*J Antimicrob Chemother* 2022; **77**: 689–694
https://doi.org/10.1093/jac/dkab425 Advance Access publication 28 November 2021

Journal of Antimicrobial Chemotherapy

# *In vitro* activity of the orally bioavailable ceftibuten/VNRX-7145 (VNRX-5236 etzadroxil) combination against a challenge set of Enterobacterales pathogens carrying molecularly characterized β-lactamase genes

**Rodrigo E. Mendes[1]*, Paul R. Rhomberg[1], Amy A. Watters[1] and Mariana Castanheira[1]**

[1]*JMI Laboratories, North Liberty, IA, USA*

*Corresponding author. E-mail: rodrigo-mendes@jmilabs.com

*Received 3 September 2021; accepted 21 October 2021*

**Objectives:** This study assessed the activity of ceftibuten, ceftibuten combined with the active form (VNRX-5236) of the β-lactamase inhibitor VNRX-7145 and comparators against a challenge set of Gram-negative pathogens.

**Methods:** Two hundred and five Enterobacterales carrying plasmid AmpC (53 isolates), ESBL (50), KPC (50), OXA-48-like (49) or OXA-48-like with KPC (3) encoding genes were selected. Susceptibility was determined by broth microdilution. VNRX-5236 and avibactam were tested at a fixed concentration of 4 mg/L.

**Results:** Ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.12/1 mg/L) MIC values were 256-fold lower than those of ceftibuten ($MIC_{50/90}$ 32/256 mg/L) for all Enterobacterales and 2- to 4-fold lower than those of ceftazidime/avibactam ($MIC_{50/90}$ 0.5/2 mg/L). For isolates producing a plasmid-encoded AmpC, VNRX-5236 decreased ceftibuten MIC ($MIC_{50/90}$ 0.12/1 mg/L) by at least 512-fold compared with ceftibuten ($MIC_{50/90}$ 128/>256 mg/L). Ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.06/0.12 mg/L) and meropenem ($MIC_{50/90}$ ≤0.03/0.06 mg/L; 100% susceptible) showed comparable activities against ESBL isolates and these agents had $MIC_{90}$ values 4- to 8-fold lower than that of ceftazidime/avibactam ($MIC_{50/90}$ 0.25/0.5 mg/L; 100% susceptible). Ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.12/0.5 mg/L) had the lowest MIC for KPC producers, followed by ceftazidime/avibactam ($MIC_{50/90}$ 2/4 mg/L; 98.0% susceptible). The same $MIC_{90}$ values were obtained for ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.25/1 mg/L) and ceftazidime/avibactam ($MIC_{50/90}$ 1/1 mg/L; 100.0% susceptible) for isolates carrying $bla_{OXA\text{-}48\text{-}like}$. VNRX-5236 decreased the ceftibuten MIC at least 16-fold for three isolates carrying $bla_{OXA\text{-}48\text{-}like}$ and $bla_{KPC}$.

**Conclusions:** VNRX-5236 rescued the *in vitro* activity of ceftibuten against Enterobacterales carrying common serine β-lactamases, including ESBL, AmpC and the KPC and OXA-48-like carbapenemases. Ceftibuten/VNRX-5236 may have potential as an oral treatment for infections caused by resistant Enterobacterales, while sparing carbapenems.

## Introduction

The emergence and spread of Enterobacterales producing ESBL and carbapenemase enzymes have threatened the utility of agents within the β-lactam class for treating infections caused by these organisms.[1–3] A report from the US CDC estimated that, in 2017, 197 400 infections in hospitalized patients were caused by ESBL-producing Enterobacterales and 9100 of those patients died.[4] A report from the ECDC documented that between 14.6% and 15.1% of *Escherichia coli* isolates were resistant to third-generation cephalosporins during 2015–19. This phenotype was reported to be present in 31% of *Klebsiella pneumoniae* during the same period.[5] Although carbapenem resistance remains low among *E. coli*, 8% of *K. pneumoniae* showed this phenotype in Europe,[5] whereas 2.3%, 16.2% and 15.5% of *K. pneumoniae* were carbapenem resistant in the USA, Latin America and Asia-Pacific regions during 2019, respectively.[6]

β-Lactamase inhibitors (BLIs) have long been used to restore the activity of β-lactams against isolates producing β-lactamases.[7] However, approved β-lactam/BLI combinations possess limitations due to their lack of coverage against important β-lactamase enzymes and/or lack of oral bioavailability. The only oral BLI, clavulanic acid, was approved for use with amoxicillin in 1984, but it

exhibits no useful activity against AmpC cephalosporinases, most oxacillinases or carbapenemases. The orally bioavailable etzadroxil prodrug, VNRX-7145, is under clinical development in combination with ceftibuten, a third-generation oral cephalosporin. VNRX-7145 undergoes biotransformation to the active inhibitor, VNRX-5236, which is a new broad-spectrum cyclic boronate BLI.[8] This study assessed the *in vitro* activity of ceftibuten alone, the investigational ceftibuten/VNRX-5236 combination and comparator agents against a challenge set of MDR Gram-negative pathogens.

## Materials and methods

### Bacterial isolates

A set of 198 non-duplicate single-patient Enterobacterales (11 species) isolates were collected from patients in European and US medical centres in 2015–16 through the SENTRY Antimicrobial Surveillance Program (Table 1). Seven isolates collected from Latin American and Asia-Pacific hospitals were added, for an overall total of 205 isolates studied. These isolates were selected for their plasmid AmpC (53 isolates), ESBL (50), KPC (50), OXA-48-like (49) or OXA-48-like in combination with KPC (3) encoding genes (see below). Identification was confirmed for these Enterobacterales by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany).

### Antimicrobial susceptibility testing

Isolates were tested for susceptibility by broth microdilution following CLSI M07 guidelines.[9] MIC values of comparator agents were interpreted using CLSI and EUCAST breakpoint criteria.[10,11] Ceftibuten breakpoints published by EUCAST were applied to ceftibuten/VNRX-5236 for comparative purposes of *in vitro* testing results only, since no breakpoints were available for ceftibuten/VNRX-5236 during the preparation of this manuscript. Frozen-form broth microdilution panels were manufactured by JMI Laboratories (North Liberty, IA, USA) using fresh cation-adjusted Mueller–Hinton broth. VNRX-5236 and avibactam BLI agents were provided by Venatorx Pharmaceuticals Inc. (Malvern, PA, USA) and tested at a fixed concentration of 4 mg/L. Quality assurance was performed by concurrently testing CLSI-recommended quality control reference strains *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and ATCC BAA-1705, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213. This study was performed prior to the formal CLSI approval of quality control MIC ranges for ceftibuten/VNRX-5236; however, all MIC values obtained for ATCC BAA-1705 were within the currently approved range.

### Characterization of resistance mechanisms by next-generation sequencing

Total genomic DNA was extracted using a fully automated Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, OH, USA), which was used as input material for library construction. DNA libraries were prepared using the Nextera™ library construction protocol (Illumina, San Diego, CA, USA) following the instructions of the manufacturer and were sequenced on MiSeq Sequencer platforms (Illumina) at JMI Laboratories. FASTQ format sequencing files for each sample set were assembled independently using *de novo* assembler SPAdes 3.11.1. An in-house software was applied to align the assembled sequences against a curated database containing known β-lactamase resistance genes with the purpose of screening.[12] Isolates classified within the AmpC or ESBL group carried exclusively plasmid-encoded AmpC or ESBL genes only. Similarly, those isolates carrying $bla_{KPC}$ were classified as KPC, whereas those carrying $bla_{OXA-48-like}$ genes were categorized accordingly. A *K. pneumoniae* carrying $bla_{OXA-48}$ and $bla_{KPC-3}$, a *K. pneumoniae* carrying $bla_{OXA-232}$ and $bla_{KPC-2}$ and a *Citrobacter freundii* species complex carrying $bla_{OXA-48}$ and $bla_{KPC-2}$ were included and analysed as a separate group. In addition, some isolates classified within the KPC and OXA-48-like subsets also carried ESBL and/or plasmid AmpC genes. Information related to β-lactamase gene content can be found in Table S1 (available as Supplementary data at *JAC* Online).

## Results and discussion

Overall, ceftibuten/VNRX-5236 inhibited 94.6% of isolates in the challenge set at ≤1 mg/L (Table 1). Eight isolates displayed ceftibuten/VNRX-5236 MIC values of 2–8 mg/L and carried carbapenemases ($bla_{OXA-48}$ or $bla_{KPC}$) with multiple ESBL genes, except for two isolates, each with $bla_{CMY-2}$ or $bla_{SHV-26}$ (first-generation BLI resistant). Two *E. coli* (both carrying $bla_{CMY-42}$) and one *K. pneumoniae* ($bla_{OXA-48}$ and $bla_{DHA-1}$) displayed a ceftibuten/VNRX-5236 MIC of >32 mg/L. These three isolates showed an MDR phenotype, including a carbapenem-resistant Enterobacterales (CRE) phenotype by the *K. pneumoniae* (Table S1).

The ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.12/1 mg/L) MIC values were 256-fold lower than those of ceftibuten alone ($MIC_{50/90}$ 32/256 mg/L; 40.5% susceptible) for all Enterobacterales and 2- to 4-fold lower than those of ceftazidime/avibactam ($MIC_{50/90}$ 0.5/2 mg/L) (Tables 1 and 2). Meropenem ($MIC_{50/90}$ 0.25/32 mg/L; 58.5–64.9% susceptible) and piperacillin/tazobactam ($MIC_{50/90}$ >64/>64 mg/L; 34.1–38.0% susceptible) had limited activity against this challenge set, as did all other comparators, including oral agents (≤29.9% susceptible) (Table 2).

When tested against the subset of isolates producing plasmid-encoded AmpC, VNRX-5236 in combination with ceftibuten ($MIC_{50/90}$ 0.12/1 mg/L) decreased MIC values by at least 512-fold compared with ceftibuten alone ($MIC_{50/90}$ 128/>256 mg/L) (Tables 1 and 2). Ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.12/1 mg/L; 94.3% inhibited at ≤1 mg/L), ceftazidime/avibactam ($MIC_{50/90}$ 0.25/2 mg/L; 96.2% susceptible) and meropenem ($MIC_{50/90}$ ≤0.03/0.06 mg/L; 98.1%–100% susceptible) were active *in vitro* against isolates producing plasmid-encoded AmpC. Other agents tested had suboptimal activities against this subset (Table 2).

Ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.06/0.12 mg/L; 98.0% inhibited at ≤1 mg/L) and meropenem ($MIC_{50/90}$ ≤0.03/0.06 mg/L; 100% susceptible) had similar MIC values for the subset of ESBL isolates, with $MIC_{90}$ values 4- to 8-fold lower than that of ceftazidime/avibactam ($MIC_{50/90}$ 0.25/0.5 mg/L; 100% susceptible) (Table 2). All but four KPC-producing strains were inhibited by ≤1 mg/L ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.12/0.5 mg/L; 92.0% inhibited at ≤1 mg/L) (Table 1). Ceftazidime/avibactam ($MIC_{50/90}$ 2/4 mg/L; 98.0% susceptible) was also highly active against the subset of KPC producers (Table 2). Similarly, ceftibuten/VNRX-5236 ($MIC_{50/90}$ 0.25/1 mg/L; 93.9% inhibited at ≤1 mg/L) and ceftazidime/avibactam ($MIC_{50/90}$ 1/1 mg/L; 100.0% susceptible) were both active against the subset of isolates carrying $bla_{OXA-48-like}$ and both agents had $MIC_{90}$ values of 1 mg/L. Moreover, VNRX-5236 decreased the ceftibuten MIC at least 16-fold when tested against three isolates carrying both $bla_{OXA-48-like}$ and $bla_{KPC}$ genes, with all three isolates exhibiting ceftibuten/VNRX-5236 MICs of 0.5–1 mg/L (Table 1).

Ceftibuten/VNRX-5236 demonstrated potent activity overall and against each of these important subsets of clinical isolates, inhibiting all but 5.4% of strains at ≤1 mg/L, regardless of β-lactamase content. The challenge set of isolates carried the most common β-lactamase encoding genes, including the $bla_{CTX}$ group that

**Table 1.** Antimicrobial activity of agents tested against the main organisms and organism groups

| Organism/group (no. of isolates) | No. of isolates and cumulative % inhibited at MIC (mg/L) of: | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | >[a] | $MIC_{50}$ | $MIC_{90}$ |
| Enterobacteriaceae (205)[b] | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | 4 | 2 | 6 | 3 | 19 | 25 | 24 | 18 | 27 | 18 | 28 | 17 | 14 | 32 | 256 |
| | | | | | 2.0 | 2.9 | 5.9 | 7.3 | 16.6 | 28.8 | 40.5 | 49.3 | 62.4 | 71.2 | 84.9 | 93.2 | 100.0 | | |
| ceftibuten/VNRX-5236[c] | 12 | 17 | 38 | 52 | 35 | 29 | 11 | 2 | 5 | 1 | 0 | 0 | | | | | 3 | 0.12 | 1 |
| | 5.9 | 14.1 | 32.7 | 58.0 | 75.1 | 89.3 | 94.6 | 95.6 | 98.0 | 98.5 | 98.5 | 98.5 | | | | | 100.0 | | |
| AmpC (53) | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | | | | | | 1 | 1 | 2 | 4 | 4 | 18 | 12 | 11 | 128 | >256 |
| | | | | | | | | | | 1.9 | 3.8 | 7.5 | 15.1 | 22.6 | 56.6 | 79.2 | 100.0 | | |
| ceftibuten/VNRX-5236[c] | 1 | 1 | 8 | 19 | 12 | 5 | 4 | 0 | 1 | 0 | 0 | 0 | | | | | 2 | 0.12 | 1 |
| | 1.9 | 3.8 | 18.9 | 54.7 | 77.4 | 86.8 | 94.3 | 94.3 | 96.2 | 96.2 | 96.2 | 96.2 | | | | | 100.0 | | |
| ESBL (50) | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | 4 | 2 | 5 | 1 | 8 | 11 | 11 | 3 | 4 | 1 | | | | 4 | 16 |
| | | | | | 8.0 | 12.0 | 22.0 | 24.0 | 40.0 | 62.0 | 84.0 | 90.0 | 98.0 | 100.0 | | | | | |
| ceftibuten/VNRX-5236[c] | 9 | 8 | 18 | 11 | 2 | 1 | 0 | 1 | | | | | | | | | | 0.06 | 0.12 |
| | 18.0 | 34.0 | 70.0 | 92.0 | 96.0 | 98.0 | 98.0 | 100.0 | | | | | | | | | | | |
| KPC[d] (50) | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | | | | | 7 | 7 | 10 | 5 | 13 | 3 | 2 | 2 | 1 | 16 | 64 |
| | | | | | | | | | 14.0 | 28.0 | 48.0 | 58.0 | 84.0 | 90.0 | 94.0 | 98.0 | 100.0 | | |
| ceftibuten/VNRX-5236[c] | 1 | 4 | 5 | 16 | 10 | 9 | 1 | 0 | 3 | 1 | | | | | | | | 0.12 | 0.5 |
| | 2.0 | 10.0 | 20.0 | 52.0 | 72.0 | 90.0 | 92.0 | 92.0 | 98.0 | 100.0 | | | | | | | | | |
| OXA-48-like[e] (49) | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | | | 1 | 2 | 4 | 6 | 2 | 7 | 4 | 10 | 8 | 3 | 2 | 32 | 128 |
| | | | | | | | 2.0 | 6.1 | 14.3 | 26.5 | 30.6 | 44.9 | 53.1 | 73.5 | 90.0 | 95.9 | 100.0 | | |
| ceftibuten/VNRX-5236[c] | 1 | 4 | 7 | 6 | 11 | 12 | 5 | 1 | 1 | 0 | 0 | 0 | 0 | | | | 1 | 0.25 | 1 |
| | 2.0 | 10.2 | 24.5 | 36.7 | 59.2 | 83.7 | 93.9 | 95.9 | 98.0 | 98.0 | 98.0 | 98.0 | 98.0 | | | | 100.0 | | |
| OXA-48 and KPC[f] (3) | | | | | | | | | | | | | | | | | | | |
| ceftibuten | | | | | | | | | | | | 1 | 2 | | | | | – | – |
| | | | | | | | | | | | | 33.3 | 100.0 | | | | | | |
| ceftibuten/VNRX-5236[c] | | | | | | | 2 | 1 | | | | | | | | | | – | – |
| | | | | | | | 66.7 | 100.0 | | | | | | | | | | | |

[a]Represents an MIC >256 mg/L for ceftibuten alone or >32 mg/L for ceftibuten/VNRX-5236.
[b]Includes *C. freundii* (4 isolates), *Enterobacter cloacae* (8), *E. coli* (71), *Hafnia alvei* (1), *Klebsiella oxytoca* (3), *K. pneumoniae* (106), *Pluralibacter gergoviae* (1), *Proteus mirabilis* (6), *Providencia stuartii* (1), *Raoultella ornithinolytica* (2) and *Serratia marcescens* (2).
[c]VNRX-5236 was tested at a fixed concentration of 4 mg/L. VNRX-5236 is the active BLI of the orally available VNRX-7145 product.
[d]Includes KPC-2, KPC-3, KPC-4 and KPC-6 encoding gene variants.
[e]Includes OXA-48, OXA-163, OXA-232 and OXA-244 encoding gene variants.
[f]Includes a *K. pneumoniae* carrying $bla_{OXA-48}$ and $bla_{KPC-3}$ (ceftibuten/VNRX-5236 MIC 0.5 mg/L), a *K. pneumoniae* carrying $bla_{OXA-232}$ and $bla_{KPC-2}$ (ceftibuten/VNRX-5236 MIC 0.5 mg/L) and a *C. freundii* species complex carrying $bla_{OXA-48}$ and $bla_{KPC-2}$ (ceftibuten/VNRX-5236 MIC 1 mg/L).

is currently carried by the vast majority of *E. coli* and *K. pneumoniae* isolates resistant to third-generation cephalosporins.[1] In addition, ceftibuten/VNRX-5236 showed potent activity against isolates producing (co-producing) the serine carbapenemases KPC and OXA-48-like, which have become prevalent worldwide and endemic in certain regions.[2,13–15] The orally bioavailable ceftibuten/VNRX-7145 combination may be a valuable option for treating infections caused by MDR Enterobacterales carrying ESBL, AmpC and/or serine carbapenemase genes. Pending results from clinical efficacy and safety studies, this combination may provide the convenience of switching to oral administration with the accordingly recognized benefits.[16]

It is important to note that the isolates included in this study were selected solely based on the presence of specific β-lactamases and do not represent the true distribution of these organisms and resistance mechanisms in clinical or community settings. Also, isolates carrying genes encoding Ambler class B carbapenemases were excluded due to the known lack of inhibition by VNRX-5236.[8] Moreover, the data presented here did not assess resistance mechanisms other than the presence of β-lactamase encoding genes.[17] These additional mechanisms of resistance or a combination thereof probably could explain the elevated MIC values (i.e. >32 mg/L) observed for ceftibuten/VNRX-5236 against a small number of isolates in this challenge set. The investigational

**Table 2.** Antimicrobial activity of ceftibuten, ceftibuten/VNRX-5236 and comparator agents against selected Enterobacterales and resistant subsets

| | MIC (mg/L) | | | CLSI[a] | | | EUCAST[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| Antimicrobial agent | $MIC_{50}$ | $MIC_{90}$ | range | %S | %I | %R | %S | %I | %R |
| Enterobacterales (205) | | | | | | | | | |
| ceftibuten[b] | 32 | 256 | ≤0.12 to >256 | 40.5 | 8.8 | 50.7 | 7.3 | | 92.7 |
| ceftibuten/VNRX-5236[b] | 0.12 | 1 | ≤0.015 to >32 | | | | 94.6 | | 5.4 |
| ceftazidime/avibactam | 0.5 | 2 | 0.06 to 32 | 98.5 | | 1.5 | 98.5 | | 1.5 |
| piperacillin/tazobactam | >64 | >64 | 0.5 to >64 | 38.0 | 10.7 | 51.2 | 34.1 | | 65.9 |
| ceftazidime | 64 | >256 | 0.25 to >256 | 7.8 | 7.3 | 84.9 | 4.9 | 2.9 | 92.2 |
| cefepime | 16 | >256 | ≤0.12 to >256 | 27.8 | 10.2[e] | 62.0 | 22.9 | 9.3 | 67.8 |
| meropenem | 0.25 | 32 | ≤0.03 to >64 | 58.5 | 6.3 | 35.1 | 64.9 | 8.3 | 26.8 |
| levofloxacin | 16 | >16 | 0.03 to >16 | 22.4 | 5.9 | 71.7 | 22.4 | 5.9 | 71.7 |
| trimethoprim/ sulfamethoxazole | >4 | >4 | ≤0.5 to >4 | 29.9 | | 70.1 | 29.9 | 4.4 | 65.7 |
| AmpC (53) | | | | | | | | | |
| ceftibuten[b] | 128 | >256 | 4 to >256 | 3.8 | 3.8 | 92.5 | 0.0 | | 100.0 |
| ceftibuten/VNRX-5236[b] | 0.12 | 1 | ≤0.015 to >32 | | | | 94.3 | | 5.7 |
| ceftazidime/avibactam | 0.25 | 2 | 0.06 to 32 | 96.2 | | 3.8 | 96.2 | | 3.8 |
| piperacillin/tazobactam | 8 | >64 | 1 to >64 | 73.6 | 15.1 | 11.3 | 67.9 | | 32.1 |
| ceftazidime | 32 | 256 | 4 to >256 | 1.9 | 5.7 | 92.5 | 0.0 | 1.9 | 98.1 |
| cefepime | 0.5 | 4 | ≤0.12 to 32 | 83.0 | 11.3[e] | 5.7 | 79.2 | 11.3 | 9.4 |
| meropenem | ≤0.03 | 0.06 | ≤0.03 to 2 | 98.1 | 1.9 | 0.0 | 100.0 | 0.0 | 0.0 |
| levofloxacin | 0.5 | >16 | 0.03 to >16 | 50.9 | 9.4 | 39.6 | 50.9 | 9.4 | 39.6 |
| trimethoprim/ sulfamethoxazole | 1 | >4 | ≤0.5 to >4 | 52.8 | | 47.2 | 52.8 | 0.0 | 47.2 |
| ESBL (50) | | | | | | | | | |
| ceftibuten[b] | 4 | 16 | ≤0.12 to 64 | 84.0 | 6.0 | 10.0 | 24.0 | | 76.0 |
| ceftibuten/VNRX-5236[b] | 0.06 | 0.12 | ≤0.015 to 2 | | | | 98.0 | | 2.0 |
| ceftazidime/avibactam | 0.25 | 0.5 | 0.06 to 2 | 100.0 | | 0.0 | 100.0 | | 0.0 |
| piperacillin/tazobactam | 4 | 64 | 0.5 to >64 | 76.0 | 18.0 | 6.0 | 68.0 | | 32.0 |
| ceftazidime | 16 | 128 | 0.25 to >256 | 20.0 | 18.0 | 62.0 | 12.0 | 8.0 | 80.0 |
| cefepime | 16 | 128 | 1 to >256 | 14.0 | 20.0[e] | 66.0 | 2.0 | 20.0 | 78.0 |
| meropenem | ≤0.03 | 0.06 | ≤0.03 to 0.25 | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| levofloxacin | 16 | >16 | 0.03 to >16 | 22.0 | 4.0 | 74.0 | 22.0 | 4.0 | 74.0 |
| trimethoprim/ sulfamethoxazole | >4 | >4 | ≤0.5 to >4 | 32.0 | | 68.0 | 32.0 | 2.0 | 66.0 |
| KPC[c] (50) | | | | | | | | | |
| ceftibuten[b] | 16 | 64 | 2 to >256 | 48.0 | 10.0 | 42.0 | 0.0 | | 100.0 |
| ceftibuten/VNRX-5236[b] | 0.12 | 0.5 | ≤0.015 to 8 | | | | 92.0 | | 8.0 |
| ceftazidime/avibactam | 2 | 4 | 0.25 to 16 | 98.0 | | 2.0 | 98.0 | | 2.0 |
| piperacillin/tazobactam | >64 | >64 | 16 to >64 | 2.0 | 4.0 | 94.0 | 0.0 | | 100.0 |
| ceftazidime | 256 | >256 | 8 to >256 | 0.0 | 2.0 | 98.0 | 0.0 | 0.0 | 100.0 |
| cefepime | 64 | >256 | 1 to >256 | 2.0 | 4.0[e] | 94.0 | 2.0 | 0.0 | 98.0 |
| meropenem | 16 | >64 | 0.06 to >64 | 6.0 | 10.0 | 84.0 | 16.0 | 26.0 | 58.0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| levofloxacin | 16 | >16 | 0.25 to >16 | 4.0 | 8.0 | 88.0 | 4.0 | 8.0 | 88.0 |
| trimethoprim/ sulfamethoxazole | >4 | >4 | ≤0.5 to >4 | 18.4 | | 81.6 | 18.4 | 10.2 | 71.4 |
| OXA-48-like[d] (49) | | | | | | | | | |
| ceftibuten[b] | 32 | 256 | 0.5 to >256 | 30.6 | 14.3 | 55.1 | 6.1 | | 93.9 |
| ceftibuten/VNRX-5236[b] | 0.25 | 1 | ≤0.015 to >32 | | | | 93.9 | | 6.1 |
| ceftazidime/avibactam | 1 | 1 | 0.12 to 2 | 100.0 | | 0.0 | 100.0 | | 0.0 |
| piperacillin/tazobactam | >64 | >64 | 64 to >64 | 0.0 | 6.1 | 93.9 | 0.0 | | 100.0 |
| ceftazidime | 128 | 256 | 0.5 to >256 | 10.2 | 4.1 | 85.7 | 8.2 | 2.0 | 89.8 |
| cefepime | 128 | >256 | 0.5 to >256 | 10.2 | 6.1[e] | 83.7 | 6.1 | 6.1 | 87.8 |
| meropenem | 8 | 32 | 0.12 to 64 | 30.6 | 14.3 | 55.1 | 44.9 | 8.2 | 46.9 |
| levofloxacin | 16 | >16 | 0.03 to >16 | 12.2 | 2.0 | 85.7 | 12.2 | 2.0 | 85.7 |
| trimethoprim/ sulfamethoxazole | >4 | >4 | ≤0.5 to >4 | 16.3 | | 83.7 | 16.3 | 6.1 | 77.6 |
| OXA-48-like and KPC[f] (3) | | | | | | | | | |
| ceftibuten[b] | – | – | 16 to 32 | | | | | | |
| ceftibuten/VNRX-5236[b] | – | – | 0.5 to 1 | | | | | | |
| ceftazidime/avibactam | – | – | 0.5 to 4 | | | | | | |
| piperacillin/tazobactam | – | – | >64 | | | | | | |
| ceftazidime | – | – | 32 to 256 | | | | | | |
| cefepime | – | – | 64 to >256 | | | | | | |
| meropenem | – | – | 32 to >64 | | | | | | |
| levofloxacin | – | – | 8 to >16 | | | | | | |
| trimethoprim/ sulfamethoxazole | – | – | >4 | | | | | | |

[a]MIC values were interpreted using CLSI and EUCAST criteria (2021), where breakpoints are available.
[b]VNRX-5236 was tested at a fixed concentration of 4 mg/L; ceftibuten/VNRX-5236 used the EUCAST ceftibuten breakpoints for Enterobacterales infections originating in the urinary tract (susceptible ≤1 mg/L/resistant >1 mg/L; based on a once-daily 400 mg oral dose of ceftibuten) for comparison purposes of *in vitro* testing results only; VNRX-5236 is the active BLI of the orally bioavailable etzadroxil prodrug, VNRX-7145.
[c]Includes KPC-2, KPC-3, KPC-4 and KPC-6 encoding gene variants.
[d]Includes OXA-48, OXA-163, OXA-232 and OXA-244 encoding gene variants.
[e]Intermediate interpreted as susceptible-dose dependent.
[f]Includes a *K. pneumoniae* carrying $bla_{OXA-48}$ and $bla_{KPC-3}$ (ceftibuten/VNRX-5236 MIC 0.5 mg/L), a *K. pneumoniae* carrying $bla_{OXA-232}$ and $bla_{KPC-2}$ (ceftibuten/VNRX-5236 MIC 0.5 mg/L) and a *C. freundii* species complex carrying $bla_{OXA-48}$ and $bla_{KPC-2}$ (ceftibuten/VNRX-5236 MIC 1 mg/L).

BLI VNRX-5236 rescued the *in vitro* activity of ceftibuten against Enterobacterales carrying common β-lactamases. This combination may have potential clinical utility when treating patients with infections caused by resistant Enterobacterales, while sparing the use of carbapenem agents.

## Funding

This study was performed by JMI Laboratories and supported by Venatorx Pharmaceuticals Inc., which included funding for services related to preparing this manuscript. Discovery and development of VNRX-7145 are supported by NIH R01 grant 1R01AI111539 and by NIAID contract HHSN272201600029C.

## Transparency declarations

JMI Laboratories was contracted to perform services in 2020 for Affinity Biosensors, Allergan, Amicrobe, Inc., Amplyx Pharma, Artugen Therapeutics USA, Inc., Astellas, Basilea, Beth Israel Deaconess Medical Center, BIDMC, bioMerieux, Inc., BioVersys Ag, Bugworks, Cidara, Cipla, Contrafect, Cormedix, Crestone, Inc., Curza, CXC7, Entasis, Fedora Pharmaceutical, Fimbrion Therapeutics, Fox Chase, GlaxoSmithKline, Guardian Therapeutics, Hardy Diagnostics, IHMA, Janssen Research & Development, Johnson & Johnson, Kaleido Biosciences, KBP Biosciences, Luminex, Matrivax, Mayo Clinic, Medpace, Meiji Seika Pharma Co., Ltd, Melinta, Menarini, Merck, Meridian Bioscience Inc., Micromyx, MicuRx, N8 Medical, Nabriva, National Institutes of Health, National University of Singapore, North Bristol NHS Trust, Novome Biotechnologies, Paratek, Pfizer, Prokaryotics Inc., QPEX Biopharma, Rhode Island Hospital, RIHML, Roche, Roivant, Salvat, Scynexis, SeLux Diagnostics, Shionogi, Specific Diagnostics, Spero, SuperTrans Medical LT, T2 Biosystems, The University of Queensland, Thermo Fisher Scientific, Tufts Medical Center, Universite de Sherbrooke, University of Iowa, University of Iowa Hospitals and Clinics, University of Wisconsin, UNT System College of Pharmacy, URMC, UT Southwestern, Venatorx, Viosera Therapeutics and Wayne State University. There are no speakers' bureaus or stock options to declare.

## Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

## References

**1** Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC Antimicrob Resist* 2021; **3**: dlab092.

**2** Papp-Wallace KM, Endimiani A, Taracila MA *et al.* Carbapenems: past, present, and future. *Antimicrob Agents Chemother* 2011; **55**: 4943–60.

**3** Bush K, Bradford PA. Epidemiology of β-lactamase-producing pathogens. *Clin Microbiol Rev* 2020; **33**: e00047-19.

**4** CDC. Antibiotic Resistance Threats in the United States, 2019. 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf.

**5** ECDC. Antimicrobial Resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report for 2019. 2020. https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2019.pdf.

**6** JMI Laboratories. SENTRY Antimicrobial Surveillance Program. 2021. https://sentry-mvp.jmilabs.com/.

**7** Tooke CL, Hinchliffe P, Bragginton EC *et al.* β-Lactamases and β-lactamase inhibitors in the 21st century. *J Mol Biol* 2019; **431**: 3472–500.

**8** Chatwin CL, Hamrick JC, Trout REL *et al.* 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; **65**: e0055221.

**9** CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Eleventh Edition: M07*. 2018.

**10** CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-First Edition: M100*. 2021.

**11** EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. 2021. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf.

**12** Mendes RE, Jones RN, Woosley LN *et al.* Application of next-generation sequencing for characterization of surveillance and clinical trial isolates: analysis of the distribution of β-lactamase resistance genes and lineage background in the United States. *Open Forum Infect Dis* 2019; **6** Suppl 1: S69–78.

**13** ECDC. Rapid Risk Assessment: Increase in OXA-244-Producing *Escherichia coli* in the European Union/European Economic Area and the UK Since 2013, First Update. 2021. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-increase-oxa-244-producing-escherichia-coli-eu-eea.

**14** ECDC. Risk Assessment: Emergence of Hypervirulent *Klebsiella pneumoniae* ST23 Carrying Carbapenemase Genes in UE/EEA Countries. 2021. https://www.ecdc.europa.eu/en/publications-data/risk-assessment-emergence-hypervirulent-klebsiella-pneumoniae-eu-eea.

**15** Hirvonen VHA, Spencer J, van der Kamp MW. Antimicrobial resistance conferred by OXA-48 β-lactamases: towards a detailed mechanistic understanding. *Antimicrob Agents Chemother* 2021; **65**: e00184-21.

**16** Mertz D, Koller M, Haller P *et al.* Outcomes of early switching from intravenous to oral antibiotics on medical wards. *J Antimicrob Chemother* 2009; **64**: 188–99.

**17** Rice LB. Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. *Mayo Clin Proc* 2012; **87**: 198–208.