In vitro Activity of Gepotidacin against *Klebsiella pneumoniae*, Including Molecularly Characterized Fluoroquinolone Not Susceptible Subsets Causing Urinary Tract Infections in the United States (2019–2022)

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Digital poster

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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, a 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 uUTIs.[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 antibiotics against *Klebsiella pneumoniae*, including molecularly characterized fluoroquinolone (FQ) not susceptible isolates collected from UTI patients in the United States.

Materials and Methods

Bacterial isolates

- A total of 2,001 *K. pneumoniae* 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 of FQ-resistance determinants

- *K. pneumoniae* with MIC ≥0.5 µg/mL for ciprofloxacin and/or ≥1 µg/mL for levofloxacin (not susceptible to either agent based on CLSI criteria)[8] were selected for screening of FQ resistance mechanism.
- Isolates were subjected to genome sequencing, followed by screening for mutations in the quinolone resistance-determining regions (QRDR) of GyrA, GyrB, ParC, and ParE and plasmid-mediated FQ resistance (PMQR) genes.
- Total genomic DNA from the selected isolates was extracted using the 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 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 software was applied to align the assembled sequences against a comprehensive in-house database containing known plasmid-encoded FQ resistance genes and reference GyrA, GyrB, ParC, and ParE sequences from a susceptible control strain.[9]
- Isolates not susceptible to FQ with wildtype sequences for QRDR and absence of PMQR genes were subjected to expression analysis of efflux-pumps AcrAB-TolC and OqxAB.

Results

- Overall, gepotidacin ($MIC_{50/90}$, 4/16 µg/mL) inhibited 94.9% of all isolates at MIC of ≤16 µg/mL (Table 1).
- Gepotidacin had an MIC_{50} value of 4 µg/mL and an MIC_{90} value of 8 µg/mL when tested against FQ susceptible isolates (Table 1).
  - Other oral agents tested were active against FQ susceptible isolates with susceptibility rates ≥90%, except for nitrofurantoin (37.4% susceptible) (Table 2).
- A total of 14.5% (291/2,001) of isolates met the MIC criteria for screening of FQ resistance mechanisms (Tables 1 and 2).
- Gepotidacin showed an MIC_{50} value of 16 µg/mL and an MIC_{90} value of 32 µg/mL against FQ not susceptible isolates (Table 1).
  - Other agents tested had susceptibilities of 5.8–48.5% against FQ not susceptible strains (Table 2).
- The majority (61.2%; 178/291) of *K. pneumoniae* isolates not susceptible to FQ showed wildtype QRDR sequences (Tables 1 and 2).
  - Among FQ not susceptible isolates with wildtype QRDR sequences, 64.6% (115/178) carried PMQR genes. The remaining 63 (35.4%) isolates did not harbor PMQR genes but had elevated expression of *oqxAB* and/or *acrAB*, except for 4 isolates.
- Gepotidacin demonstrated similar activity against FQ not susceptible isolates with wildtype QRDR and the presence of PMQR genes ($MIC_{50/90}$, 16/64 µg/mL) or isolates with wildtype QRDR and without PMQR genes ($MIC_{50/90}$, 32/32 µg/mL) (Tables 1 and 2).
  - Comparator agents had limited activity with low susceptibility rates against isolates with wildtype QRDR sequences, except for amoxicillin-clavulanate (95.2% susceptible) and cefazolin (90.3% susceptible), which were active against the subset of isolates with wildtype QRDR sequences and absence of PMQR genes (Table 2).
- A total of 38.8% (113/291) FQ not susceptible *K. pneumoniae* isolates showed mutations in the QRDR sequences (Tables 1 and 2). Within this subset, 63 (55.8%) isolates carried PMQR genes, whereas these genes were absent in 50 (44.2%) isolates.
- Gepotidacin had consistent MIC_{90} results of 16 µg/mL against isolates that carried QRDR mutations and were negative for PMQR genes, whereas other agents had susceptibilities of ≤68% (Table 2).

Table 1. Frequency distribution of gepotidacin MIC values against FQ susceptible and FQ not susceptible *K. pneumoniae* from the United States

Phenotype/genotype (No. tested)	No. 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	27	187	1,112	430	136	80	21	1	4	16
	0.3	1.7	11.0	66.6	88.1	94.9	98.9	99.9	100		
FQ-S (1,710)	6	19	172	1,080	361	60	10	2		4	8
	0.4	1.5	11.5	74.7	95.8	99.3	99.9	100			
FQ-NS (291)	1	8	15	32	69	76	70	19	1	16	32
	0.3	3.1	8.2	19.2	43.0	69.1	93.1	99.7	100		
Wildtype QRDR (178)[a]		1	0	4	42	54	61	15	1	16	32
		0.6	0.6	2.8	26.4	56.7	91.0	99.4	100		
PMQR gene-negative (63)[b]				1	0	15	43	4		32	32
				1.6	1.6	25.4	93.7	100			
PMQR gene-positive (115)		1	0	3	42	39	18	11	1	16	64
		0.9	0.9	3.5	40.0	73.9	89.6	99.1	100		
Non-wildtype QRDR (113)[c]	1	7	15	28	27	22	9	4		8	32
	0.9	7.1	20.4	45.1	69.0	88.5	96.5	100			
PMQR gene-negative (50)[d]	1	5	9	9	14	10	2			8	16
	2.0	12.0	30.0	48.0	76.0	96.0	100				
GyrA (S83I); ParC (S80I) (22)		3	6	3	7	3				4	16
		13.6	40.9	54.5	86.4	100					
Other QRDR genotypes (28)[e]	1	2	3	6	7	7	2			8	16
	3.6	10.7	21.4	42.9	67.9	92.9	100				
PMQR gene-positive (63)		2	6	19	13	12	7	4		8	32
		3.2	12.7	42.9	63.5	82.5	93.7	100			

FQ-S, fluoroquinolone susceptible; FQ-NS, fluoroquinolone not susceptible; QRDR, quinolone resistance determining region; PMQR, plasmid-mediated quinolone resistance; GyrA, DNA gyrase subunit A; ParC, DNA topoisomerase IV subunit A.
[a] Includes isolates wildtype for QRDR, with and without plasmid-mediated FQ resistance genes.
[b] Includes isolates negative for *aac(6')-Ib-cr* and/or *qnr*. All but 3 isolates had elevated expression of *oqxAB* and/or *acrAB*.
[c] Includes isolates with alteration within QRDR, with and without plasmid-mediated FQ resistance genes.
[d] Isolates negative for plasmid-mediated FQ resistance genes and with non-wildtype QRDR sequences.
[e] Includes 12 QRDR genotypes other than GyrA (S83I) and ParC (S80I).

Table 2. Activity of gepotidacin and comparator agents against FQ susceptible and not susceptible *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
All (2,001)	4/16 (—)	2/8 (90.0)	1/>32 (88.8)	0.015/0.5 (86.3)	≤0.12/>4 (84.7)	64/>128 (34.0)
FQ-S (1,710)	4/8 (—)	2/4 (97.1)	1/2 (97.6)	0.015/0.03 (100)	≤0.12/0.5 (93.8)	64/128 (37.4)
FQ-NS (291)	16/32 (—)	16/32 (48.5)	>32/>32 (37.2)	2/>4 (5.8)	>4/>4 (30.9)	128/>128 (13.7)
Wildtype QRDR (178)[a]	16/32 (—)	8/16 (61.2)	>32/>32 (46.3)	0.5/4 (7.9)	>4/>4 (38.2)	128/>128 (14.6)
PMQR gene-negative (63)[b]	32/32 (—)	4/8 (95.2)	4/16 (90.3)	0.5/1 (14.3)	2/>4 (74.6)	>128/>128 (0.0)
PMQR gene-positive (115)	16/64 (—)	16/32 (42.6)	>32/>32 (22.6)	2/4 (4.3)	>4/>4 (18.3)	64/>128 (22.6)
Non-wildtype QRDR (113)[c]	8/32 (—)	16/>32 (28.3)	>32/>32 (23.0)	>4/>4 (2.7)	>4/>4 (19.5)	128/>128 (12.4)
PMQR gene-negative (50)[d]	8/16 (—)	16/>32 (46.0)	>32/>32 (36.0)	>4/>4 (6.0)	>4/>4 (30.0)	128/>128 (10.0)
GyrA (S83I); ParC (S80I) (22)	4/16 (—)	>32/>32 (18.2)	>32/>32 (18.2)	>4/>4 (0.0)	>4/>4 (18.2)	>128/>128 (0.0)
Other QRDR genotypes (28)[e]	8/16 (—)	8/32 (67.9)	8/>32 (50.0)	2/>4 (10.7)	>4/>4 (39.3)	128/>128 (17.9)
PMQR gene-positive (63)	8/32 (—)	16/32 (14.3)	>32/>32 (12.7)	>4/>4 (0.0)	>4/>4 (11.1)	128/>128 (14.3)

FQ-S, fluoroquinolone susceptible; FQ-NS, fluoroquinolone not susceptible; QRDR, quinolone resistance determining region; PMQR, plasmid-mediated quinolone resistance; GyrA, DNA gyrase subunit A; ParC, DNA topoisomerase IV subunit A; GEP, gepotidacin; AMC, amoxicillin-clavulanate; CFZ, cefazolin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; CLSI breakpoints applied; "—" breakpoint not available.
[a] Includes isolates wildtype for QRDR, with and without plasmid-mediated FQ resistance genes.
[b] Includes isolates negative for *aac(6')-Ib-cr* and/or *qnr*. All but 3 isolates had elevated expression of *oqxAB* and/or *acrAB*.
[c] Includes isolates with alteration within QRDR, with and without plasmid-mediated FQ resistance genes.
[d] Isolates negative for plasmid-mediated FQ resistance genes and with non-wildtype QRDR sequences.
[e] Includes 12 QRDR genotypes other than GyrA (S83I) and ParC (S80I).

Conclusions

- Gepotidacin showed activity against FQ susceptible and FQ not susceptible *K. pneumoniae* UTI isolates from the US, whereas standard antibiotics tested show limited activity against the FQ not susceptible set (<49% susceptibility).
  - In this study, gepotidacin $MIC_{50/90}$ values were higher for FQ not susceptible *K. pneumoniae* in comparison to FQ susceptible isolates (16/32 µg/ml versus 4/8 µg/ml).
  - Gepotidacin MIC values do not appear to be substantially affected by QRDR mutations alone. There is a trend towards higher gepotidacin MICs with the presence of PMQR and/or overexpression of efflux-pump genes.
- These data support the development of gepotidacin for the treatment of uUTI caused by *K. pneumoniae* in the US.

Disclosures

REM, AS, JHK, and SRA are employees of Element Iowa City (JMI Laboratories). RK and DT are employees of GSK group of companies. This study at Element Iowa City was supported by GSK. Element received compensation fees for services in relation to preparing the abstract and poster. This project has been funded in whole or in part with federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under OTA Agreement No. HHSO100201300011C.

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Presentation number P1061
IDWeek 2024 | October 16–19, 2024 | Los Angeles, California