

Impact of Variations in Susceptibility Testing Parameter on the *In Vitro* Activity of Ceftibuten in Combination with VNRX-7145

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INTRODUCTION

VenatoRx is developing VNRX-7145, a novel oral beta-lactamase inhibitor in combination with ceftibuten for the treatment of gram-negative infections. VNRX-7145 undergoes biotransformation *in vivo* to yield the active beta-lactamase inhibitor, VNRX-5236. We compared standard broth microdilution (BMD), broth macrodilution (BMaD) and agar dilution (AD) testing methods and determined the effect of modification of testing parameters on the BMD minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of ceftibuten alone and ceftibuten combined with a fixed concentration of 4 µg/mL VNRX-5236 (CTB/VNRX-5236).

MATERIALS & METHODS

- BMD MIC and MBC testing was performed using standard CLSI broth and agar dilution methods [1,2,3] for three quality control strains: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC BAA-1705, and four clinical strains: *E. coli* 1261278 (CTX-M15,KPC-3); *E. coli* 1480076 (CMY-2); *K. pneumoniae* 1266420 (CTX-M15); *K. pneumoniae* 1434760 (CTX-M15, OXA-48).
- The effect of different variables within the testing system were evaluated including medium supplements such as polysorbate-80 (P-80) (0.002% V/V), pooled human serum (50%), human serum albumin (HSA) (4 g/dL), and pooled human urine (100%). In addition, variations of cations, pH, inoculum, temperature and incubation time were evaluated.
- Ceftibuten hydrate (Sigma-Aldrich, Batch 12243) was tested at a range of 0.06 – 128 µg/mL alone and in combination with VNRX-5236 (VenatoRx Pharmaceuticals, Batch RT00097-126) at a fixed concentration of 4 µg/mL.

RESULTS

Table 1. Comparison of broth microdilution, broth macrodilution and agar diffusion MICs

Organism ID	Minimum Inhibitory Concentration (µg/mL)											
	Standard BMD				Macro Broth Dilution				Agar dilution			
	CAMHB Lot 1		CAMHB Lot 1		CAMHB Lot 1		CAMHB Lot 1		CAMHB Lot 1		CAMHB Lot 1	
	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	0.25	0.12	0.25	≤0.06	0.5	0.25	0.5	0.25	0.5	≤0.06	0.25	≤0.06
<i>K. pneumoniae</i> ATCC 700603	1	0.25	0.5	0.25	1	0.25	1	0.25	1	0.25	1	0.25
<i>K. pneumoniae</i> ATCC BAA 1705	16	0.25	16	0.25	16	0.25	16	0.25	8	0.25	8	0.25
<i>K. pneumoniae</i> 1434760	>128	0.5	>128	0.5	128	1	128	1	64	0.5	128	0.5
<i>E. coli</i> 1261278	64	0.25	64	0.25	64	0.25	64	0.25	32	0.12	32	0.12
<i>E. coli</i> 1480076	128	0.25	128	0.25	>128	0.5	>128	0.25	>128	0.25	>128	0.25
<i>K. pneumoniae</i> 1266420	64	0.12	64	0.12	64	0.12	64	0.25	32	0.12	32	0.12

Table 3. Effect of pH on BMD MIC and MBC

Organism ID	MIC or MBC	Macrobroth (µg/mL)							
		pH 5		pH 6		pH 7.2		pH 8	
		CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	MIC	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.12
	MBC	1	0.5	0.5	0.5	1	0.25	0.5	0.12
<i>K. pneumoniae</i> ATCC 700603	MIC	4	8	1	1	1	0.25	1	0.25
	MBC	64	8	8	2	1	1	1	0.25
<i>K. pneumoniae</i> ATCC BAA 1705	MIC	32	4	16	1	16	0.25	8	0.25
	MBC	128	8	16	1	16	0.5	16	0.25
<i>K. pneumoniae</i> 1434760	MIC	>128	4	>128	2	>128	1	128	1
	MBC	>128	8	>128	2	>128	1	128	1
<i>E. coli</i> 1261278	MIC	64	1	64	1	64	0.25	32	0.25
	MBC	128	1	128	1	64	0.25	32	0.25
<i>E. coli</i> 1480076	MIC	128	1	>128	1	>128	0.5	>128	0.5
	MBC	128	2	>128	1	>128	1	>128	0.5
<i>K. pneumoniae</i> 1266420	MIC	>128	8	128	2	64	0.25	32	0.12
	MBC	>128	16	>128	2	64	0.5	32	0.12

Table 5. Effect of cations on BMD MIC

Organism ID	MIC (µg/mL)			
	CAMHB		CAMHB, no cations	
	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	0.25	≤0.06	0.25	≤0.06
<i>K. pneumoniae</i> ATCC 700603	0.5	0.25	1	0.25
<i>K. pneumoniae</i> ATCC BAA 1705	16	0.25	16	0.25
<i>K. pneumoniae</i> 1434760	128	0.5	128	0.5
<i>E. coli</i> 1261278	32	0.25	64	0.25
<i>E. coli</i> 1480076	128	0.5	>128	0.25
<i>K. pneumoniae</i> 1266420	64	0.12	64	0.12

Table 7. Effect of inoculum concentration on BMD MIC

Organism ID	BMD MIC (µg/ml)										
	10 ⁴ cfu/mL		10 ⁵ cfu/mL		10 ⁶ cfu/mL		10 ⁷ cfu/mL		10 ⁸ cfu/mL	10 ⁸ cfu/mL	10 ⁸ cfu/mL (hazy endpoint)
	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	0.25	≤0.06	0.25	0.12	0.25	0.12	0.5	0.12	0.5	0.12	>128
<i>K. pneumoniae</i> ATCC 700603	0.5	0.25	0.5	0.25	1	0.25	1	0.25	>128	8	128
<i>K. pneumoniae</i> ATCC BAA 1705	8	0.25	16	0.25	16	0.25	16	0.25	>128	32	>128
<i>K. pneumoniae</i> 1434760	64	0.5	128	0.5	>128	0.5	>128	0.5	>128	64	128
<i>E. coli</i> 1261278	32	0.12	32	0.12	64	0.25	64	0.12	128	0.25	64
<i>E. coli</i> 1480076	128	0.25	128	0.25	128	0.25	128	0.5	>128	0.5	32
<i>K. pneumoniae</i> 1266420	32	0.12	32	0.12	64	0.12	64	0.12	96	16	64

Table 2. Comparison of MIC and MBC

Organism ID	MIC or MBC	Standard BMD (µg/mL)				Macro Broth Dilution (µg/mL)			
		CAMHB Lot 1		CAMHB Lot 2		CAMHB Lot 1		CAMHB Lot 2	
		CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	MIC	0.25	0.12	0.25	≤0.06	0.5	0.25	0.5	0.25
	MBC	0.25	0.12	0.25	≤0.06	0.5	0.25	0.5	0.25
<i>K. pneumoniae</i> ATCC 700603	MIC	1	0.25	0.5	0.25	1	0.25	1	0.25
	MBC	1	0.25	1	0.25	1	0.25	1	0.25
<i>K. pneumoniae</i> ATCC BAA 1705	MIC	16	0.25	16	0.25	16	0.25	16	0.25
	MBC	32	0.25	16	0.25	16	0.25	16	0.5
<i>K. pneumoniae</i> 1434760	MIC	>128	0.5	>128	0.5	128	1	128	1
	MBC	>128	1	>128	0.5	>128	1	>128	1
<i>E. coli</i> 1261278	MIC	64	0.25	64	0.25	64	0.25	64	0.25
	MBC	64	0.25	64	0.25	128	0.5	64	0.5
<i>E. coli</i> 1480076	MIC	128	0.25	128	0.25	>128	0.5	>128	0.25
	MBC	>128	0.25	>128	0.25	>128	0.5	>128	0.25
<i>K. pneumoniae</i> 1266420	MIC	64	0.12	64	0.12	64	0.12	64	0.25
	MBC	64	0.12	64	0.12	128	0.12	128	0.25

Table 4. Effect of human serum albumin (HSA), pooled human serum, and polysorbate-80 (P-80)

Organism ID	MIC or MBC	BMD MIC (µg/mL)							
		Standard BMD		+ 0.002% P-80		+ 50 % Human Serum		+4 g/dL HSA	
		CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	MIC	0.25	≤0.06	0.5	0.12	0.5	≤0.06	1	0.5
	MBC	0.25	≤0.06	0.5	0.12	1	0.12	2	0.5
<i>K. pneumoniae</i> ATCC 700603	MIC	0.5	0.25	0.5	0.25	1	0.25	2	0.5
	MBC	1	0.5	0.5	0.25	2	0.5	4	1
<i>K. pneumoniae</i> ATCC BAA 1705	MIC	16	0.25	16	0.25	16	0.12	32	1
	MBC	16	0.25	32	0.5	64	1	32	1
<i>K. pneumoniae</i> 1434760	MIC	128	0.5	128	0.5	16	0.25	>128	2
	MBC	>128	1	>128	0.5	64	1	>128	2
<i>E. coli</i> 1261278	MIC	32	0.25	64	0.25	16	0.25	64	1
	MBC	64	0.25	64	0.25	>128	2	128	1
<i>E. coli</i> 1480076	MIC	128	0.5	>128	0.5	128	0.5	128	1
	MBC	>128	0.5	>128	0.5	>128	1	>128	2
<i>K. pneumoniae</i> 1266420	MIC	64	0.12	64	0.12	4	0.12	128	0.5
	MBC	64	0.25	64	0.12	16	0.25	128	0.5

Table 6. Effect of incubation conditions on BMD MIC

Organism ID	Variation in Incubation Time-MIC (µg/ml)																					
	16 hours				18 hours				20 hours				24 hours				48 hours					
	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236		
<i>E. coli</i> ATCC 25922	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06
<i>K. pneumoniae</i> ATCC 700603	0.5	0.25	0.5	0.25	1	0.25	1	0.25	1	0.25	1	0.25	1	0.25	1	0.25	1	0.25	1	0.25	1	0.25
<i>K. pneumoniae</i> ATCC BAA 1705	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25	16	0.25
<i>K. pneumoniae</i> 1434760	128	0.5	128	0.5	128	0.5	>128	0.5	>128	1	128	0.5	128	0.5	128	0.5	128	0.5	128	0.5	128	0.5
<i>E. coli</i> 1261278	32	0.25	32	0.25	32	0.25	32	0.25	64	0.25	128	0.25	32	0.12	32	0.12	32	0.12	32	0.12	32	0.12
<i>E. coli</i> 1480076	128	0.5	128	0.5	>128	0.5	>128	0.5	>128	0.5	>128	0.5	>128	0.25	128	0.5	128	0.5	128	0.5	128	0.25
<i>K. pneumoniae</i> 1266420	64	0.12	64	0.12	64	0.12	64	0.12	64	0.12	64	0.12	32	0.12	32	0.12	32	0.25	32	0.12	32	0.12

Table 8. Effect of pooled human urine on BMD MIC

Organism ID	MIC (µg/ml)					
	CAMHB Lot 1		Urine pH 6		Urine pH 7.2	
	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236	CTB	CTB/VNRX 5236
<i>E. coli</i> ATCC 25922	0.25	0.12	0.25	0.12	0.12	≤0.06
<i>K. pneumoniae</i> ATCC 700603	0.5	0.25	1	0.5	0.5	0.12
<i>K. pneumoniae</i> ATCC BAA 1705	8	0.25	2	0.5	2	≤0.06
<i>K. pneumoniae</i> 1434760	>128	0.5	>128	1	128	0.5
<i>E. coli</i> 1261278	64	0.25	64	0.		