ARGONAUT-IV: Susceptibility of Carbapenem-resistant *Klebsiellae* to Ceftibuten/VNRX-5236

Andrew R. Mack [1,2], Christopher R. Bethel [2], Steven Marshall [2], Robin Patel [3], David van Duin [4], Vance G. Fowler [5], Daniel D. Rhoads [6], Michael R. Jacobs [6], Focco van den Akker [1], David A. Six [7], Greg Moeck [7], Krisztina M. Papp-Wallace [1,2], Robert A. Bonomo [1,2]

1 Case Western Reserve University, Cleveland, OH, USA.
2 Veterans Affairs Northeast Ohio Healthcare System, Cleveland, OH, USA.
3 Mayo Clinic, Rochester, MN, USA.
4 University of North Carolina, Chapel Hill, NC, USA.
5 Duke University Medical Center, Durham, NC, USA
6 University Hospitals Cleveland Medical Center, Cleveland, OH, USA.
7 Venatorx Pharmaceuticals, Inc., Malvern, PA, USA.

INTRODUCTION

Figure 1: CDC Threat Assessment for Carbapenem-Resistant Enterobacteriaceae, the family under which *Klebsiella* falls (1).

***Klebsiella* pneumoniae and β-lactamases**

- *K. pneumoniae* is a gram-negative pathogen responsible for a wide variety of serious infections
- Carbapenem resistance in *Klebsiella* spp. arises through mutational and acquired mechanisms, poses major clinical challenges, and is considered an "urgent threat" by the CDC (1).
- *Klebsiella pneumoniae* carbapenemase (KPC) is a class A β-lactamase capable of hydrolyzing all classes of β-lactam antibiotics.

Ceftibuten and VNRX-5236/VNRX-7145

- Ceftibuten is an oral cephalosporin antibiotic.
- VNRX-5236 is a bicyclic boronate β-lactamase inhibitor (BLI) with activity against all classes of serine β-lactamases (2).
- VNRX-7145 (VNRX-5236 etzadroxil) is currently in clinical development as an orally bioavailable BL/BLI inhibitor combination partnered with ceftibuten (CTB).

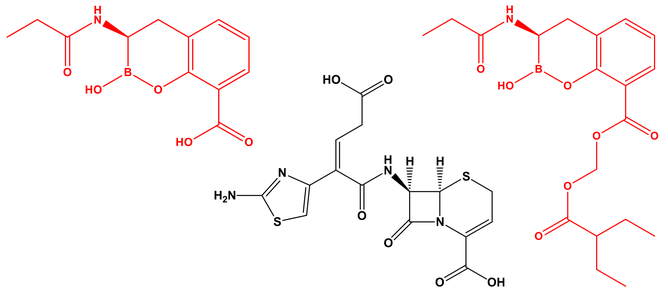

Figure 2: Structures of VNRX-5236 (β-lactamase inhibitor; red, left), ceftibuten (β-lactam antibiotic; black, center), and VNRX-7145 (β-lactamase inhibitor; red, right; prodrug of VNRX-5236).

Materials and Methods

Bacterial Strains

A selection of 200 *K. pneumoniae* strains collected as part of the Antibiotic Resistance Leadership Group's (ARLG) Consortium on Resistance against Carbapenems in *Klebsiella* (CRACKLE) study were tested (3). Of these, 193 contained a class A *Klebsiella pneumoniae* carbapenemase (KPC), 1 contained a class B New Delhi Metallo-β-Lactamase (NDM), and 6 contained the carbapenem-hydrolyzing oxacillinase (OXA) variant OXA-48. ATCC control strains were used for quality control purposes. All CRACKLE strains have previously been subjected to whole genome sequencing and the data is available through the National Center for Biotechnology Information (NCBI) under BioProject numbers PRJNA339843 and PRJNA433394.

Minimum Inhibitory Concentrations (MICs)

MICs were determined using the ThermoFisher Sensititre system with custom assay panels and were read visually. This methodology is based on the CLSI M07 Reference Microdilution Susceptibility Method. Breakpoints were interpreted using the CLSI M100 standard, except for ceftibuten and ceftibuten/VNRX-5236 for which a provisional non-urinary tract breakpoint of 1 µg/ml was used.

RESULTS

MICs Reveal High Levels of CTB/VNRX-5236 Activity and Potentiation of CTB by VNRX-5236

MIC (µg/ml)	AMK	CST	CAZ	CZA	FEP	MEM	MVB	CTB	CTB/VNRX-5236	TGC
0.03	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	117 (58.5%)	0 (0%)	0 (0%)	0 (0%)
0.06	0 (0%)	0 (0%)	0 (0%)	7 (3.5%)	0 (0%)	0 (0%)	18 (9%)	0 (0%)	0 (0%)	0 (0%)
0.12	0 (0%)	0 (0%)	0 (0%)	6 (3%)	0 (0%)	0 (0%)	5 (2.5%)	0 (0%)	114 (57%)	0 (0%)
0.25	0 (0%)	31 (15.5%)	0 (0%)	12 (6%)	0 (0%)	0 (0%)	11 (5.5%)	3 (1.5%)	25 (12.5%)	0 (0%)
0.5	9 (4.5%)	100 (50%)	2 (1%)	55 (27.5%)	0 (0%)	2 (1%)	18 (9%)	1 (0.5%)	18 (9%)	24 (12%)
1	25 (12.5%)	17 (8.5%)	0 (0%)	60 (30%)	0 (0%)	3 (1.5%)	18 (9%)	5 (2.5%)	28 (14%)	95 (47.5%)
2	20 (10%)	6 (3%)	0 (0%)	45 (22.5%)	3 (1.5%)	9 (4.5%)	4 (2%)	6 (3%)	10 (5%)	58 (29%)
4	13 (6.5%)	7 (3.5%)	0 (0%)	10 (5%)	3 (1.5%)	21 (10.5%)	0 (0%)	17 (8.5%)	1 (0.5%)	19 (9.5%)
8	14 (7%)	39 (19.5%)	1 (0.5%)	1 (0.5%)	21 (10.5%)	165 (82.5%)	9 (4.5%)	39 (19.5%)	2 (1%)	4 (2%)
16	39 (19.5%)		2 (1%)	4 (2%)	32 (16%)			60 (30%)	0 (0%)	
32	70 (35%)		195 (97.5%)		141 (70.5%)			69 (34.5%)	2 (1%)	
64	10 (5%)									

Table 1: MIC distribution for *K. pneumoniae*, (n = 200). AMK, amikacin; CST, colistin; CAZ, ceftazidime; CZA, ceftazidime/avibactam; FEP, cefepime; MEM, meropenem; MVB, meropenem/vaborbactam; CTB, ceftibuten; TGC, tigecycline.

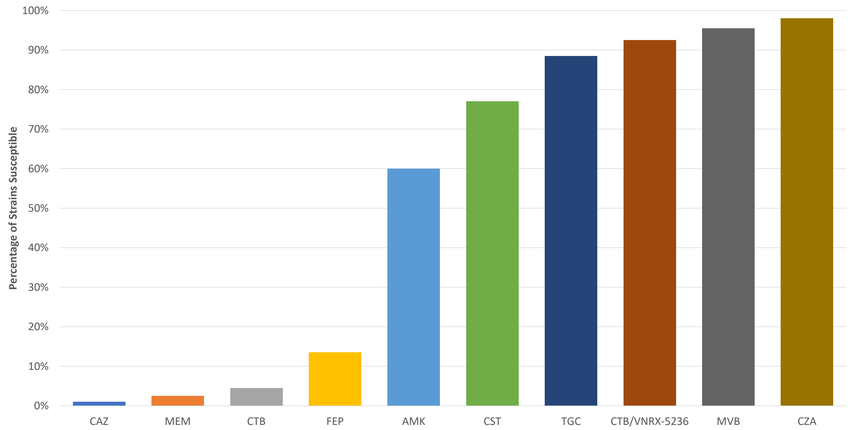

Figure 3: Percent of *K. pneumoniae* strains susceptible, (n = 200). AMK, amikacin; CST, colistin; CAZ, ceftazidime; CZA, ceftazidime/avibactam; FEP, cefepime; MEM, meropenem; MVB, meropenem/vaborbactam; CTB, ceftibuten; TGC, tigecycline.

VNRX-5236 Restores CTB Activity Against Carbapenem-resistant *K. pneumoniae*

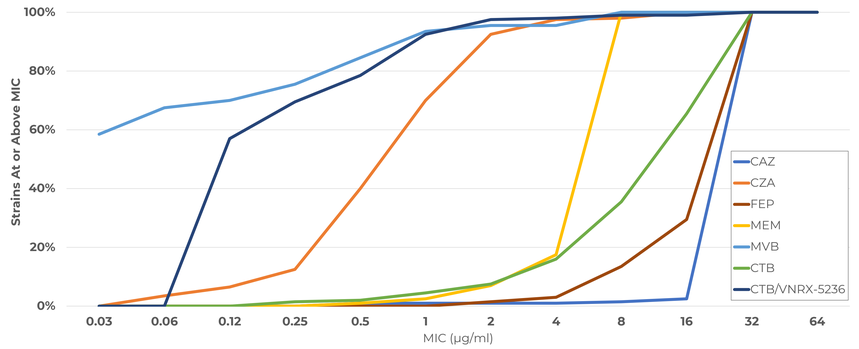

Figure 4: Cumulative percentage of *K. pneumoniae* isolates (n=200) inhibited at each MIC. AMK, amikacin; CST, colistin; CAZ, ceftazidime; CZA, ceftazidime/avibactam; FEP, cefepime; MEM, meropenem; MVB, meropenem/vaborbactam; CTB, ceftibuten; TGC, tigecycline.

	AMK	CST	CAZ	CZA	FEP	MEM	MVB	CTB	CTB/VNRX-5236	TGC
S Breakpoint	≤ 16	≤ 2	≤ 4	≤ 8/4	≤ 8	≤ 1	≤ 4/8	≤ 1	≤ 1/4	≤ 2
MIC_{50}	16	0.5	>16	1	>32	>4	≤ 0.03	≤ 0.12	≤0.12	1
MIC_{90}	32	>4	>16	2	>32	>4	1	8	1	4
% S	60	77	1	98	13.5	2.5	99.5	4.5	92.5	88.5

Table 2: MIC_{50} and MIC_{90} values and CLSI breakpoints for *K. pneumoniae*, (n = 200). AMK, amikacin; CST, colistin; CAZ, ceftazidime; CZA, ceftazidime/avibactam; FEP, cefepime; MEM, meropenem; MVB, meropenem/vaborbactam; CTB, ceftibuten; TGC, tigecycline. The breakpoint for CST is intermediate as no susceptible breakpoint is available. A provisional CTB breakpoint for non-urinary tract infections is used for CTB and CTB/VNRX-5236.

Many MVB Resistant and Intermediate Strains are Inhibited by ≤1 mcg/mL CTB/VNRX-5236

Strain	AMK	CST	CAZ	CAZ-AVI	FEP	MEM	MEM-VAB	CTB	CTB/VNRX-5236	TGC
4	16	1	> 16	4/4	> 16	> 4	> 4/8	> 16	1/4	1
64	16	0	> 16	> 8/4	> 16	> 4	0.5/8	> 16	4/4	4
89	32	1	> 16	2/4	> 16	> 4	> 4/8	> 16	2/4	2
128	> 32	1	> 16	> 8/4	> 16	> 4	> 4/8	> 16	> 16/4	1
129	> 32	2	> 16	0.5/4	> 16	> 4	> 4/8	> 16	0.25/4	1
140	2	1	> 16	> 8/4	8	2	<= 0.03/8	> 16	<= 0.12/4	1
141	1	1	> 16	> 8/4	> 16	> 4	> 4/8	> 16	> 16/4	1
192	> 32	1	1	0.25/4	2	> 4	> 4/8	0.25	0.25/4	1
193	> 32	1	1	0.25/4	2	> 4	> 4/8	0.25	0.25/4	1
196	> 32	1	> 16	1/4	> 16	> 4	> 4/8	> 16	1/4	2
197	> 32	1	> 16	1/4	> 16	> 4	> 4/8	> 16	0.5/4	1

Table 3: MIC comparison for *K. pneumoniae* strains resistant to CZA and/or intermediate or resistant to MVB. AMK, amikacin; CST, colistin; CAZ, ceftazidime; CZA, ceftazidime/avibactam; FEP, cefepime; MEM, meropenem; MVB, meropenem/vaborbactam; CTB, ceftibuten; TGC, tigecycline.

CONCLUSIONS

- VNRX-5236 enhanced the activity of CTB against the 200 *K. pneumoniae* isolates tested, with <=1 mcg/mL CTB/VNRX-5236 inhibiting 92.5% of these carbapenem-resistant isolates.
- With the prodrug (VNRX-7145) allowing for oral administration, ceftibuten/VNRX-5236 represents a potential option for step-down therapy of carbapenem resistant *K. pneumoniae* infections.

REFERENCES

1. Centers for Disease Control and Prevention. (2019). Antibiotic Resistance Threats in the United States, 2019.
2. Chatwin CL, Hamrick JC, Trout REL, Myers CL, Cusick SM, Weiss WJ, Pulse ME, Xerri L, Burns CJ, Moeck G, Daigle DM, John K, Uehara T, Pevear DC. Microbiological Characterization of VNRX-5236, a Broad-Spectrum β-Lactamase Inhibitor for Rescue of the Orally Bioavailable Cephalosporin Ceftibuten as a Carbapenem-Sparing Agent against Strains of Enterobacterales Expressing Extended-Spectrum β-Lactamases and Serine Carbapenemases. Antimicrob Agents Chemother. 2021 Jul 16;65(8):e0055221.
3. van Duin D, Perez F, Rudin SD, Cober E, Hanrahan J, Ziegler J, Webber R, Fox J, Mason P, Richter SS, Cline M, Hall GS, Kaye KS, Jacobs MR, Kalayjian RC, Salata RA, Segre JA, Conlan S, Evans S, Fowler VG Jr, Bonomo RA. Surveillance of carbapenem-resistant Klebsiella pneumoniae: tracking molecular epidemiology and outcomes through a regional network. Antimicrob Agents Chemother. 2014 Jul;58(7):4035-41.

ACKNOWLEDGEMENTS

This project was sponsored by Venatorx Pharmaceuticals, Inc. and funded in part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272201600029C and Grant 5R44AI109879-04A1. Additional funds and/or facilities provided by the Cleveland Department of Veterans Affairs to R.A.B. and K.M.P.-W., the Veterans Affairs Merit Review Program Awards 1I01BX001974 (R.A.B.) and 1I01BX002872 (K.M.P.-W.) from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development, the Geriatric Research Education and Clinical Center VISN 10 (R.A.B.) and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Numbers R21AI114508, R01AI100560, R01AI063517, and R01AI072219 to R.A.B. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Veterans Affairs.