4		1	4	4	
4	5	16	3	3	10	5	3	2		4	7	2
8	10	14	17	1	19	1				8	12	

16	10	11	5	3	10	6	3		2	3	4	
32	8	8	11	9	5	4	1			6	5	
64	20	7	24	9	5	11	12			7	5	
128	28	12	21	13	1	20	16			12	1	
>128	29	4	24	72	6	52	46			4	6	
Proportion (%) susceptible based upon prospective β-lactam breakpoint of:												
1 mg/L	0.0	23.6	0.0	0.0	40.0	1.8	22.7	98.2	97.3	15.8	22.8	96.5
2 mg/L	0.0	34.5	4.5	0.0	49.1	10.0	26.4	98.2	98.2	22.8	29.8	96.5
4 mg/L	4.5	49.1	7.3	2.7	58.2	14.5	29.1	100.0	98.2	29.8	42.1	100.0
8 mg/L	13.6	61.8	22.7	3.6	75.5	15.5	29.1	100.0	98.2	43.9	63.2	100.0

Abbreviations: AVI, avibactam; AZT, aztreonam; CPM, cefepime, MEM, meropenem; NAC, nacubactam

Table 5. MIC distributions of aztreonam alone and in combination against aztreonam-susceptible (MIC ≤2 mg/L), nacubactam-resistant (MIC >32 mg/L) MBL producers

	No isolates with indicated aztreonam MIC (mg/L), in the presence of:				
MIC (mg/L)	No DBO	Nacubactam 1 mg/L	Nacubactam 2 mg/L	Nacubactam 4 mg/L	Avibactam 4 mg/L
≤0.03	1	13	23	24	4
0.06	4	11	4	3	9
0.125	6	2	1	1	10
0.25	9	3	1	1	4
0.5	2				2
1	3				
2	4				