Synergistic effect of clinically available 8-lactamases inhibitors on cefiderocol activity against carbapenemase-producing Gram-negative organisms

<u>Gabriele Bianco^{1,2}</u>, Sara Comini^{1,2}, Paolo Gaibani³, Matteo Boattini^{1,2}, Giuliana Banche², Cristina Costa^{1,2}, Patrice Nordmann^{4,5}, Rossana Cavallo^{1,2}

¹Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy; ²Department of Public Health and Paediatrics, University of Turin, Italy, ³Operative unit of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; ⁴Medical and Molecular Microbiology, Faculty of Fribourg, Switzerland; ⁵National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland.

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 piuD and pirR, pirA and piuA, and cirA 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 βlactamase 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 \leq 0.5), additive (FICI >0.5 and \leq 1), indifferent ((FICI >1 and \leq 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 cefiderocolresistant, 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-41producing *K. pneumoniae* (N435), KPC-50-producing *K. pneumoniae* (N859), KPC-53-producing *K. pneumoniae* (CAZ59BO), KPC-66producing *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**).

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

• 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**).

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

KPC-3-producing-K.p. KPC_TO1

Image Nerma Control Norma Control Con	Strain	Species	Sequence typing	Carbapenemase gene	Other β-lactamases genes	MIC (mg/L)					FICI and interpretation			
biller of the second						CFDC	CFDC+TAZ	CFDC+AVI	CFDC+VAB	CDFC+REL	CFDC+TAZ	CFDC+AVI	CFDC+VAB	CDFC+REL
Delta J. production S. 1.2 Max. Max. Max. S. 1.1 L <thl< th=""> <thl< th=""> L</thl<></thl<>	Ambler class A													
1110 Lambander Mag.:	BO318KP	K. pneumoniae	ST-512	$bla_{\rm KPC-3}$	$bla_{\text{TEM-1}}, bla_{\text{SHV-11}}$	4	2	1	1	1	<u>0.56</u>	0.25	0.31	0.31
SHO A. parameter · Margent Data of Margent Data o	N1118	K. pneumoniae	-	$bla_{\rm KPC-2}$	$bla_{\rm SHV-11}$	0.12	0.12	<=0.007	0.015	0.015	1.06	0.18	0.24	0.24
Add Set D K prominent ST 10 Bits, mark product Bits, mar	N2350	K. pneumoniae	-	$bla_{\rm KPC-3}$	$bla_{\rm SHV-11}, bla_{ m OXA-9}$	2	2	<=0.007	0.25	0.125	1.06	0.25	0.37	0.31
Aff Affer Aff Affer Affer Affer Affer Affer Affer 	CAZ156BO	K. pneumoniae	ST-101	$bla_{\rm KPC-3}$	bla _{SHV-156}	1	0.5	0.125	0.06	0.125	<u>0.56</u>	0.19	0.12	0.19
XM3 SC X prometers S131 Mage: Ma	BAT16KP	K. pneumoniae	ST-512	$bla_{\rm KPC-3}$	$bla_{\text{TEM-1}}, bla_{\text{SHV-11}}$	1	1	0.06	0.25	0.06	1.03	0.12	0.31	0.12
RF TOTR production6.1.316.4.306.4.30.1.30.7.40.0130.1.20.1.40.4.50.1.00.1.30.1	BAT15KP	K. pneumoniae	ST-512	$bla_{\rm KPC-3}$	$bla_{\text{TEM-1}}, bla_{\text{SHV-11}}$	1	1	0.12	0.25	0.12	1.06	0.18	0.31	0.18
CIC_D01 K. prosenses 57.2.1 Mager, Mager, Mager, Mager, O 0.15 0.15 0.15 0.16 0.16 0.08 0.08 0.09 0 CIPD07 K. prosenses 57.12 Mager,	KPC_TO5	K. pneumoniae	ST-512	$bla_{\rm KPC-3}$	$bla_{\mathrm{TEM} ext{-}1\mathrm{A}}, bla_{\mathrm{OXA} ext{-}9}, bla_{\mathrm{SHV} ext{-}11}$	0.5	0.25	0.015	0.12	0.015	<u>0.56</u>	0.09	0.3	0.09
GPO7 K. ressension S1519 Biograph Mage, Biograph Diage, Biograph <thd< td=""><td>KPC_TO1</td><td>K. pneumoniae</td><td>ST-512</td><td>$bla_{\rm KPC-3}$</td><td>$bla_{\mathrm{TEM} ext{-}1\mathrm{A}}, bla_{\mathrm{OXA} ext{-}9}, bla_{\mathrm{SHV} ext{-}11}$</td><td>0.12</td><td>0.06</td><td><=0.007</td><td>0.015</td><td>0.015</td><td><u>0.56</u></td><td>0.12</td><td>0.19</td><td>0.19</td></thd<>	KPC_TO1	K. pneumoniae	ST-512	$bla_{\rm KPC-3}$	$bla_{\mathrm{TEM} ext{-}1\mathrm{A}}, bla_{\mathrm{OXA} ext{-}9}, bla_{\mathrm{SHV} ext{-}11}$	0.12	0.06	<=0.007	0.015	0.015	<u>0.56</u>	0.12	0.19	0.19
GP200A. promounderS10-100MongateMongateMongateS10-100C125C125C120C-0117C1012C.0117C1013C.0117C1013C013C1017C1013C1017C1017C1013C1017	KPB07	K. pneumoniae	ST-1519	$bla_{\rm KPC-3}$	$bla_{\mathrm{TEM-1}}, bla_{\mathrm{OXA-9}}, bla_{\mathrm{SHV-11}}$	0.25	0.25	0.015	0.06	0.06	1.06	0.18	0.30	0.30
GPR05X. promonale Y. StratterStratter Magener, Magener, Mag	KPB09	K. pneumoniae	ST-1519	$bla_{\rm KPC-3}$	$bla_{\mathrm{TEM-1}}, bla_{\mathrm{OXA-9}}, bla_{\mathrm{SHV-11}}$	0.125	0.125	<=0.007	0.015	0.015	1.06	0.18	0.18	0.18
CHOPEK pressureK	KPB013	K. pneumoniae	ST-1519	$bla_{\rm KPC-3}$	$bla_{\text{OXA-9}}, bla_{\text{SHV-11}}$	0.125	0.125	<=0.007	0.015	<=0.007	1.06	0.18	0.18	0.12
QRC T0.1K parameteS 1512Marger, Marger, Ma	KPB02	K. pneumoniae	ST-1519	$bla_{ m KPC-36}$	$bla_{\mathrm{TEM-1}}, bla_{\mathrm{OXA-9}}, bla_{\mathrm{SHV-11}}$	0.25	0.25	<=0.007	0.06	0.06	1.06	0.28	0.30	0.30
W1245000°Xxxx	KPC_TO3	K. pneumoniae	ST-512	$bla_{ m KPC-66}$	$bla_{\mathrm{TEM-1A}}, bla_{\mathrm{OXA-9}}, bla_{\mathrm{SHV-11}}$	0.5	<=0.007	<=0.007	0.015	0.015	0.26	0.08	0.09	0.09
4435 <i>E</i> generation (manual) <i>Manual</i> (manual)	BOT-EMOKP	K. pneumoniae	ST-1519	$bla_{\rm KPC-31}, bla_{\rm KPC-3}$	$bla_{\mathrm{TEM-1}}, bla_{\mathrm{OXA-9}}, bla_{\mathrm{SHV-11}}$	8	2	0.5	0.25	0.5	0.31	0.12	0.09	0.12
859£ generative81512Singer (approximation of a strain	N435	K. pneumoniae	-	bla _{KPC-41}	$bla_{\rm SHV-11}, bla_{\rm TEM-1}$	4	0.5	0.12	0.12	0.5	0.13	0.09	0.09	0.19
Zeymannian CarponitaStableJohngarn Margan <td>N859</td> <td>K. pneumoniae</td> <td>ST-512</td> <td>$bla_{\rm KPC-50}$</td> <td>$bla_{\rm SHV-11}$</td> <td>16</td> <td>16</td> <td>4</td> <td>1</td> <td>4</td> <td>1.06</td> <td>0.31</td> <td>0.12</td> <td>0.31</td>	N859	K. pneumoniae	ST-512	$bla_{\rm KPC-50}$	$bla_{\rm SHV-11}$	16	16	4	1	4	1.06	0.31	0.12	0.31
Kyone week ST256 More week M	CAZ59BO	K. pneumoniae	ST-512	bla _{KPC-53}	$bla_{\text{TFM-1}}, bla_{\text{SHV-11}}$	2	0.5	0.125	0.125	0.125	0.31	0.13	0.13	0.13
941 2 2 0 0 0 0 0.0 0.0 0.0 0.00 <t< td=""><td>TOBC1</td><td>K.pneumoniae</td><td>ST-258</td><td>bla_{kPC 21}</td><td>16</td><td>16</td><td>4</td><td>1</td><td>0.5</td><td>1</td><td>0.31</td><td>0.12</td><td>0.09</td><td>0.12</td></t<>	TOBC1	K.pneumoniae	ST-258	bla _{kPC 21}	16	16	4	1	0.5	1	0.31	0.12	0.09	0.12
value value <th< td=""><td>890</td><td>P. aeruginosa</td><td>-</td><td>bla_{KPC-2}</td><td>$bla_{\text{TEM-1}}$</td><td>0.25</td><td>0.25</td><td>0.03</td><td>0.06</td><td>0.06</td><td>1.06</td><td>0.18</td><td>0.30</td><td>0.30</td></th<>	890	P. aeruginosa	-	bla _{KPC-2}	$bla_{\text{TEM-1}}$	0.25	0.25	0.03	0.06	0.06	1.06	0.18	0.30	0.30
SMM <i>L</i> . odd ST 457 <i>Magnace Magnace Magnace</i>	Ambler class B													
NY100 L. odi NI dage Magges Magges Magges L. odi L. Di Li L. Di L. Di	N590	E. coli	ST-167	$bla_{\rm NDM-5}$	$bla_{ m CMY-42}$	4	4	2	4	2	1.06	<u>0.56</u>	1.06	0.56
24.322 <i>L</i> , <i>col</i> - <i>bbanyat bbanyat bbanyat</i>	N1700	E. coli	ST-69	$bla_{\text{NDM-1}}$	$bla_{ m CMY-4},$ $bla_{ m CTX-M-15},$ $bla_{ m OXA-10},$ $bla_{ m TEM-1B}$	2	0.5	0.06	1	1	0.31	0.28	<u>0.56</u>	<u>0.56</u>
Z2732 E ofil - Øriggen Ørigen Ørigen Ørigen <thørig< td=""><td>N2352</td><td>E. coli</td><td>-</td><td>$bla_{\rm NDM-5}$</td><td>$bla_{\mathrm{CTX-M-15}}, bla_{\mathrm{OXA-1}}, bla_{\mathrm{TEM-190}}$</td><td>0.5</td><td>0.25</td><td>0.25</td><td>0.25</td><td>0.5</td><td><u>0.56</u></td><td><u>0.62</u></td><td><u>0.56</u></td><td>1.06</td></thørig<>	N2352	E. coli	-	$bla_{\rm NDM-5}$	$bla_{\mathrm{CTX-M-15}}, bla_{\mathrm{OXA-1}}, bla_{\mathrm{TEM-190}}$	0.5	0.25	0.25	0.25	0.5	<u>0.56</u>	<u>0.62</u>	<u>0.56</u>	1.06
Link	R2752	E.coli	-	$bla_{ m VIM-34}$	$bla_{\mathrm{TEM-1}}$	0.06	0.03	0.03	0.06	0.03	<u>0.56</u>	<u>0.75</u>	1.06	<u>0.56</u>
N102 K presumation ST-147 blagggett blagggett blagggett 8 4 2 4 4 6 6 0.10 0.26 0.10 0.26	N1491	E. cloacae	ST-78	$bla_{\rm NDM-1}$	$bla_{ m ACT-24}, bla_{ m CTX-M-15}, bla_{ m TEM-1}, bla_{ m OXA-1}$	4	4	2	4	4	1.06	0.62	1.06	1.06
DDDC2 k preamoning S1:47 bis_{DD11}	N1692	K .pneumoniae	ST-147	$bla_{\rm NDM-1}$	$bla_{ m CTX-M-15}, bla_{ m OXA-140}, bla_{ m OXA-9}, bla_{ m SHV-11}$	8	4	2	4	4	<u>0.56</u>	0.31	<u>0.56</u>	<u>0.56</u>
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	TOBC2	K .pneumoniae	ST-147	$bla_{ m NDM-1}$	$bla_{\text{CTX-M-15}}, bla_{\text{OXA-1}}, bla_{\text{OXA-9}}, bla_{\text{SHV-11}}, bla_{\text{TEM-1}}$	16	8	8	8	8	<u>0.56</u>	<u>0.56</u>	<u>0.56</u>	<u>0.56</u>
Vills P. aeruginosa - biogram biogram biogram 025 0.05	N1697	C. freundi	-	$bla_{\rm NDM-1}$	$bla_{\rm OXA-1}, bla_{\rm SHV-12}$	2	2	1	1	1	<u>0.62</u>	<u>0.56</u>	<u>0.56</u>	<u>0.56</u>
N1330 P. arruginons ST-235 bloganobic bloganobic 0.05 0.05 0.06	N1215	P. aeruginosa	-	$bla_{\rm VIM-2}$	$bla_{\mathrm{OXA-486}}, bla_{\mathrm{PDC-3}}, bla_{\mathrm{PER-1}}, bla_{\mathrm{OXA-4}}$	0.25	0.125	0.125	0.25	0.06	<u>0.56</u>	<u>0.56</u>	1.06	0.30
N124 P. aeruginosa S1-11 Magarina M	N1539	P. aeruginosa	ST-235	$bla_{\rm NDM-1}$	bla_{PAO}, bla_{OXA-50}	0.06	0.06	0.06	0.06	0.06	1.06	1.06	1.06	1.06
N1744 R æruginosa ST-2613 blassain blassain blassain 1 0.5 0.5 1.06 0.56 <	N1244	P. aeruginosa	ST-111	$bla_{\rm IMP-18}$	$bla_{PDC-3}, bla_{OXA-2}, bla_{OXA-50}$	0.03	0.015	0.015	0.03	0.03	0.56	0.56	1.06	1.06
Number class B - D ST-52 ba_{000+9} ba_{000+9} ba_{00C+9} ba_{000+9} ba_{00C+9}	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	<u>0.56</u>	<u>0.56</u>
Name out is include out if the out is include out if the out is include out is	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
VALOG A. Bullmahnin - BulloxAs,33 Bull	12004	A . h				0	0	Q	Q	0	1.06	1.06	1.06	1.06
Number class D <i>E. coli</i> ST-38 <i>bla</i> _{QAA18} <i>bla</i> _{CTKM-27} 0.25 0.03 0.013 0.013 0.18 0.12 0.30 0.015 N1067 <i>E. coli</i> ST-38 <i>bla</i> _{QAA248} <i>bla</i> _{CTKM-27} 0.12 0.015 0.03 0.015 0.19 0.37 0.31 0.015 N1067 <i>K. pneumonia</i> ST-10 <i>bla</i> _{QAA248} <i>bla</i> _{CTKM-27} 0.12 0.015 0.12 0.066 0.09 0.18 0.30 0.30 N1067 <i>K. pneumonia</i> ST-10 <i>bla</i> _{QAA248} <i>bla</i> _{CTKM-27} 0.12 0.12 0.12 0.066 0.09 0.18 0.30 0.30 0.17 0.12 0.25 0.56 <td>N2004</td> <td>A. Duumunnu</td> <td>-</td> <td>$Dia_{\rm NDM-1}, Dia_{\rm OXA-23}$</td> <td>$Dia_{OXA-66}, Dia_{ADC-30}$</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>1.00</td> <td>1.00</td> <td>1.00</td> <td>1.00</td>	N2004	A. Duumunnu	-	$Dia_{\rm NDM-1}, Dia_{\rm OXA-23}$	$Dia_{OXA-66}, Dia_{ADC-30}$	0	0	0	0	0	1.00	1.00	1.00	1.00
K1067 E. coli ST-38 bla _{0XXL38} bla _{CTXM-27} 0.25 0.03 0.015 0.06 0.03 0.18 0.12 0.30 0.015 K1085 E. coli ST-38 bla _{0XA-24} bla _{CTXM-15} /bla _{SW11} 0.12 0.015 0.03 0.03 0.015 0.19 0.37 0.31 0.31 0.31 K1091 K. pneumoniae ST-11 bla _{0XA-24} bla _{CTXM-15} /bla _{SW11} 0.5 0.06 0.015 0.12 0.06 0.03 0.015 0.06 0.09 0.37 0.31 0.31 0.31 K1011 K pneumoniae ST-12 bla _{0XA-38} bla _{0TXM-66} 0.25 0.12 0.12 0.12 0.25 0.56 0.	Ambler class D													
K1085 E. coli ST-38 bla _{QXA-244} bla _{CTX.M-27} 0.12 0.015 0.03 0.015 0.19 0.37 0.31 0.31 K1091 K. pneumoniae ST-11 bla _{QXA-48} bla _{QXA-48} bla _{QXA-46} 0.5 0.06 0.015 0.12 0.06 0.09 0.18 0.30 0.5 K612 A. baumannii ST-2 bla _{QXA-30} bla _{QXA-66} 0.25 0.12 0.12 0.12 0.25 0.5 0.5 0.56 0.	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
K1091K. pneumoniaeST-11 bla_{0XA-48} $bla_{0TXA-15}$ bla_{0RA-65} 0.66 0.015 0.12 0.06 0.09 0.18 0.30 0.56 N612A. baumanniiST-2 bla_{0XA-23} $bla_{0AC-25ike}, bla_{0XA66}$ 0.25 0.12 0.12 0.12 0.25 0.56	N1085	E. coli	ST-38	$bla_{\rm OXA-244}$	bla _{CTX-M-27}	0.12	0.015	0.03	0.03	0.015	0.19	0.37	0.31	0.19
No12 A. baumannii ST-2 bla $_{0XA-23}$ bla $_{ADC-25like}$, bla $_{0XA-66}$ 0.25 0.12 0.12 0.25 </td <td>N1091</td> <td>K. pneumoniae</td> <td>ST-11</td> <td>$bla_{ m OXA-48}$</td> <td>$bla_{ m CTX-M-15}, bla_{ m SHV-11}$</td> <td>0.5</td> <td>0.06</td> <td>0.015</td> <td>0.12</td> <td>0.06</td> <td>0.09</td> <td>0.18</td> <td>0.30</td> <td>0.18</td>	N1091	K. pneumoniae	ST-11	$bla_{ m OXA-48}$	$bla_{ m CTX-M-15}, bla_{ m SHV-11}$	0.5	0.06	0.015	0.12	0.06	0.09	0.18	0.30	0.18
N774 A. baumannii ST-2 bla _{0XA-40} bla _{0XA-25like} , bla _{0XA-66} 1 0.5 0.5 1 0.5 0.62 0.62 0.56 1 N1183 A. baumannii ST-2 bla _{0XA-23} bla _{0XA-25} bla _{0XA-66} 0.25 0.12 0.12 0.25 0.12 0.56 0.56 0.56 1.06 0.56 NCBB0432 A. baumannii ST-195 bla _{0XA-23} bla _{TEM-1} 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.12 0.12 0.66 0.56	N612	A. baumannii	ST-2	bla _{OXA-23}	$bla_{\rm ADC-25like}, bla_{\rm OXA-66}$	0.25	0.12	0.12	0.12	0.25	<u>0.56</u>	<u>0.56</u>	0.56	1.06
N1183 A. baumannii ST-2 bla _{0XA-23} bla _{ADC-25-like} bla _{0XA-66} 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.12 0.25 0.25 0.25 0.25 0.25 0.25	N774	A. baumannii	ST-2	$bla_{ m OXA-40}$	$bla_{\rm ADC-25like}, bla_{\rm OXA-66}$	1	0.5	0.5	1	0.5	<u>0.56</u>	<u>0.62</u>	<u>0.56</u>	1.06
A. Baumannia ST-195 bla_{0XA-23} bla_{TEM-1} 0.25 0.25 0.125 0.25	N1183	A. baumannii	ST-2	$bla_{\rm OXA-23}$	$bla_{ m ADC-25-like}, bla_{ m OXA-66}$	0.25	0.12	0.12	0.25	0.12	<u>0.56</u>	<u>0.56</u>	1.06	1.06
A. baumanii ST-195 bla_{0XA-23} bla_{TEM-1} 0.12	ACBB0432	A. baumannii	ST-195	bla _{OXA-23}	bla _{TEM-1}	0.25	0.25	0.125	0.125	0.25	1.06	<u>0.56</u>	<u>0.56</u>	1.06
A. baumaniiST-2bla _{0XA-23} bla _{ADC-25like} , bla _{0XA-66} 3232323232321.061.	BO415CRAB	A. baumannii	ST-195	bla _{OXA-23}	$bla_{\mathrm{TEM-1}}$	0.12	0.12	0.06	0.12	0.12	1.06	0.56	1.06	1.06
Reference strains Image: Second strain s	COBC3	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
ATCC 25922 E. coll - - - 0.06 0.06 0.06 0.06 0.06 1.06 1.25 1.06	Reference strains	E				0.00	0.07	0.07	0.07	0.07	1.04	1.05	1.07	1.07
ATCC 700603 K. pneumoniae - - bla_{SHV-18} 0.05 0.06 0.03 0.12 0.30 0.30 0.30 ATCC BAA-2814 K. pneumoniae - bla_{SHV-11} , bla_{TEM-1} 1 0.03 0.66 0.06 0.06 0.06 0.12 0.30 0.30 0 ATCC 27853 P. aeruginosa - - - 0.06 0.06 0.06 0.03 1.06 1.06 1.06 0.02 0.00	ATCC 25922	E. coli	-	-	-	0.06	0.06	0.06	0.06	0.06	1.06	1.25	1.06	1.06
ATCC BAA-2814 K. pneumoniae - bla_{KPC} bla_{SHV-11}, bla_{TEM-1} 1 0.03 0.6 0.06 0.15 0.56 0.12 0 ATCC 27853 P. aeruginosa - - - 0.06 0.06 0.06 0.03 1.06 1.06 0.06 0.015 0.12 0	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 27853 P. aeruginosa 0.06 0.06 0.06 1.06 1.06 1.06 0.03	ATCC BAA-2814	K. pneumoniae	-	$bla_{\rm KPC}$	$bla_{\text{SHV-11}}, bla_{\text{TEM-1}}$	1	0.03	0.5	0.06	0.06	0.15	<u>0.56</u>	0.12	0.12
	ATCC 27853	P. aeruginosa	-	-	-	0.06	0.06	0.06	0.06	0.03	1.06	1.06	1.06	<u>0.56</u>



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.

- 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.

References

- Kazmierczak KM, Tsuji M, Wise MG, Hackel M, Yamano Y, Echols R, et al. In vitro activity of cefiderocol, a siderophore cephalosporin, against a recent collection of clinically relevant carbapenem-non-susceptible Gram-negative bacilli, including serine carbapenemase- and metallo-β-lactamase-producing isolates (SIDERO-WT-2014 Study). Int J Antimicrob Agents 2019; 53:177-84. doi: 10.1016/j.ijantimicag.2018.10.007.
- Karakonstantis S, Rousaki M, Kritsotakis EI. Cefiderocol: Systematic Review of Mechanisms of Resistance, Heteroresistance and In Vivo Emergence of Resistance. Antibiotics 2022; 11:723. doi: 10.3390/antibiotics11060723.
- The European Committee on Antimicrobial Susceptibility Testing. Guidance document on broth microdilution testing of cefiderocol. 2020. Available at http://www.eucast.org.
- Doern CD. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. J Clin Microbiol 2014; 52:4124-8. doi: 10.1128/JCM.01121-14.
- NCCLS. 1999. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. Document M26-A. National Committee for Clinical Laboratory Standards, Wayne, PA.