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Evaluation of the *in vitro* activity of cefepime plus VNRX-5133 and comparative agents against carbapenem-resistant Enterobacteriaceae

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INTRODUCTION

- Carbapenem-resistant Enterobacteriaceae (CRE) continue to be an urgent threat to public health
- CRE infections are associated with high rates of patient morbidity and mortality due, in part, to the limited number of treatment options
- Newly-approved β-lactam/β-lactamase inhibitors have improved our ability to treat CRE infections; however, their *in vitro* spectrum is limited
- Ceftazidime-avibactam and meropenem-vaborbactam demonstrate high rates of *in vitro* susceptibility against KPC-producing Enterobacteriaceae, but are not active against metallo-β-lactamase (MBL)-producing Enterobacteriaceae
  - Resistance to ceftazidime-avibactam has been reported due to mutations in bla_{KPC} that encodes variant KPC enzymes
- VNRX-5133 is a new, broad-spectrum cyclic boronate β-lactamase inhibitor with direct inhibitory activity against class A, B, C, and D β-lactamases
- A combination of cefepime plus VNRX-5133 demonstrates high rates of *in vitro* activity against Enterobacteriaceae collected during surveillance studies
- The objectives of this study were to:
  1. Evaluate the *in vitro* activity of cefepime/VNRX-5133 against a well-characterized collection of CRE clinical isolates
  2. Compare the *in vitro* activity of cefepime/VNRX-5133 to comparative CRE agents, including isolates resistant to ceftazidime-avibactam or meropenem-vaborbactam

METHODS

Isolates:

- Clinically diverse CRE isolates collected from patients at UPMC over a period of 2013 – 2018 were selected based on underlying mechanisms of resistance

Susceptibility Testing:

- MICs were measured by broth microdilution using reference CLSI methods for the following agents:

β-lactam Agent	Inhibitor	Range Tested (μg/mL)
Ceftazidime	--	0.5 – 512
Ceftazidime	Plus avibactam 4μg/mL	0.25 – 256
Cefepime	--	0.25 – 256
Cefepime	Plus tazobactam 8μg/mL	0.06 – 64
Cefepime	Plus VNRX-5133 4μg/mL	0.06 – 64
Ceftolozane	Plus tazobactam 4μg/mL	0.25 – 256
Meropenem	--	0.06 – 64
Piperacillin	Plus tazobactam 4μg/mL	0.5 – 512

- Quality control (QC) strains *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853 were used throughout

Detection of and characterization of β-lactamases and porin genes:

- β-lactamase and porin genes were detected by either whole-genome sequence analysis (Illuminia), or by PCR and Sanger DNA sequencing

Laboratory transfer of bla_{KPC}

- bla_{KPC} variants were ligated into cloning vector pBCSK and introduced into laboratory strain *E. coli* TOP10

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RESULTS

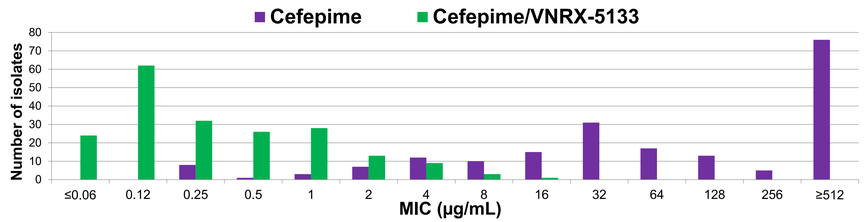
Characteristics of CRE clinical isolates

- 198 CRE clinical isolates were tested; 100% of isolates were resistant to meropenem (MER) and/or harbored a carbapenemase

Organism	n	MER MIC_{50}	MER MIC range	% MER resistant	% KPC*	% MBL
C. freundii	5	>64	0.06 - >64	80%	20%	20%
E. coli	11	4	0.06 – 16	82%	45%	0%
E. aerogenes	12	4	0.06 – 32	58%	25%	0%
E. cloacae	20	2	0.06 - >64	50%	40%	5%
K. pneumoniae	142	32	0.06 - >64	87%	95%	1%
K. oxytoca	4	32	8 – 64	100%	100%	0%
S. marcescens	4	32	4 - >64	100%	0%	0%
Total	198	32	0.06 - >64	82%	80%	2%

* 14% of isolates harbored KPC variant enzymes

Figure 1. Comparison of cefepime and cefepime plus VNRX-5133 MICs



- The MIC_{50} for cefepime and cefepime/VNRX-5133 were 64 and 0.25 μg/mL, respectively
  - Median fold-reduction in MIC following the addition of VNRX-5133 was 256-fold

Figure 2. Cefepime (left) and cefepime plus VNRX-5133 (right) susceptibility rates by organism*

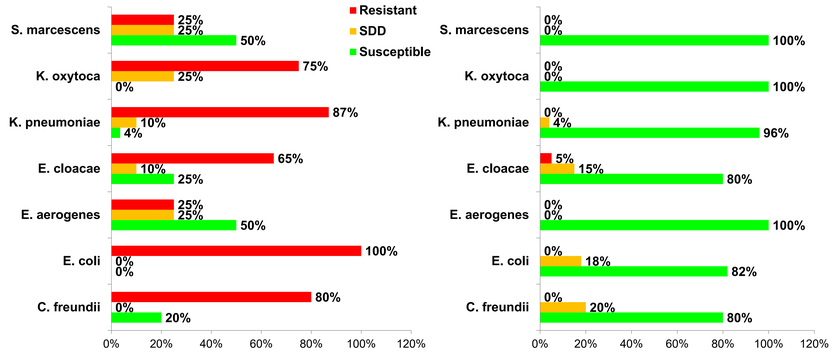


Table 1. Evaluation of MICs against cefepime plus VNRX-5133 versus comparator agents

Drug	MIC_{50}	MIC_{90}	MIC range	Percent susceptible*
Cefepime/VNRX-5133	0.25	2	≤0.06 – 16	93%* (99% ≤ 8 μg/mL)
Meropenem	32	>64	≤0.06 – >64	18%
Ceftazidime-avibactam	1	16	≤0.25 – 256	86%
Colistin	0.5	64	0.12 - >64	82%**
Fosfomycin	16	128	0.25 - >256	75%**
Tigecycline	1	2	0.06 – 8	40%**
Gentamicin	1	128	0.12 - >64	68%

***CLSI cefepime breakpoints were used (Susceptible ≤ 2 μg/mL) **EUCAST breakpoint was applied**

Cefepime/VNRX-5133 versus other agents

- Against a subset of 125 CRE isolates (91 *K. pneumoniae*, 17 *E. cloacae*, 10 *E. coli*, 7 *E. aerogenes*) cefepime/VNRX-5133 was more potent *in vitro* than comparators

Figure 3. Cumulative percentage of isolates by MIC compared by drug

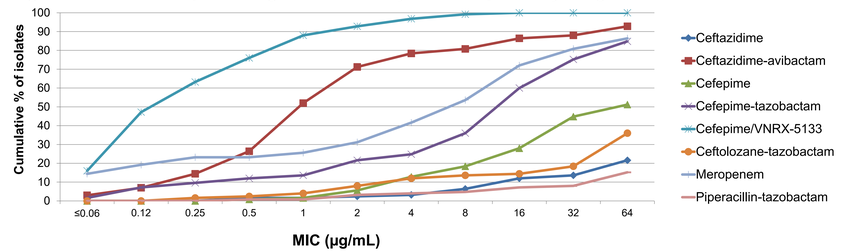


Figure 4. Impact of the addition of VRX-5133 or tazobactam on cefepime MICs*

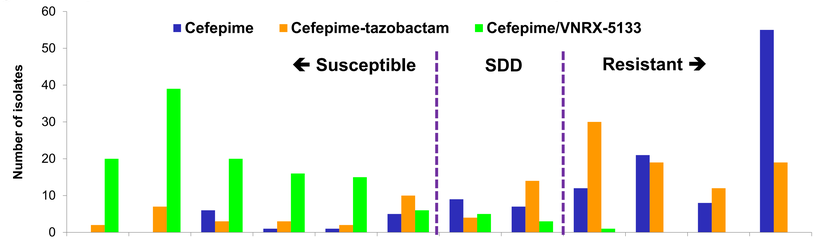
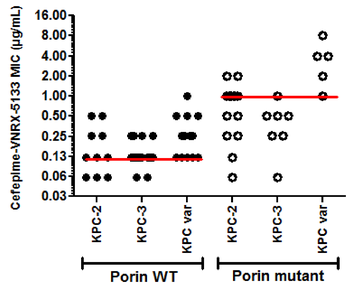


Figure 5. Cefepime/VNRX-5133 MICs by *ompK36* porin genotype and KPC variant



KPC variant cloned into E. coli TOP10	Cefepime MIC	Cefepime-VNRX MIC	Cefepime-TAZ MIC	CAZ-AVI MIC
Blank vector (neg)	≤0.25	0.12	≤0.25	0.25
KPC-2	64	0.12	16	1
KPC-3	>256	0.12	16	1
D179Y, T243M	16	0.5	1	>64
A177E, D179Y	8	0.25	≤0.25	64
D179Y	8	0.25	≤0.25	>64
169-170 EL ins	1	0.12	≤0.25	8
168-169 EL del	1	≤0.06	≤0.25	16
A177E	>256	0.12	32	4
T243A	32	≤0.06	32	2
V240G	>256	0.25	32	32

CONCLUSIONS

- **Cefepime plus VNRX-5133 demonstrates potent *in vitro* activity against clinically-diverse CRE clinical isolates**
  - **The combination retains *in vitro* activity against KPC-variant and MLB-producing CRE that are resistant to ceftazidime-avibactam**
  - **MICs do not vary by KPC-subtype among *K. pneumoniae***
- **Among KPC-producing *K. pneumoniae,* median MICs for both cefepime and cefepime/VNRX-5133 were higher against isolates with *ompK36* porin mutations suggesting that cefepime, and perhaps VNRX-5133, relies on intact porin channels for access to the periplasmic space**