

High-potency of the novel therapeutic combination meropenem/pilabactam (formerly meropenem/ANT3310) against KPC β -lactamase variants driving resistance to ceftazidime/avibactam

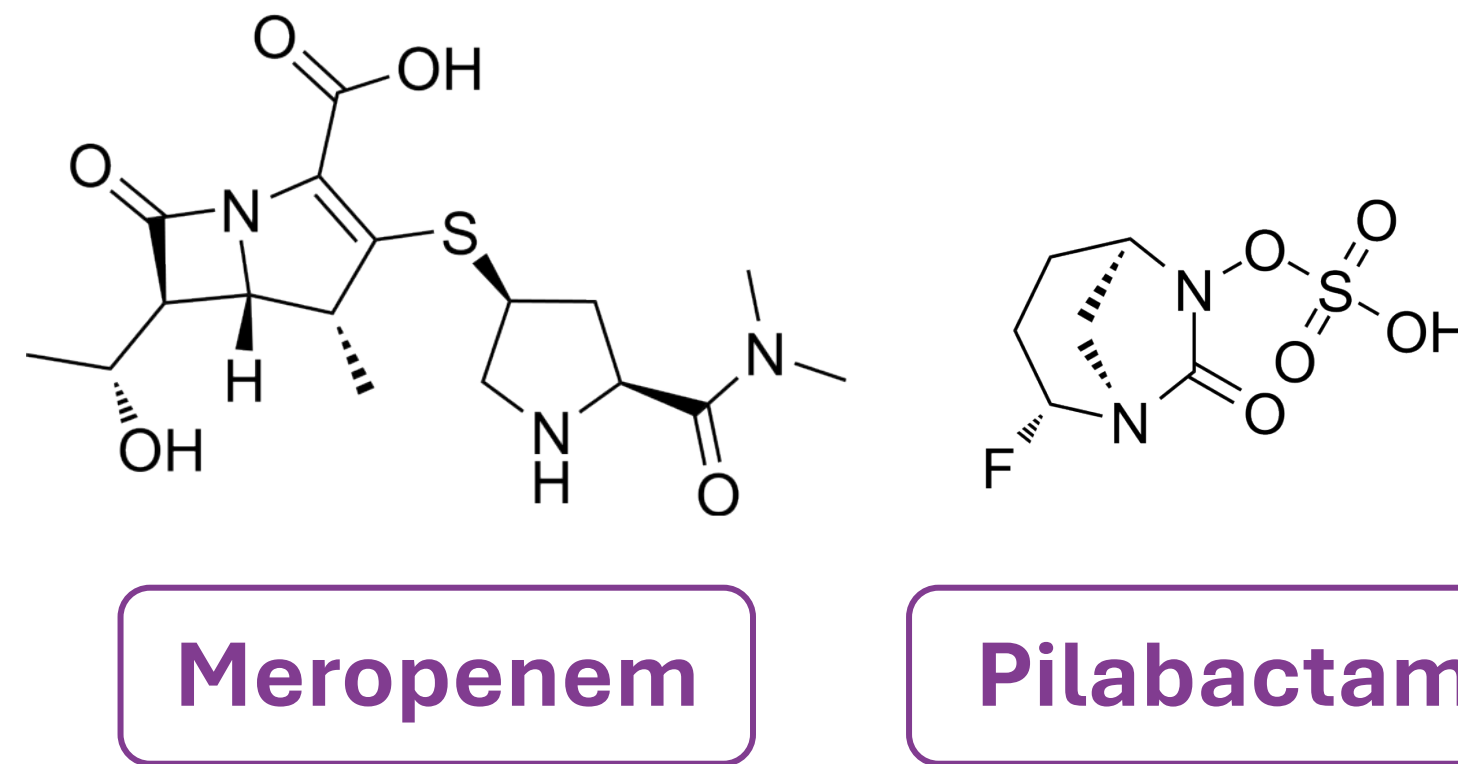
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Background

Pilabactam (formerly ANT3310) is a newly developed diazabicyclooctane (DBO) β -lactamase inhibitor in clinical trials with meropenem. It exhibits **potent activity against serine- β -lactamases, including KPC-type enzymes**. KPC variants associated with resistance to novel therapeutic combinations such as ceftazidime/avibactam are becoming a growing concern.



In this study, we evaluate the **activity of MEM/PIL** and other recently approved β -lactam/ β -lactamase inhibitor combinations against Enterobacterales strains producing **ceftazidime/avibactam-resistant KPC variants**.

Methods

- To evaluate the **activity of MEM/PIL and comparator combinations** (ceftazidime/avibactam, aztreonam/avibactam, meropenem/vaborbactam, and imipenem/relebactam), we engineered an **isogenic panel of 40 recombinant *Escherichia coli*** strains producing KPC-2, KPC-3, and 18 KPC variants (with **amino acid alterations in the Ω -loop, the loop 237-243, and the loop 266-275**) under wild-type permeability (*E. coli* TG1) and porin-deficient (*E. coli* HB4) backgrounds.
- The activity of MEM/PIL and comparators was further validated against a panel of **24 non-duplicate *Klebsiella pneumoniae* clinical isolates producing KPC-2, KPC-3 and 15 KPC variants** with substitutions within the same regions.
- Broth microdilution MICs** were performed and interpreted according to **EUCAST guidelines**. Inhibitors were tested at fixed concentrations (4 mg/L for avibactam and relebactam, and 8 mg/L for pilabactam and vaborbactam).

Results

TABLE 1. Antibiotic susceptibility data of β -lactams and β -lactam/ β -lactamase inhibitor combinations against isogenic *E. coli* TG1 and HB4 transformants expressing KPC-variants.

KPC β -lactamase	Amino acid alteration ^a	Location in sequence ^b	MIC (mg/L) ^c															
			<i>E. coli</i> TG1								<i>E. coli</i> HB4							
			CAZ (R>4)	C/A (R>8)	A/A (R>4)	IMP (R>4)	I/R (R>2)	MEM (R>8)	M/V (R>8)	M/P (R>8)	CAZ (R>4)	C/A (R>8)	A/A (R>4)	IMP (R>4)	I/R (R>2)	MEM (R>8)	M/V (R>8)	M/P (R>8)
<i>E. coli</i> TG1	-	-	0.25	≤ 0.06	≤ 0.06	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	-	-	-	-	-	-	-	
<i>E. coli</i> HB4	-	-	-	-	-	-	-	-	-	1	0.25	0.125	0.125	0.125	≤ 0.06	≤ 0.06		
KPC-2	-	-	64	0.5	0.25	8	≤ 0.06	4	≤ 0.06	≤ 0.06	512	4	0.25	8	≤ 0.06	4	≤ 0.06	
KPC-3	-	-	128	0.5	0.125	2	≤ 0.06	2	≤ 0.06	≤ 0.06	2048	16	0.25	2	≤ 0.06	2	≤ 0.06	
KPC-8	V240G	Loop 237-243	2048	8	0.25	8	≤ 0.06	4	≤ 0.06	≤ 0.06	>2048	128	2	8	≤ 0.06	4	≤ 0.06	
KPC-23	V240A	Loop 237-243	2048	2	≤ 0.06	8	0.125	8	≤ 0.06	≤ 0.06	>2048	32	1	32	0.125	64	0.5	
KPC-28	del ₂₄₂₋₂₄₃ _GT	Loop 237-243	512	32	0.5	0.125	0.125	0.125	≤ 0.06	≤ 0.06	>2048	256	2	0.125	0.125	0.125	≤ 0.06	
KPC-31	D179Y	Ω -loop	512	32	0.125	0.125	≤ 0.06	0.125	≤ 0.06	≤ 0.06	2048	256	0.5	0.125	≤ 0.06	0.125	≤ 0.06	
KPC-35	L169P	Ω -loop	128	4	≤ 0.06	0.125	0.125	0.125	≤ 0.06	≤ 0.06	1024	16	0.25	0.125	0.125	1	≤ 0.06	
KPC-39	A172T	Ω -loop	32	8	≤ 0.06	1	0.125	0.25	≤ 0.06	≤ 0.06	256	16	0.25	2	0.125	4	0.125	
KPC-47	A172T, T242A	Ω -loop and loop 237-243	256	16	≤ 0.06	1	0.125	0.5	≤ 0.06	≤ 0.06	>2048	256	0.5	8	0.125	32	0.25	
KPC-48	L169P, A172T	Ω -loop	256	64	0.125	0.125	0.125	0.125	≤ 0.06	≤ 0.06	2048	128	0.5	0.125	≤ 0.06	1	0.25	
KPC-66	del ₁₆₈₋₁₆₉ _EL	Ω -loop	256	8	≤ 0.06	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	2048	64	0.25	0.125	0.125	1	0.125	
KPC-85	A172V	Ω -loop	8	8	≤ 0.06	1	0.125	0.125	≤ 0.06	≤ 0.06	64	2	≤ 0.06	1	≤ 0.06	1	≤ 0.06	
KPC-94	L169H, del ₁₇₀ _N	Ω -loop	1024	128	2	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	>2048	1024	>64	0.125	0.125	0.5	0.125	
KPC-95	A172T, D179Y	Ω -loop	256	32	≤ 0.06	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	2048	64	0.25	≤ 0.06	≤ 0.06	0.5	0.125	
KPC-132	ins ₂₇₀ _KDDKSRAPN	Loop 266-275	256	16	0.25	1	0.125	0.125	≤ 0.06	≤ 0.06	2048	256	1	4	0.25	8	0.25	
KPC-148	ins ₂₇₅ _EAVYTRAPNKDDKYS	Loop 266-275	128	32	0.25	1	0.125	0.5	≤ 0.06	≤ 0.06	1024	256	1	4	0.25	32	0.25	
KPC-178	P174L	Ω -loop	128	8	≤ 0.06	2	0.125	0.5	≤ 0.06	≤ 0.06	256	16	0.25	2	0.125	8	0.125	
-	KPC-2 L169A	Ω -loop	16	0.5	≤ 0.06	0.5	0.125	0.125	≤ 0.06	≤ 0.06	128	16	0.125	4	0.125	16	0.25	
-	KPC-2 L169T	Ω -loop	32	0.5	≤ 0.06	1	0.125	0.25	≤ 0.06	≤ 0.06	256	16	0.25	4	0.125	16	≤ 0.06	
-	KPC-3 D179N	Ω -loop	64	1	≤ 0.06	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	256	8	0.125	0.125	≤ 0.06	≤ 0.06	≤ 0.06	

CAZ, ceftazidime; C/A, ceftazidime/avibactam; A/A, aztreonam/avibactam; IMP, imipenem; I/R, imipenem/relebactam; MEM, meropenem; M/V, meropenem/vaborbactam; M/P, pilabactam.
^a del, deletion of amino acids; ins, insertion of amino acids.
^b Location in KPC loops previously indicated by Hobson C. A. *et al.* AAC 2022. NA, not applicable.
^c EUCAST breakpoints indicated for Enterobacterales. Clinical breakpoint for meropenem/pilabactam was interpreted using that of meropenem/vaborbactam.

TABLE 2. Detailed phenotypic and genotypic features of clinical isolates carrying different KPC variants.

Strain	ST	KPC β -lactamase	MIC (mg/L) ^a								Porin genotype ^b	
			CAZ (R>4)	C/A (R>8)	A/A (R>4)	IMP (R>4)	I/R (R>2)	MEM (R>2)	M/V (R>8)	M/P (R>2)	OmpK35	OmpK36
ARGA00246	307	KPC-2	128	1	0.25	8	≤ 0.06	8	≤ 0.06	≤ 0.06	-	-
ARGA00247	512	KPC-3	1024	4	0.25	16	0.25	16	≤ 0.06	≤ 0.06	C66fs	-
HUPM-3	512	KPC-8	2048	16	1	32	0.125	64	1	0.25	E89*	ins ₁₁₄ _GD
HUPM-36	512	KPC-8	2048	8	0.5	4	≤ 0.06	4	≤ 0.06	0.125	E89*	-
20220166	512	KPC-23	1024	16	0.25	4	≤ 0.06	4	≤ 0.06	≤ 0.06	C66fs	-
231992	512	KPC-28	2048	128	4	0.125	≤ 0.06	1	0.5	0.25	C66fs	ins ₁₁₄ _GD
20211109	512	KPC-31	2048	128	0.5	0.125	0.125	2	1	0.25	C66fs	-
966	512	KPC-31	2048	256	1	≤ 0.06	≤ 0.06	4	2	0.5	C66fs	ins ₁₁₄ _GD
175871	512	KPC-31	2048	512	2	0.125	≤ 0.06	4	2	1	C66fs	ins ₁₁₄ _GD
183483	512	KPC-31	2048	256	1	≤ 0.06	≤ 0.06	2	1	0.25	C66fs	ins ₁₁₄ _GD
196226	512	KPC-31	2048	128	1	≤ 0.06	≤ 0.06	4	1	0.5	C66fs	ins ₁₁₄ _GD
KPLA	258	KPC-35	1024	32	0.25	0.25	0.25	1	0.5	0.125	C66fs	ins ₁₁₄ _GD
183111	512	KPC-39	1024	64	1	16	0.25	16	2	0.25	C66fs	ins ₁₁₄ _GD
183860	512	KPC-47	1024	64	1	16	0.5	32	0.5	0.25	C66fs	ins ₁₁₄ _GD
183358	512	KPC-48	2048	128	2	4	0.5	8	1	0.5	C66fs	ins ₁₁₄ _GD
20220294	512	KPC-66	1024	32	0.125	0.25	0.25	4	1	0.25	C66fs	ins ₁₁₄ _GD
202826	512	KPC-85	1024	32	0.5	32	0.125	64	1	0.25	C66fs	ins ₁₁₄ _GD
171513	512	KPC-94	2048	512	64	0.125	0.125	1	1	0.25	C66fs	ins ₁₁₄ _GD
180510	512	KPC-95	2048	512	4	8	0.5	32	1	1	C66fs	ins ₁₁₄ _GD
20220366	512	KPC-132	1024	64	0.25	4	0.25	16	2	0.5	C66fs	ins ₁₁₄ _GD
213842	512	KPC-132	1024	64	1	8	0.25	16	1	1	C66fs	ins ₁₁₄ _GD
214802	512	KPC-132	1024	64	1	4	0.125	16	0.5	0.5	C66fs	ins ₁₁₄ _GD
196228	512	KPC-148	1024	128	1	8	0.125	32	1	0.5	C66fs	ins ₁₁₄ _GD
231595	512	KPC-178	512	32	0.5	16	0.125	32	0.5	0.25	C66fs	ins ₁₁₄ _GD

CAZ, ceftazidime; C/A, ceftazidime/avibactam; IMP, imipenem; I/R, imipenem/relebactam; MEM, meropenem; M/V, meropenem/vaborbactam; M/P, pilabactam.
^a EUCAST breakpoints indicated for Enterobacterales. Clinical breakpoint for meropenem/pilabactam was interpreted using that of meropenem/vaborbactam.
^b fs, frameshift; ins, insertion of amino acids. *, premature stop codon.

MEM/PIL demonstrated potent activity against all strains and retained activity against all ceftazidime/avibactam-resistant KPC variants, including those with substitutions in the Ω -loop and surrounding loops.

In the **isogenic panel**, pilabactam reduced meropenem MICs to ≤ 0.06 mg/L irrespective of the permeability background and was **more active than all comparator combinations**, which showed MIC ranges in the porin-deficient *E. coli* HB4 background of 8-1024, 0.125-128, ≤ 0.06 -0.25 and ≤ 0.06 -0.5 mg/L for ceftazidime/avibactam, aztreonam/avibactam, imipenem/relebactam and meropenem/vaborbactam, respectively (TABLE 1).

Against **clinical isolates**, MEM/PIL demonstrated **outstanding activity**, with MIC_{50/90} of 0.25/1 mg/L, similar to imipenem/relebactam (MIC_{50/90}=0.125/0.5 mg/L) and superior to aztreonam/avibactam and meropenem/vaborbactam (MIC_{50/90}=1/4 and 1/2 mg/L, respectively) (TABLE 2).

Conclusions

- MEM/PIL consistently restored meropenem activity against KPC-producing Enterobacterales**, including ceftazidime/avibactam-resistant variants, and **outperformed or matched currently available β -lactam/ β -lactamase inhibitor combinations**, even in porin-deficient backgrounds.
- These findings support **further clinical development of MEM/PIL** to address emerging ceftazidime/avibactam resistance in KPC-producing Enterobacterales.