Synergistic effect of clinically available β-lactamases inhibitors on cefiderocol activity against carbapenemase-producing Gram-negative organisms

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Background

- Activity of cefiderocol has been investigated in large international surveillance studies, revealing promising results on the *in vitro* activity against meropenem-non-susceptible Gram-negative isolates [1].
- Based on the mechanism of cefiderocol, mutations affecting the iron transporter systems are associated with clinical resistance. Mutations in *piu*D and *pir*R, *pir*A and *piu*A, and *cir*A have been identified in *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* clinical isolates, respectively [2].
- However, the role of several β-lactamases in reduced susceptibility or resistance to cefiderocol has been supported by several recent reports [2].

The purpose of this study was to investigate the in vitro impact of clinically available β-lactamase inhibitors on cefiderocol activity against diverse carbapenemase-producing Gram-negative characterized strains.

Material and methods

- A collection of 44 well-characterized Gram-negative isolates obtained from various clinical sources and countries were included.
- Cefiderocol antimicrobial susceptibility was evaluated by reference broth microdilution using iron depleted cation adjusted Muller Hinton broth following EUCAST guidelines [3].
- The chequerboard microdilution panel method was used to determine the synergy of tazobactam, avibactam, vaborbactam and relebactam in combination with cefiderocol. Concentration of inhibitors was set at 4 mg/L and the final concentration of cefiderocol ranged from 0.007 to 16 mg/L. The fractional inhibitory concentration (FIC) was calculated with the formula: MIC of drug A or B in combination/MIC of drug A or B alone; the FIC index (FICI) was determined by summing the FICs of drugs A and B. According to the minimum FICI, the results of combination tests were interpreted as synergistic (FICI≤0.5), additive (FICI >0.5 and ≤1), indifferent ((FICI >1 and ≤4)), and antagonistic (>4) [4].
- Five strains were selected for subsequent time-kill curve (TKC) assays: two strains producing class A carbapenemases, one strain producing class B carbapenemase and two strains producing of class D carbapenemase.

Results

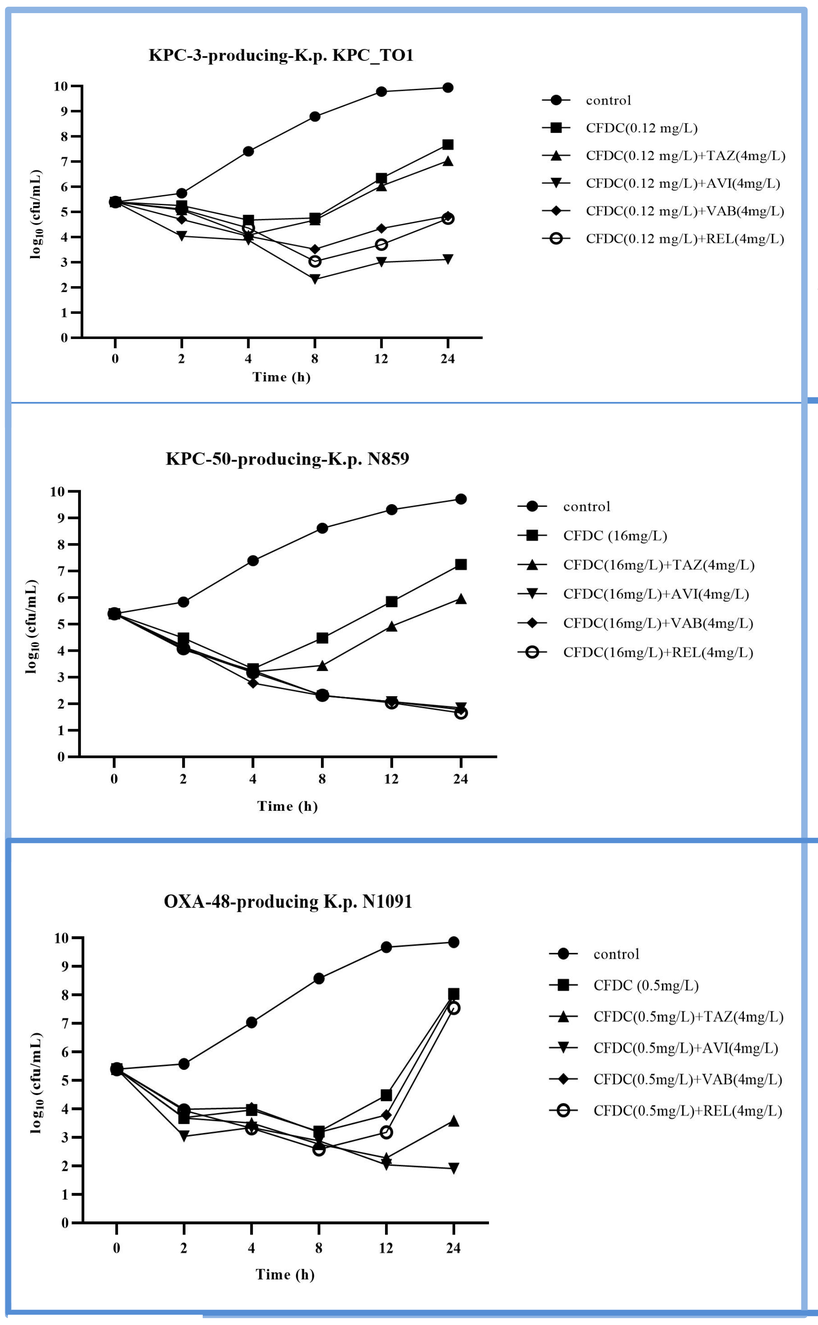
- Thirty-two out of the 44 strains were susceptible to cefiderocol (MICs range: 0.03-2 mg/L). Twelve strains were cefiderocol-resistant, of which five were KPC-producing *K. pneumoniae* (MICs range 4-16 mg/L), four were Enterobacterales NDM-producers (MICs range 4-16 mg/L), and three were *A. baumannii* NDM/ OXA-23-like or OXA-23-like producers (MICs range: 8-16 mg/L) (**Table 1**).
- Avibactam, vaborbactam and relebactam combined with cefiderocol had a synergistic effect on all KPC producers, regardless of other β-lactamases co-expressed (4- to 256-fold reduction of cefiderocol MICs) (**Table 1**).
- Synergistic effect of tazobactam was only observed on KPC-41-producing *K. pneumoniae* (N435), KPC-50-producing *K. pneumoniae* (N859), KPC-53-producing *K. pneumoniae* (CAZ59BO), KPC-66-producing *K. pneumoniae* (KPC_TO3) and KPC-31-producing *K. pneumoniae* (BOT-EMOKP and TO-BC1).
- Among the 12 metallo-β-lactamases producers, β-lactamase inhibitors combined with cefiderocol had a very low synergistic effect rate: FICI values < 0.5 were observed for combinations including avibactam, tazobactam or relebactam only on three strains co-expressing metallo-β-lactamases (NDM or VIM) and various other β-lactamases belonging to Ambler classes A, C and D (**Table 1**).
- No synergistic effect was observed for all OXA-carbapenemase producing *A. baumannii* strains, including those co-producers of NDM enzymes (**Table 1**).
- All β-lactamases inhibitors combined with cefiderocol showed synergistic effect on OXA-48-like-producing *E. coli* or *K. pneumoniae* (**Table 1**).

Table 1: FICIs of tazobactam, avibactam, vaborbactam and relebactam in combination with cefiderocol against carbapenemase-producing Gram-negative strains.

Strain	Species	Sequence typing	Carbapenemase gene	Other β-lactamases genes	MIC (mg/L)					FICI and interpretation			
					CFDC	CFDC+TAZ	CFDC+AVI	CFDC+VAB	CDFC+REL	CFDC+TAZ	CFDC+AVI	CFDC+VAB	CDFC+REL
Ambler class A													
BO318KP	*K. pneumoniae*	ST-512	bla_{KPC-3}	bla_{TEM-1}, bla_{SHV-11}	4	2	1	1	1	0.56	**0.25**	**0.31**	**0.31**
N1118	*K. pneumoniae*	-	bla_{KPC-2}	bla_{SHV-11}	0.12	0.12	<=0.007	0.015	0.015	1.06	**0.18**	**0.24**	**0.24**
N2350	*K. pneumoniae*	-	bla_{KPC-3}	bla_{SHV-11}, bla_{OXA-9}	2	2	<=0.007	0.25	0.125	1.06	**0.25**	**0.37**	**0.31**
CAZ156BO	*K. pneumoniae*	ST-101	bla_{KPC-3}	$bla_{SHV-156}$	1	0.5	0.125	0.06	0.125	0.56	**0.19**	**0.12**	**0.19**
BAT16KP	*K. pneumoniae*	ST-512	bla_{KPC-3}	bla_{TEM-1}, bla_{SHV-11}	1	1	0.06	0.25	0.06	1.03	**0.12**	**0.31**	**0.12**
BAT15KP	*K. pneumoniae*	ST-512	bla_{KPC-3}	bla_{TEM-1}, bla_{SHV-11}	1	1	0.12	0.25	0.12	1.06	**0.18**	**0.31**	**0.18**
KPC_TO5	*K. pneumoniae*	ST-512	bla_{KPC-3}	bla_{TEM-1A}, bla_{OXA-9}, bla_{SHV-11}	0.5	0.25	0.015	0.12	0.015	0.56	**0.09**	**0.3**	**0.09**
KPC_TO1	*K. pneumoniae*	ST-512	bla_{KPC-3}	bla_{TEM-1A}, bla_{OXA-9}, bla_{SHV-11}	0.12	0.06	<=0.007	0.015	0.015	0.56	**0.12**	**0.19**	**0.19**
KPB07	*K. pneumoniae*	ST-1519	bla_{KPC-3}	bla_{TEM-1}, bla_{OXA-9}, bla_{SHV-11}	0.25	0.25	0.015	0.06	0.06	1.06	**0.18**	**0.30**	**0.30**
KPB09	*K. pneumoniae*	ST-1519	bla_{KPC-3}	bla_{TEM-1}, bla_{OXA-9}, bla_{SHV-11}	0.125	0.125	<=0.007	0.015	0.015	1.06	**0.18**	**0.18**	**0.18**
KPB013	*K. pneumoniae*	ST-1519	bla_{KPC-3}	bla_{OXA-9}, bla_{SHV-11}	0.125	0.125	<=0.007	0.015	<=0.007	1.06	**0.18**	**0.18**	**0.12**
KPB02	*K. pneumoniae*	ST-1519	bla_{KPC-36}	bla_{TEM-1}, bla_{OXA-9}, bla_{SHV-11}	0.25	0.25	<=0.007	0.06	0.06	1.06	**0.28**	**0.30**	**0.30**
KPC_TO3	*K. pneumoniae*	ST-512	bla_{KPC-66}	bla_{TEM-1A}, bla_{OXA-9}, bla_{SHV-11}	0.5	<=0.007	<=0.007	0.015	0.015	**0.26**	**0.08**	**0.09**	**0.09**
BOT-EMOKP	*K. pneumoniae*	ST-1519	bla_{KPC-31}, bla_{KPC-3}	bla_{TEM-1}, bla_{OXA-9}, bla_{SHV-11}	8	2	0.5	0.25	0.5	**0.31**	**0.12**	**0.09**	**0.12**
N435	*K. pneumoniae*	-	bla_{KPC-41}	bla_{SHV-11}, bla_{TEM-1}	4	0.5	0.12	0.12	0.5	**0.13**	**0.09**	**0.09**	**0.19**
N859	*K. pneumoniae*	ST-512	bla_{KPC-50}	bla_{SHV-11}	16	16	4	1	4	1.06	**0.31**	**0.12**	**0.31**
CAZ59BO	*K. pneumoniae*	ST-512	bla_{KPC-53}	bla_{TEM-1}, bla_{SHV-11}	2	0.5	0.125	0.125	0.125	**0.31**	**0.13**	**0.13**	**0.13**
TOBC1	*K,pneumoniae*	ST-258	bla_{KPC-31}	16	16	4	1	0.5	1	**0.31**	**0.12**	**0.09**	**0.12**
R90	*P. aeruginosa*	-	bla_{KPC-2}	bla_{TEM-1}	0.25	0.25	0.03	0.06	0.06	1.06	**0.18**	**0.30**	**0.30**
Ambler class B													
N590	*E. coli*	ST-167	bla_{NDM-5}	bla_{CMY-42}	4	4	2	4	2	1.06	0.56	1.06	0.56
N1700	*E. coli*	ST-69	bla_{NDM-1}	bla_{CMY-4}, $bla_{CTX-M-15}$, bla_{OXA-10}, bla_{TEM-1B}	2	0.5	0.06	1	1	**0.31**	**0.28**	0.56	0.56
N2352	*E. coli*	-	bla_{NDM-5}	$bla_{CTX-M-15}$, bla_{OXA-1}, $bla_{TEM-190}$	0.5	0.25	0.25	0.25	0.5	0.56	0.62	0.56	1.06
R2752	*E.coli*	-	bla_{VIM-34}	bla_{TEM-1}	0.06	0.03	0.03	0.06	0.03	0.56	0.75	1.06	0.56
N1491	*E. cloacae*	ST-78	bla_{NDM-1}	bla_{ACT-24}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{OXA-1}	4	4	2	4	4	1.06	0.62	1.06	1.06
N1692	*K .pneumoniae*	ST-147	bla_{NDM-1}	$bla_{CTX-M-15}$, $bla_{OXA-140}$, bla_{OXA-9}, bla_{SHV-11}	8	4	2	4	4	0.56	**0.31**	0.56	0.56
TOBC2	*K .pneumoniae*	ST-147	bla_{NDM-1}	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{OXA-9}, bla_{SHV-11}, bla_{TEM-1}	16	8	8	8	8	0.56	0.56	0.56	0.56
N1697	*C. freundi*	-	bla_{NDM-1}	bla_{OXA-1}, bla_{SHV-12}	2	2	1	1	1	0.62	0.56	0.56	0.56
N1215	*P. aeruginosa*	-	bla_{VIM-2}	$bla_{OXA-486}$, bla_{PDC-3}, bla_{PER-1}, bla_{OXA-4}	0.25	0.125	0.125	0.25	0.06	0.56	0.56	1.06	**0.30**
N1539	*P. aeruginosa*	ST-235	bla_{NDM-1}	bla_{PAO}, bla_{OXA-50}	0.06	0.06	0.06	0.06	0.06	1.06	1.06	1.06	1.06
N1244	*P. aeruginosa*	ST-111	bla_{IMP-18}	bla_{PDC-3}, bla_{OXA-2}, bla_{OXA-50}	0.03	0.015	0.015	0.03	0.03	0.56	0.56	1.06	1.06
N1744	*P. aeruginosa*	ST-2613	bla_{NDM-1}	$bla_{OXA-488}$, $bla_{PAO-like}$	1	1	0.5	0.5	0.5	1.06	0.56	0.56	0.56
Ambler class B + D													
N1898	*A. baumannii*	ST-52	bla_{NDM-9}, bla_{OXA-58}	$bla_{ADC-158}$, bla_{OXA-98}	16	8	16	8	16	0.56	1.06	0.56	1.06
N2004	*A. baumannii*	-	bla_{NDM-1}, bla_{OXA-23}	bla_{OXA-66}, bla_{ADC-30}	8	8	8	8	8	1.06	1.06	1.06	1.06
Ambler class D													
N1067	*E. coli*	ST-38	$bla_{OXA-181}$	$bla_{CTX-M-27}$	0.25	0.03	0.015	0.06	0.03	**0.18**	**0.12**	**0.30**	**0.18**
N1085	*E. coli*	ST-38	$bla_{OXA-244}$	$bla_{CTX-M-27}$	0.12	0.015	0.03	0.03	0.015	**0.19**	**0.37**	**0.31**	**0.19**
N1091	*K. pneumoniae*	ST-11	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{SHV-11}	0.5	0.06	0.015	0.12	0.06	**0.09**	**0.18**	**0.30**	**0.18**
N612	*A. baumannii*	ST-2	bla_{OXA-23}	$bla_{ADC-25like}$, bla_{OXA-66}	0.25	0.12	0.12	0.12	0.25	0.56	0.56	0.56	1.06
N774	*A. baumannii*	ST-2	bla_{OXA-40}	$bla_{ADC-25like}$, bla_{OXA-66}	1	0.5	0.5	1	0.5	0.56	0.62	0.56	1.06
N1183	*A. baumannii*	ST-2	bla_{OXA-23}	$bla_{ADC-25-like}$, bla_{OXA-66}	0.25	0.12	0.12	0.25	0.12	0.56	0.56	1.06	1.06
ACBB0432	*A. baumannii*	ST-195	bla_{OXA-23}	bla_{TEM-1}	0.25	0.25	0.125	0.125	0.25	1.06	0.56	0.56	1.06
BO415CRAB	*A. baumannii*	ST-195	bla_{OXA-23}	bla_{TEM-1}	0.12	0.12	0.06	0.12	0.12	1.06	0.56	1.06	1.06
TOBC3	*A. baumannii*	ST-2	bla_{OXA-23}	$bla_{ADC-25like}$, bla_{OXA-66}	32	32	32	32	32	1.06	1.06	1.06	1.06
Reference strains													
ATCC 25922	*E. coli*	-	-	-	0.06	0.06	0.06	0.06	0.06	1.06	1.25	1.06	1.06
ATCC 700603	*K. pneumoniae*	-	-	bla_{SHV-18}	0.25	0.015	0.06	0.06	0.03	**0.12**	**0.30**	**0.30**	**0.18**
ATCC BAA-2814	*K. pneumoniae*	-	bla_{KPC}	bla_{SHV-11}, bla_{TEM-1}	1	0.03	0.5	0.06	0.06	**0.15**	0.56	**0.12**	**0.12**
ATCC 27853	*P. aeruginosa*	-	-	-	0.06	0.06	0.06	0.06	0.03	1.06	1.06	1.06	0.56

Red shading indicates resistance. Green shading indicates synergistic effect. Underline character indicate additive effect. Abbreviations: CFDC, cefiderocol; TAZ, tazobactam; AVI, avibactam; VAB, vaborbactam; REL, relebactam; FICI: Fractional inhibitory concentration index.

Figure 1: Time kill curves of cefiderocol alone and combined with tazobactam, avibactam, vaborbactam and relebactam.



- The TKC further displayed that avibactam, vaborbactam and relebactam, but not tazobactam, combined with cefiderocol had a synergistic effect on KPC_TO1 (bla_{KPC-3}) and N859 (bla_{KPC-50}). Likewise, the TKC analysis confirmed the indifferent effect of all β-lactamases inhibitors on cefiderocol activity against NDM and OXA-23 producers (**Figure 1**).
- Overall, time kill experiments showed synergistic bactericidal effects by combinations involving avibactam and relebactam: both combinations in *K. pneumoniae* N859 (bla_{KPC-50}) and cefiderocol plus avibactam in *K. pneumoniae* N1091 (bla_{OXA-48}) [5]. In these cases, bacterial cell viability in the cefiderocol/β-lactamase inhibitor combination continuously decreased within 24h of incubation (**Figure 1**).

Conclusions

In conclusion, our results support the role of carbapenemases and other β-lactamases as a source of reduced susceptibility to cefiderocol and consequently, the potential contribution of β-lactamase inhibitors for recovering the efficacy of cefiderocol. The addition of clinically available serine β-lactamase inhibitors to cefiderocol might represent an important development in the formulation to increase its spectrum and therapeutic efficacy, and to limit *in vivo* emergence of resistance. From our results, avibactam might be the best partner as it has a broader spectrum of action and provided bactericidal synergistic effect in combination with cefiderocol.

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