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In Vitro Activity of Nacubactam in Combination with Aztreonam or Cefepime against Beta-lactamase-positive Enterobacterales Isolates

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INTRODUCTION

Nacubactam (OP0595) is a novel dual-action diazabicyclooctane with beta-lactamase inhibition and direct antibacterial properties. The present study reports the susceptibility of beta-lactamase-positive *Enterobacterales* to nacubactam alone and as a 1:1 fixed ratio combination with aztreonam or cefepime.

MATERIALS & METHODS

A set of 375 clinical isolates from various worldwide locations collected between 2016 and 2018 (Figure 1) representing a range of genotypes including extended-spectrum beta-lactamases (ESBL), metallo beta-lactamases (MBL), AmpCs, OXA carbapenemases and KPC carbapenemases were evaluated (Table 1). Genotypes were determined by multiplex PCR, as described previously [1]. MIC determinations were performed by broth microdilution [2] and interpretative categories were applied using EUCAST guidelines, where breakpoints were available [3].

Table 1. Breakdown of beta-lactamase by species tested

Category	β-lactamase	Species: *Enterobacter* spp.	*Escherichia coli*	*Klebsiella pneumoniae*	*Morganella morganii*	ALL
ESBL (n=110)	CTX-M-1		5			5
	CTX-M-12			2		2
	CTX-M-134		1			1
	CTX-M-14		5			5
	CTX-M-15	5		5		15
	CTX-M-189		1			1
	CTX-M-2		5	1		6
	CTX-M-218		1			1
	CTX-M-22		1			1
	CTX-M-223		1			1
	CTX-M-24		5			5
	CTX-M-27		5	5		10
	CTX-M-3		5	1		6
	CTX-M-55		5			5
	CTX-M-65		4			4
	CTX-M-71		1			1
	CTX-M-8		5			5
	CTX-M-82		1			1
	CTX-M-9		1			1
	SHV-12	3	5	5		13
	SHV-18			1		1
	SHV-2		1	5		6
	SHV-2A			5		5
	SHV-31			2		2
	SHV-38			1		1
	SHV-7			2		2
	TEM-10		1			1
	TEM-19		1			1
	TEM-52		1			1
	VEB-19		1			1
MBL (n=42)	IMP-4	3		2		5
	IMP-8	2	1			3
	NDM-1	3		9		12
	NDM-5		3			3
	NDM-7		4	3		7
	NDM-9			1		1
	NDM-TYPE	1	1			2
	VIM-1	2		5		7
	VIM-4	1				1
	VIM-57		1			1
AmpC (n=104)	CMY-140		1			1
	CMY-141		1	1		2
	CMY-142		1			1
	CMY-145		3			3
	CMY-146		4			4
	CMY-148		3			3
	CMY-153		1			1
	CMY-16			2		2
	CMY-161		1			1
	CMY-163		1			1
	CMY-165		1			1
	CMY-2		11	6		17
	CMY-2 + DHA-1		1	2		3
	CMY-4		4	5		9
	CMY-42		11			11
	CMY-42 + DHA-1		2			2
	CMY-48		1			1
	CMY-6			1		1
	CMY-TYPE		10			10
	DHA-1	3	5	15		23
	DHA-4				1	1
	FOX-5			6		6
OXA (n=54)	OXA-163			2		2
	OXA-181	1	5	8		14
	OXA-232		4	6		10
	OXA-244		1	1		2
	OXA-48	5	8	13		26
KPC (n=65)	KPC-2	7	10	15		32
	KPC-3	2	8	18		28
	KPC-31			1		1
	KPC-4			1		1
	KPC-46			1		1
	KPC-5	1				1
	KPC-TYPE			1		1
	Grand Total	**39**	**175**	**160**	**1**	**375**

RESULTS

Table 2. Summary MIC and susceptibility data for nacubactam combinations and comparators against all *Enterobacterales* and separate β-lactamase category

Category	Drug	MIC 50	MIC 90	MIN	MAX	EUCAST Breakpoints (S|I|R)*	% S	% I	% R
All isolates (n=375)	Aztreonam	16	>64	≤0.06	>64	≤1 | 2-4 | >4	25.3	10.4	64.3
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**≤0.015**	**8**	-	-	-	-
	Cefepime	4	>64	0.03	>64	≤1 | 2-4 | >4	33.6	18.1	48.3
	Cefepime/nacubactam (1:1 ratio)	**0.5**	**2**	**0.03**	**32**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	0.5	>64	≤0.015	>64	≤8 | - | >8	87.7	-	12.3
	Meropenem/vaborbactam [fixed 8 mg/L]	0.06	32	0.03	>32	≤8 | - | >8	88.5	-	11.5
	Nacubactam	**1**	**4**	**0.5**	**>32**	-	-	-	-
ESBL (n=110)	Aztreonam	32	>64	0.25	>64	≤1 | 2-4 | >4	7.3	16.4	76.4
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**0.06**	**4**	-	-	-	-
	Cefepime	8	>64	0.03	>64	≤1 | 2-4 | >4	16.4	25.5	58.2
	Cefepime/nacubactam (1:1 ratio)	**0.5**	**2**	**0.03**	**4**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	0.5	2	≤0.015	8	≤8 | - | >8	100.0	-	0.0
	Meropenem/vaborbactam [fixed 8 mg/L]	0.06	0.12	0.03	4	≤8 | - | >8	100.0	-	0.0
	Nacubactam	**1**	**2**	**0.5**	**>32**	-	-	-	-
MBL (n=42)	Aztreonam	0.25	8	≤0.06	64	≤1 | 2-4 | >4	76.2	11.9	11.9
	Aztreonam/nacubactam (1:1 ratio)	**0.25**	**1**	**≤0.015**	**2**	-	-	-	-
	Cefepime	64	>64	0.25	>64	≤1 | 2-4 | >4	2.4	4.8	92.9
	Cefepime/nacubactam (1:1 ratio)	**2**	**8**	**0.12**	**32**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	>64	>64	≤0.015	>64	≤8 | - | >8	4.8	-	95.2
	Meropenem/vaborbactam [fixed 8 mg/L]	>32	>32	0.06	>32	≤8 | - | >8	31.0	-	69.0
	Nacubactam	**2**	**8**	**1**	**>32**	-	-	-	-
AmpC (n=104)	Aztreonam	16	>64	≤0.06	>64	≤1 | 2-4 | >4	7.7	14.4	77.9
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**2**	**0.03**	**8**	-	-	-	-
	Cefepime	1	16	0.03	>64	≤1 | 2-4 | >4	61.5	17.3	21.2
	Cefepime/nacubactam (1:1 ratio)	**0.25**	**2**	**0.03**	**4**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	0.5	8	≤0.015	>64	≤8 | - | >8	97.1	-	2.9
	Meropenem/vaborbactam [fixed 8 mg/L]	0.06	0.12	0.03	>32	≤8 | - | >8	98.1	-	1.9
	Nacubactam	**2**	**4**	**0.5**	**>32**	-	-	-	-
OXA (n=54)	Aztreonam	0.12	8	≤0.06	32	≤1 | 2-4 | >4	87.0	1.9	11.1
	Aztreonam/nacubactam (1:1 ratio)	**0.12**	**0.5**	**≤0.015**	**1**	-	-	-	-
	Cefepime	0.5	8	0.06	16	≤1 | 2-4 | >4	75.9	13.0	11.1
	Cefepime/nacubactam (1:1 ratio)	**0.25**	**2**	**0.06**	**2**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	0.5	1	0.06	2	≤8 | - | >8	100.0	-	0.0
	Meropenem/vaborbactam [fixed 8 mg/L]	1	>32	0.12	>32	≤8 | - | >8	81.5	-	18.5
	Nacubactam	**2**	**4**	**1**	**>32**	-	-	-	-
KPC (n=65)	Aztreonam	>64	>64	8	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Aztreonam/nacubactam (1:1 ratio)	**1**	**2**	**0.25**	**2**	-	-	-	-
	Cefepime	32	>64	1	>64	≤1 | 2-4 | >4	3.1	20.0	76.9
	Cefepime/nacubactam (1:1 ratio)	**1**	**2**	**0.12**	**4**	-	-	-	-
	Ceftazidime/avibactam [fixed 4 mg/L]	1	4	≤0.015	64	≤8 | - | >8	95.4	-	4.6
	Meropenem/vaborbactam [fixed 8 mg/L]	0.06	2	0.03	>32	≤8 | - | >8	96.9	-	3.1
	Nacubactam	**2**	**2**	**0.5**	**16**	-	-	-	-

*S, susceptible; I, intermediate; R, resistant

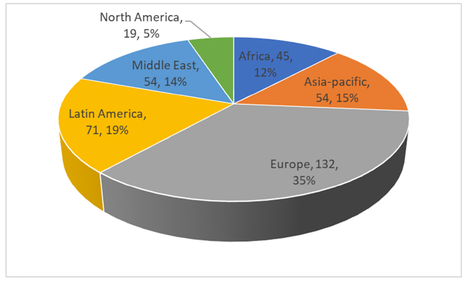

Figure 1. Geographical distribution of the isolates tested

Table 3. Summary MIC and susceptibility data for aztreonam or cefepime combinations with nacubactam by genotype & species

Organism/ Category	Drug	MIC 50	MIC 90	MIN	MAX	EUCAST Breakpoints (S|I|R)*	% S	% I	% R
Enterobacter spp. MBL (n=12)	Aztreonam	2	64	≤0.06	64	≤1 | 2-4 | >4	41.7	16.7	41.7
	Aztreonam/nacubactam (1:1 ratio)	**0.25**	**0.5**	**0.06**	**1**	-	-	-	-
	Cefepime	32	>64	0.25	>64	≤1 | 2-4 | >4	8.3	8.3	83.3
	Cefepime/nacubactam (1:1 ratio)	2	4	0.12	4	-	-	-	-
	Nacubactam	**1**	**2**	**1**	**2**	-	-	-	-
Enterobacter spp. KPC (n=10)	Aztreonam	>64	>64	64	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Aztreonam/nacubactam (1:1 ratio)	**1**	**1**	**0.5**	**2**	-	-	-	-
	Cefepime	16	>64	8	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Cefepime/nacubactam (1:1 ratio)	**1**	**2**	**0.5**	**2**	-	-	-	-
	Nacubactam	**2**	**2**	**1**	**2**	-	-	-	-
E. coli ESBL (n=67)	Aztreonam	32	>64	0.25	>64	≤1 | 2-4 | >4	4.5	19.4	76.1
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**0.06**	**4**	-	-	-	-
	Cefepime	8	>64	0.03	>64	≤1 | 2-4 | >4	16.4	25.4	58.2
	Cefepime/nacubactam (1:1 ratio)	0.5	2	0.03	4	-	-	-	-
	Nacubactam	**1**	**2**	**0.5**	**8**	-	-	-	-
E. coli MBL (n=10)	Aztreonam	0.25	4	≤0.06	4	≤1 | 2-4 | >4	70.0	30.0	0.0
	Aztreonam/nacubactam (1:1 ratio)	**0.12**	**1**	**≤0.015**	**2**	-	-	-	-
	Cefepime	>64	>64	32	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Cefepime/nacubactam (1:1 ratio)	**2**	**4**	**2**	**4**	-	-	-	-
	Nacubactam	**1**	**2**	**1**	**4**	-	-	-	-
E. coli AmpC (n=62)	Aztreonam	32	>64	≤0.06	>64	≤1 | 2-4 | >4	6.5	9.7	83.9
	Aztreonam/nacubactam (1:1 ratio)	**1**	**4**	**0.03**	**8**	-	-	-	-
	Cefepime	1	16	0.03	32	≤1 | 2-4 | >4	50.0	19.4	30.6
	Cefepime/nacubactam (1:1 ratio)	0.25	2	0.03	4	-	-	-	-
	Nacubactam	**1**	**4**	**0.5**	**>32**	-	-	-	-
E. coli OXA (n=18)	Aztreonam	0.12	0.5	≤0.06	1	≤1 | 2-4 | >4	100.0	0.0	0.0
	Aztreonam/nacubactam (1:1 ratio)	**0.12**	**0.25**	**0.06**	**0.25**	-	-	-	-
	Cefepime	0.25	0.5	0.12	1	≤1 | 2-4 | >4	100.0	0.0	0.0
	Cefepime/nacubactam (1:1 ratio)	**0.12**	**0.5**	**0.12**	**0.5**	-	-	-	-
	Nacubactam	**1**	**2**	**1**	**2**	-	-	-	-
E. coli KPC (n=18)	Aztreonam	>64	>64	8	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**0.25**	**1**	-	-	-	-
	Cefepime	4	>64	1	>64	≤1 | 2-4 | >4	11.1	38.9	50.0
	Cefepime/nacubactam (1:1 ratio)	0.25	1	0.12	2	-	-	-	-
	Nacubactam	**1**	**2**	**0.5**	**2**	-	-	-	-
K. pneumoniae ESBL (n=35)	Aztreonam	32	>64	0.25	>64	≤1 | 2-4 | >4	14.3	14.3	71.4
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**0.12**	**2**	-	-	-	-
	Cefepime	8	>64	0.25	>64	≤1 | 2-4 | >4	17.1	28.6	54.3
	Cefepime/nacubactam (1:1 ratio)	**0.5**	**1**	**0.12**	**2**	-	-	-	-
	Nacubactam	**2**	**2**	**1**	**>32**	-	-	-	-
K. pneumoniae MBL (n=20)	Aztreonam	0.12	0.5	≤0.06	0.5	≤1 | 2-4 | >4	100.0	0.0	0.0
	Aztreonam/nacubactam (1:1 ratio)	**0.12**	**0.25**	**0.06**	**0.5**	-	-	-	-
	Cefepime	64	>64	4	>64	≤1 | 2-4 | >4	0.0	5.0	95.0
	Cefepime/nacubactam (1:1 ratio)	4	16	2	32	-	-	-	-
	Nacubactam	**2**	**8**	**1**	**>32**	-	-	-	-
K. pneumoniae AmpC (n=38)	Aztreonam	8	64	1	>64	≤1 | 2-4 | >4	7.9	23.7	68.4
	Aztreonam/nacubactam (1:1 ratio)	**0.5**	**1**	**0.12**	**2**	-	-	-	-
	Cefepime	0.5	4	0.03	>64	≤1 | 2-4 | >4	76.3	15.8	7.9
	Cefepime/nacubactam (1:1 ratio)	**0.25**	**2**	**0.03**	**4**	-	-	-	-
	Nacubactam	**2**	**4**	**0.5**	**>32**	-	-	-	-
K. pneumoniae OXA (n=30)	Aztreonam	0.25	1	≤0.06	8	≤1 | 2-4 | >4	90.0	3.3	6.7
	Aztreonam/nacubactam (1:1 ratio)	**0.12**	**0.5**	**≤0.015**	**1**	-	-	-	-
	Cefepime	0.5	8	0.06	16	≤1 | 2-4 | >4	63.3	16.7	20.0
	Cefepime/nacubactam (1:1 ratio)	0.25	2	0.06	2	-	-	-	-
	Nacubactam	**2**	**8**	**1**	**>32**	-	-	-	-
K. pneumoniae KPC (n=37)	Aztreonam	>64	>64	8	>64	≤1 | 2-4 | >4	0.0	0.0	100.0
	Aztreonam/nacubactam (1:1 ratio)	**1**	**2**	**0.5**	**2**	-	-	-	-
	Cefepime	>64	>64	2	>64	≤1 | 2-4 | >4	0.0	16.2	83.8
	Cefepime/nacubactam (1:1 ratio)	**1**	**4**	**0.25**	**4**	-	-	-	-
	Nacubactam	**2**	**4**	**1**	**16**	-	-	-	-

*S, susceptible; I, intermediate; R, resistant

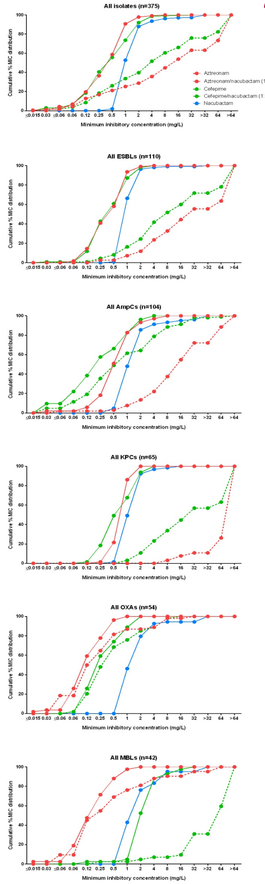

Figure 2. Cumulative MIC distribution for aztreonam, cefepime, nacubactam, aztreonam/nacubactam (1:1 ratio) and cefepime/nacubactam (1:1 ratio) against all *Enterobacterales* combined and separate β-lactamase categories.

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RESULTS SUMMARY

- The collection of 375 isolates as a whole were highly resistant to aztreonam and cefepime (Table 2), but the addition of nacubactam lowered the MIC distribution of both agents (Table 2 & Figure 2).
- This beneficial effect of nacubactam was also observed when isolates from each individual β-lactamase category were analysed (Table 2 & Figure 2).
- Nacubactam alone also had activity, which was consistent against the separate β-lactamase categories (Table 2 & Figure 2).
- Approximately 12% of all the isolates were resistant to ceftazidime/avibactam and meropenem/vaborbactam and both beta-lactamase inhibitor combinations had higher MIC_{90} values than aztreonam/nacubactam and cefepime/nacubactam (Table 2).
- As expected, ceftazidime/avibactam and meropenem/vaborbactam were poorly active against the MBL isolates.
- Aztreonam alone showed good activity against MBL and OXA isolates which was further enhanced by the addition of nacubactam (Table 2 & Figure 2) reducing MIC_{90} from 8 mg/L to 1 or 0.5 mg/L, respectively (Table 2).
- Similarly, cefepime was active against OXA isolates but activity improved with the addition of nacubactam reducing MIC_{90} from 8 mg/L to 2 mg/L (Table 2)
- The activity of aztreonam/nacubactam and cefepime/nacubactam was consistent against all individual species tested (Table 3).

CONCLUSIONS

- The addition of nacubactam protected the activity of cefepime against ESBL-, MBL-, AmpC-, OXA- and KPC-producing *Enterobacterales*.
- Nacubactam was also able to restore the activity of aztreonam against ESBL-, AmpC-, and KPC-producing *Enterobacterales* and enhance the activity of aztreonam against MBL- and OXA-producing *Enterobacterales* due to the direct action of nacubactam.
- These data support the further development of nacubactam for clinical use.

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