In vitro Activity of Gepotidacin against *Klebsiella pneumoniae*, Including Molecularly Characterized ESBL and Carbapenemase Positive Subsets Causing Urinary Tract Infections in the United States (2019–2022)

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Introduction

- Gepotidacin is a novel, bactericidal, first-in-class triazaacenaphthylene antibacterial that inhibits bacterial DNA replication by a unique mechanism of action, distinct binding site[1, 2] and provides well-balanced inhibition of two different type II topoisomerase enzymes in most pathogens causing uncomplicated urinary tract infections (uUTI) and *Neisseria gonorrhoeae*.[3, 4]
- Results from two phase 3 clinical trials demonstrated the efficacy of gepotidacin for the treatment of uUTI.[5] More recently, gepotidacin met its primary efficacy endpoint of non-inferiority in a phase 3 trial comparing gepotidacin with intramuscular ceftriaxone plus oral azithromycin combination for the treatment of urogenital gonorrhea.[6]
- This study reports the activity of gepotidacin and other oral antibacterials against *Klebsiella pneumoniae*, including molecularly characterized ESBL and carbapenemase producing isolates collected from UTI patients in the United States.

Materials and Methods

Bacterial isolates

- A total of 2,001 *K. pneumoniae* isolates collected from 73 sites located in 9 US Census Regions as part of the gepotidacin uropathogen global surveillance study were included (2019–2022).
- Only consecutive isolates responsible for UTI (1 per patient infection episode) were included.
- Bacterial identification was confirmed by standard algorithms supported by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Antimicrobial susceptibility testing

- Isolates were tested for susceptibility to gepotidacin and comparator oral antibacterial agents recommended for treatment of UTI by broth microdilution following Clinical and Laboratory Standards Institute (CLSI) M07 (2024) guidelines.[7]
- Frozen-form broth microdilution panels were manufactured by Element Iowa City (JMI Laboratories, North Liberty, Iowa, USA) with cation-adjusted Mueller-Hinton broth according to CLSI guidelines.[7, 8]
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.[8]

Screening for β-lactamase genes

- *K. pneumoniae* with MIC of ≥2 µg/mL for aztreonam, ceftazidime, ceftriaxone, or meropenem were defined as presumptive ESBL and/or carbapenemase producers and selected for screening of β-lactamase genes.
- Isolates were subjected to genome sequencing, and screening of plasmid-mediated AmpC (pAmpC), ESBL, and carbapenemase genes.
- Total genomic DNA was extracted on the Thermo Scientific KingFisher Flex Magnetic Particle Processor (Cleveland, OH, USA), and was used as input material for library construction.
- DNA libraries were prepared using the Nextera library or Illumina DNA Prep construction protocol (Illumina, San Diego, CA, USA) following the manufacturer's instructions and were sequenced on MiSeq or NextSeq Sequencer platforms at JMI Laboratories.
- FASTQ format sequencing files for each sample set were assembled independently using the *de novo* assembler SPAdes 3.11.0. An in-house developed software was applied to align the assembled sequences against a comprehensive in-house database containing known β-lactam resistance genes.[9]

Table 1. Frequency distribution of gepotidacin MIC values against *K. pneumoniae* and ESBL and/or carbapenemase positive subsets from the United States

Phenotype/genotype (No. tested)	Number and cumulative % of isolates inhibited at MIC (µg/mL) of:									MIC (µg/mL)	
	0.5	1	2	4	8	16	32	64	>64	MIC_{50}	MIC_{90}
All (2,001)	7 0.3	27 1.7	187 11.0	1,112 66.6	430 88.1	136 94.9	80 98.9	21 99.9	1 100	4	16
ESBL MIC screen-negative (1,781)	7 0.4	24 1.7	170 11.3	1,067 71.2	362 91.5	80 96.0	60 99.4	10 99.9	1 100	4	8
ESBL MIC screen-positive[a] (220)		3 1.4	17 9.1	45 29.5	68 60.5	56 85.9	20 95.0	11 100		8	32
ESBL/pAmpC (172)		2 1.2	13 8.7	37 30.2	51 59.9	45 86.0	15 94.8	9 100		8	32
CTX-M[b] (167)		2 1.2	13 9.0	35 29.9	50 59.9	43 85.6	15 94.6	9 100		8	32
pAmpC[c] (5)				2 40.0	1 60.0	2 100				8	—
Other[d] (12)			1 8.3	0 8.3	4 41.7	5 83.3	1 91.7	1 100		16	32
Carbapenemase[e] (20)			2 10.0	5 35.0	10 85.0	2 95.0	0 95.0	1 100		8	16
Negative[f] (16)		1 6.2	1 12.5	3 31.2	3 50.0	4 75.0	4 100			8	32

MIC, minimal inhibitory concentration; ESBL, extended-spectrum β-lactamase; "—", MIC_{90} value not available if <10 isolates.
[a] Includes isolates with aztreonam, ceftazidime, ceftriaxone, or meropenem MICs of ≥2 µg/mL.
[b] Includes 3 $bla_{CTX-M-13}$, 6 $bla_{CTX-M-14}$, 156 $bla_{CTX-M-15}$, and 1 $bla_{CTX-M-55}$; and 1 $bla_{CTX-M-55}$ and $bla_{CTX-M-65}$.
[c] Includes plasmidic AmpC: 2 bla_{DHA-1}, 2 bla_{DHA-1} and 1 bla_{CMY-2}, and 1 bla_{CMY-2}.
[d] Includes 1 $bla_{CTX-M-14}$ and bla_{SHV-27}, 4 $bla_{CTX-M-15}$ and bla_{SHV-12}, 1 $bla_{CTX-M-15}$ and bla_{SHV-12}, 3 bla_{SHV-12}, 1 bla_{SHV-2} and bla_{SHV-30}, 1 bla_{SHV-27}, and 1 bla_{SHV-42}. (ESBL SHV variant)
[e] Includes 9 bla_{KPC-2}, 9 bla_{KPC-3}, and 2 bla_{NDM-1}.
[f] Includes isolates where only narrow-spectrum β-lactamase genes were detected, and pAmpC, ESBL, or carbapenemase genes were not found.

Results

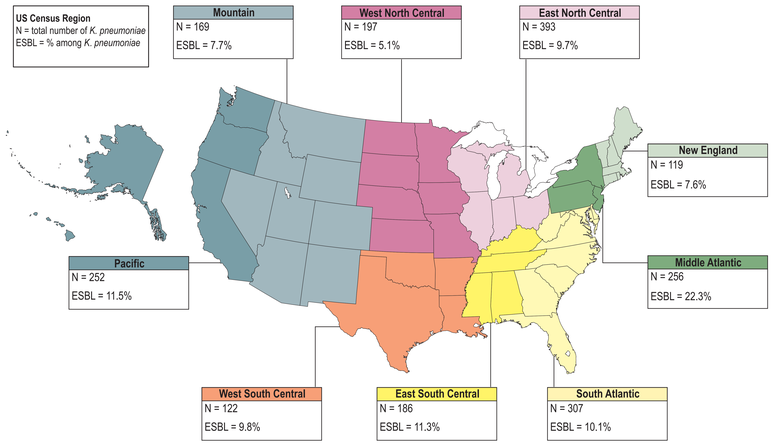
- Overall, gepotidacin ($MIC_{50/90}$, 4/16 µg/mL) inhibited 94.9% of all isolates at MIC of ≤16 µg/mL (Table 1).
- A total of 89.0% (1,781/2,001) *K. pneumoniae* isolates did not meet the criteria for screening of β-lactamase genes (presumptive ESBL negative) (Table 1).
  - Gepotidacin MIC_{50} and MIC_{90} values were 4 µg/mL and 8 µg/mL, respectively, against this group of isolates (Table 1).
  - All oral comparator agents showed activity (≥92.8% susceptible), except for nitrofurantoin (35.7% susceptible) (Table 2).
- In general, a presumptive ESBL and/or carbapenemase phenotype was noted in 11.0% (220/2,001) of *K. pneumoniae*, with the highest rates observed in the Middle Atlantic (22.3%), followed by Pacific (11.5%), East (11.3%) and West (9.8%) South Central, South Atlantic (10.1%), and East North Central (9.7%) regions in the US. Other regions had rates of 5.1–7.7% (Figure 1).
  - Gepotidacin MIC_{50} and MIC_{90} values of 8 µg/mL and 32 µg/mL, respectively, were obtained against isolates with a presumptive ESBL and/or carbapenemase phenotype (Table 1).
  - Comparator agents showed susceptibilities ≤25% (Table 2).
- Most isolates with a presumptive ESBL and/or carbapenemase phenotype carried CTX-M alleles alone (75.9%; 167/220), and a small number carried combinations of bla_{CTX-M} and ESBL variants of bla_{SHV} (2.7%; 6/220), bla_{SHV} alone (2.7%; 6/220), or pAmpC alone (2.3%; 5/220) (Tables 1 and 2).
- Among screened isolates, 9.1% (20/220) carried carbapenemases, including KPC and NDM (Table 1).
- Gepotidacin had an MIC_{90} of 32 µg/mL against screened isolates carrying ESBL and pAmpC, and an MIC_{90} of 16 µg/mL against carbapenemase positive isolates. Other oral agents had limited activity (<50% susceptibility) against isolates carrying ESBL and/or pAmpC.

Table 2. Activity of gepotidacin and comparator agents against ESBL and/or carbapenemase positive subsets of *K. pneumoniae* from the United States

Phenotype/genotype (No. tested)	MIC_{50}/MIC_{90} in µg/mL (% susceptible by CLSI)					
	GEP	AMC	CFZ	CIP	SXT	NIT
ESBL MIC screen-negative (1,781)	4/8 (—)	2/4 (98.1)	1/2 (99.5)	0.015/0.06 (94.6)	≤0.12/1 (92.8)	64/>128 (35.7)
ESBL MIC screen-positive[a] (220)	8/32 (—)	16/>32 (25.0)	>32/>32 (2.7)	2/>4 (19.5)	>4/>4 (18.6)	128/>128 (20.5)
ESBL/pAmpC (172)	8/32 (—)	16/32 (25.0)	>32/>32 (0.0)	2/>4 (16.3)	>4/>4 (12.8)	128/>128 (21.5)
CTX-M[b] (167)	8/32 (—)	16/32 (25.7)	>32/>32 (0.0)	2/>4 (15.6)	>4/>4 (12.0)	128/>128 (21.6)
pAmpC[c] (5)	8/— (—)	32/— (0.0)	>32/— (0.0)	0.5/— (40.0)	>4/— (40.0)	64/— (20.0)
Other[d] (12)	16/32 (—)	16/16 (41.7)	>32/>32 (0.0)	0.5/2 (25.0)	2/>4 (50.0)	64/>128 (25.0)
Carbapenemase[e] (20)	8/16 (—)	>32/>32 (0.0)	>32/>32 (0.0)	>4/>4 (10.0)	>4/>4 (5.0)	>128/>128 (5.0)
Negative[f] (16)	8/32 (—)	16/32 (43.8)	32/>32 (37.5)	0.12/2 (62.5)	0.5/>4 (75.0)	128/>128 (25.0)

MIC, minimal inhibitory concentration; ESBL, extended-spectrum β-lactamase; GEP, gepotidacin; AMC, amoxicillin-clavulanate; CFZ, cefazolin (used as a surrogate for oral cephalosporins); CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; "—", MIC_{90} value not available if <10 isolates or breakpoint not available.
[a] Includes isolates with aztreonam, ceftazidime, ceftriaxone, or meropenem MICs of ≥2 µg/mL.
[b] Includes 3 $bla_{CTX-M-13}$, 6 $bla_{CTX-M-14}$, 156 $bla_{CTX-M-15}$, 1 $bla_{CTX-M-55}$, and 1 $bla_{CTX-M-55}$ and $bla_{CTX-M-65}$.
[c] Includes plasmidic AmpC: 2 bla_{DHA-1}, 2 bla_{DHA-1} and 1 bla_{CMY-2}, and 1 bla_{CMY-2}.
[d] Includes 1 $bla_{CTX-M-14}$ and bla_{SHV-27}, 4 $bla_{CTX-M-15}$ and bla_{SHV-12}, 1 $bla_{CTX-M-15}$ and bla_{SHV-12}, 3 bla_{SHV-12}, 1 bla_{SHV-2} and bla_{SHV-30}, 1 bla_{SHV-27}, and 1 bla_{SHV-42}. (ESBL SHV variant)
[e] Includes 9 bla_{KPC-2}, 9 bla_{KPC-3}, and 2 bla_{NDM-1}.
[f] Includes isolates where only narrow-spectrum β-lactamase genes were detected, and pAmpC, ESBL, or carbapenemase genes were not found.

Figure 1. Proportions of presumptive ESBL-producing *K. pneumoniae* causing UTI in the 9 US Census Regions



Conclusions

- Gepotidacin showed activity against UTI-causing *K. pneumoniae* in the US medical centers including against isolates carrying ESBL, pAmpC, and/or carbapenemases.
- These data support the development of gepotidacin as a potential treatment option for uUTI caused by *K. pneumoniae* in the United States, including when other oral treatment options have limited activity due to drug resistance.

Disclosures

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