Activity of gepotidacin against *Klebsiella pneumoniae*, including molecularly characterized fluoroquinolone not susceptible subsets causing urinary tract infections in Europe and adjacent regions (2019–2022)

Gepotidacin demonstrated activity against FQ-susceptible and FQ-not susceptible *K. pneumoniae* causing UTIs in Europe, in particular against isolates carrying QRDR mutations, where common oral antibiotics showed limited activity.

Digital poster

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Introduction

- Gepotidacin is a novel, bactericidal, first-in-class triazaacenaphthylene antibiotic that inhibits bacterial DNA replication by a unique mechanism of action and distinct binding site, providing a well-balanced inhibition of two different Type II topoisomerase enzymes.
- Gepotidacin has shown *in vitro* activity against most strains of target pathogens, such as *Escherichia coli*, *Staphylococcus saprophyticus*, and *Neisseria gonorrhoeae*, including those resistant to current antibiotics.
- Results from two phase 3 clinical trials demonstrated the efficacy of gepotidacin for the treatment of uncomplicated urinary tract infections (uUTIs). 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.
- This study reports on the *in vitro* activity of gepotidacin and other oral antibiotics against *Klebsiella pneumoniae*, including molecularly characterized fluoroquinolone (FQ) not susceptible isolates collected from patients with UTI.

Materials and Methods

Bacterial organisms

- A total of 807 *K. pneumoniae* causing UTI during 2019–2022 in 38 sites in 17 European countries, Israel and Turkiye were included as part of the gepotidacin uropathogen global surveillance study.
- Bacterial identification was confirmed by standard algorithms supported by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution and agar dilution following Clinical and Laboratory Standards Institute (CLSI) M07 guidelines.
- Interpretation of MIC results was performed using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, except for amoxicillin-clavulanate MIC values that were interpreted using CLSI breakpoints.

Screening of resistance determinants

- *K. pneumoniae* with MIC ≥0.5 mg/L for ciprofloxacin and/or ≥1 mg/L for levofloxacin (not susceptible to either agent based on CLSI/EUCAST criteria) were selected for FQ resistance mechanism screening. Isolates were subjected to genome sequencing, followed by screening of plasmid-mediated FQ resistance genes and mutations in the quinolone resistance-determining regions (QRDR) of GyrA, GyrB, ParC and ParE.

Materials and Methods, *continued*

- Selected isolates had total genomic DNA extracted by the fully automated Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, OH, USA), which was used to generate 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.

Results

- Gepotidacin ($MIC_{50/90}$, 4/16 mg/L) inhibited 91.6% of all isolates at MIC of ≤16 mg/L.
- Gepotidacin had an MIC_{50} value of 4 mg/L and an MIC_{90} value of 8 mg/L when tested against FQ-susceptible isolates (Table).
- A total of 40.4% (326/807) of isolates were not susceptible to FQ, meeting the MIC criteria for screening of FQ resistance mechanisms.
- Gepotidacin showed an MIC_{50} value of 8 mg/L and an MIC_{90} value of 32 mg/L against FQ not susceptible strains, whereas other agents had susceptibilities of 1.5–76.7%.
- Gepotidacin demonstrated similar activity ($MIC_{50/90}$, 16–32/32–64 mg/L) against isolates with wildtype QRDR and with or without the plasmid-mediated *qnr* resistance genes.
  - Mecillinam had susceptibilities of 81.8–90.6% against these subsets.
  - Other antibiotics had limited activity (as low as 17.2% susceptible for cefazolin, and 23.4% susceptible for trimethoprim-sulfamethoxazole).
- Gepotidacin had consistent MIC_{90} results of 16 mg/L against isolates that were *qnr*-negative with distinct QRDR mutations.
  - Other agents had susceptibilities of ≤50%, except for mecillinam (52–83%).

Table. Activity of gepotidacin and comparator agents tested against FQ-susceptible and FQ-not susceptible *K. pneumoniae* from Europe and adjacent regions (2019–2022)

Phenotype/genotype (No. tested)	MIC_{50}/MIC_{90} in mg/L (% susceptible by EUCAST)					
	GEP	A/C	CFZ	CIP	MEC	SXT
All (807)	4/16 (—)	4/>32 (63.3)	4/>32 (54.3)	0.03/>4 (60.2)	0.5/32 (87.2)	0.25/>4 (61.2)
FQ-S (481)	4/8 (—)	2/16 (90.0)	1/>32 (83.0)	0.015/0.06 (100)	0.25/4 (94.4)	≤0.12/2 (90.0)
FQ-NS (326)	8/32 (—)	16/>32 (24.5)	>32/>32 (12.0)	>4/>4 (1.5)	2/>32 (76.7)	>4/>4 (18.7)
Wildtype QRDR[a]						
Qnr-negative (11)	32/32 (—)	2/16 (81.8)	4/>32 (72.7)	0.5/0.5 (0.0)	0.5/>32 (81.8)	1/4 (72.7)
Qnr-positive (64)	16/64 (—)	8/32 (60.9)	>32/>32 (17.2)	0.5/4 (7.8)	1/8 (90.6)	>4/>4 (23.4)
Non-wildtype QRDR[b]						
All (59)	4/16 (—)	16/>32 (42.4)	>32/>32 (22.0)	>4/>4 (0.0)	4/>32 (66.1)	>4/>4 (33.9)
GyrA (S83I); ParC (S80I) (29)	4/16 (—)	16/>32 (48.3)	>32/>32 (31.0)	>4/>4 (0.0)	8/>32 (51.7)	>4/>4 (41.4)
GyrA (S83F, D87A); ParC (S80I) (18)	2/16 (—)	32/>32 (27.8)	>32/>32 (0.0)	>4/>4 (0.0)	4/>32 (83.3)	>4/>4 (22.2)
Other (12)	8/16 (—)	8/>32 (50.0)	>32/>32 (33.3)	>4/>4 (0.0)	0.5/>32 (75.0)	>4/>4 (33.3)

FQ-S, fluoroquinolone susceptible; FQ-NS, fluoroquinolone not susceptible; QRDR, quinolone resistance determining region; GEP, gepotidacin; A/C, amoxicillin-clavulanate; CFZ, cefazolin; CIP, ciprofloxacin; MEC, mecillinam; SXT, trimethoprim-sulfamethoxazole; EUCAST breakpoints applied, except for amoxicillin-clavulanate (i.e., tested at 2/1 ratio and interpreted according to CLSI); "—" breakpoint not available; GyrA, DNA gyrase subunit A; ParC, DNA topoisomerase IV subunit A.
[a] Includes only isolates wildtype for QRDR and negative for *aac*(6')-*Ib-cr*.
[b] Includes only isolates negative for *aac*(6')-*Ib-cr* and *qnr*. All non-wildtype QRDR isolates had at least 1 mutation within GyrA. GyrB, ParC or ParE. The group "Other" includes the following profiles: GyrA (D87G) (1); GyrA (S83F) (2); GyrA (S83F, D87N) and ParC (E84K) (3); GyrA (S83I), ParC (S80I) and ParE (I438L) (1); GyrA (S83I), ParC (S80I) and ParE (L417F) (1); GyrA (S83I, D87N) and ParC (S80I) (2); and GyrA (S83Y, D87N) and ParC (S80I) (2).

Conclusions

- Gepotidacin demonstrated activity against FQ-susceptible and FQ-not susceptible *K. pneumoniae* UTI isolates from Europe.
  - This activity was retained in particular against FQ-not susceptible isolates with QRDR mutations, where common oral antibiotics showed limited activity.
- Gepotidacin MIC results were not substantially affected by QRDR mutations. However, highest MIC were seen against FQ-not susceptible isolates with wildtype QRDR, especially those that were Qnr-positive, suggesting additional impacting factors not investigated here.
- These data support the development of gepotidacin for the treatment of uncomplicated UTI caused by *K. pneumoniae* in European countries and adjacent regions.

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