Gepotidacin activity against *E. coli* and *K. pneumoniae*, including molecularly characterized fluoroquinolone not susceptible subsets causing urinary tract infections in Europe and adjacent regions (2023)

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

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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 for most pathogens provides well-balanced inhibition of two different type II topoisomerase enzymes.[3]
- Gepotidacin was recently approved by the United States Food and Drug Administration for the treatment of uncomplicated urinary tract infections (uUTI).[4]
- This study reports the *in vitro* activity of gepotidacin and other oral antibiotics against *Escherichia coli* and *Klebsiella pneumoniae*, including molecularly characterized fluoroquinolone (FQ) not susceptible (NS) isolates collected from UTI patients in European countries, Israel and Turkiye.

Methods

Bacterial Isolates

- A total of 310 *E. coli* and 154 *K. pneumoniae* isolates from 32 sites in 16 European countries, Israel, and Turkiye were included in this study, as part of the SENTRY Antimicrobial Surveillance Program for 2023. Only consecutive isolates (1 per patient infection episode) responsible for UTI 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 by broth microdilution or agar dilution (i.e mecillinam) following Clinical and Laboratory Standards Institute (CLSI) M07 (2024) guidelines.[5]

Screening of FQ- resistance determinants

- *E. coli* and *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)[6] 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.
- FQ-NS isolates with wildtype sequences for QRDR and absence of PMQR genes were subjected to expression analysis of efflux-pumps AcrAB-TolC and OqxAB.

Table 1: **Frequency distribution of gepotidacin MIC values against FQ-S and FQ-NS *E. coli* and *K. pneumoniae***

Phenotype/genotype (No)	No. and cumulative % of isolates inhibited at MIC (mg/L) of:								MIC (mg/L)	
	≤0.5	1	2	4	8	16	32	64	MIC_{50}	MIC_{90}
E. coli (310)	32 10.3	126 51	110 86.5	30 96.1	4 97.4	7 99.7	1 100		1	4
FQ-S (229)	24 10.5	92 50.7	84 87.3	23 97.4	3 98.7	3 100			1	4
FQ-NS (81)	8 9.9	34 51.9	26 84	7 92.6	1 93.8	4 98.8	1 100		1	4
Non-WT QRDR[a] (76)	8 10.5	34 55.3	26 89.5	7 98.7	1 100				1	4
Non-WT QRDR and no PMQR[b] (54)	8 14.8	27 64.8	15 92.6	4 100					1	2
WT QRDR[c] (5)						4 80	1 100		-	-
K. pneumoniae (154)			6 3.9	76 53.2	38 77.9	23 92.9	8 98.1	3 100	4	16
FQ-S (103)			1 1	65 64.1	27 90.3	9 99	1 100		4	8
FQ-NS (51)			5 9.8	11 31.4	11 52.9	14 80.4	7 94.1	3 100	8	32
Non-WT QRDR[d] (26)			5 19.2	11 61.5	3 73.1	4 88.5	3 100		4	32
Non-WT QRDR and no PMQR[e] (8)			1 12.5	6 87.5	0 87.5	1 100			4	-
WT QRDR[f] (25)					8 32	10 72	4 88	3 100	16	64

FQ-S, fluoroquinolone susceptible; FQ-NS, fluoroquinolone not susceptible isolates with MIC ≥0.5 mg/L for ciprofloxacin and/or ≥ 1 mg/L for levofloxacin; QRDR, quinolone resistance determining region; "-", $MIC_{50/90}$ values not calculated due to small number of isolates.
[a] Includes all isolates with mutation in the QRDR of GyrA (DNA gyrase subunit A), ParC (DNA topoisomerase IV subunit A), and/or ParE (DNA topoisomerase IV subunit E). A total of 28.9% (22/76) of these isolates carried PMQR genes (18 *aac*(6')-*Ib-cr*; 2 *qnrB1*; 1 *qnrS1*; and 1 *qepA*).
[b] Includes isolates with mutation in the QRDR of GyrA, ParC and ParE, and excludes 22 isolates that carried PMQR genes.
[c] Includes isolates with wildtype QRDR, and all 5 strains carried *qnrS1*.
[d] Includes isolates with mutation in the QRDR of GyrA, ParC and/or ParE. A total of 69.2% (18/26) of these isolates carried PMQR genes (4 *aac*(6')-*Ib-cr*; 1 *qnrB1*; 1 *qnrS1*; 9 *aac*(6')-*Ib-cr* and *qnrB1*, 1 *aac*(6')-*Ib-cr* and *qnrB2*, and 2 *aac*(6')-*Ib-cr* and *qnrS1*.
[e] Includes isolates with mutation in the QRDR of GyrA, ParC and/or ParE and excludes 18 isolates that carried PMQR genes.
[f] Includes isolates with wildtype QRDR, and 80.0% (20/25) of these isolates carried PMQR genes. The remaining 20.0% (5/20) of isolates without PMQR genes, overexpressed OqxAB (24- to 178-fold).

Results

E. coli

- Overall, gepotidacin ($MIC_{50/90}$, 1/4 mg/L) inhibited 99.7% of all *E. coli* isolates at MIC of ≤16 mg/L (Table 1).
  - Among the comparators shown, only mecillinam and nitrofurantoin showed susceptibility rates >90% against *E. coli* isolates (Table 2).
- A total of 81 (26.1%) *E. coli* were FQ-NS, and gepotidacin had MIC_{50} and MIC_{90} values of 1 mg/L and 4 mg/L, respectively, against both the FQ-S and -NS subsets of *E. coli* (Tables 1 and 2).
  - Mecillinam and nitrofurantoin were active against 92.6% and 95.1% of FQ-NS *E. coli* clinical isolates, whereas other comparators had limited activity (≤63% susceptible) (Table 2).
- Among FQ-NS *E. coli*, 76 isolates had QRDR amino acid alterations. In addition, 28.9% (22/76) of these isolates carried PMQR genes (18 *aac*(6')-*Ib-cr*; 2 *qnrB1*; 1 *qnrS1*; and 1 *qepA*) (Table 1).
  - Gepotidacin ($MIC_{50/90}$, 1/4 mg/L) inhibited all 76 FQ-NS *E. coli* isolates with QRDR mutations at MIC of ≤16 mg/L (Table 1).
- Gepotidacin ($MIC_{50/90}$, 1/2 mg/L) inhibited all 54 FQ-NS *E. coli* isolates with QRDR mutations that were absent of PMQR genes at MIC of ≤16 mg/L (Table 1).
  - Mecillinam and nitrofurantoin also were active (90.7–94.4% susceptible) against this subset (Table 2).
- A total of 5 FQ-NS *E. coli* isolates displayed wildtype QRDR (Table 1).
  - All 5 isolates carried *qnrS1* and had gepotidacin MIC of 16–32 mg/L (Table 1).

K. pneumoniae

- In general, gepotidacin had MIC_{50} and MIC_{90} values of 4 mg/L and 16 mg/L, respectively, against all *K. pneumoniae* , and inhibited 92.9% of isolates at MIC of ≤16 mg/L (Tables 1 and 2).
  - Mecillinam showed the highest susceptibility (86.4%) among comparators.
- A total of 33.1% (51/154) of *K. pneumoniae* isolates were FQ-NS (Tables 1 and 2).
- Gepotidacin showed MIC_{50} and MIC_{90} values of 4 mg/L and 8 mg/L, respectively, against FQ-S *K. pneumoniae*; whereas MIC_{50} and MIC_{90} values of 8 mg/L and 32 mg/L, respectively, against FQ-NS isolates (Tables 1 and 2).
  - Ciprofloxacin (100%) and mecillinam (93.2%) showed the highest susceptibilities against FQ-S isolates, but susceptibilities ≤72.5% were noted for comparators shown against the FQ-NS subset (Table 2).
- Among all 51 FQ-NS *K. pneumoniae*, 26 (51%) carried non-wildtype and 25 (49%) carried wildtype QRDR sequences, regardless of the presence of PMQR (Table 1).
  - Among the 26 FQ-NS *K. pneumoniae* with QRDR mutations, 14 (53.8%) isolates carried *qnr* genes and 4 (15.4%) isolates had *aac*(6')-*Ib-cr*.
  - Gepotidacin inhibited 88.5% of the 26 FQ-NS *K. pneumoniae* with QRDR mutations at MIC of ≤16 mg/L.
  - A small subset of 8 FQ-NS *K. pneumoniae* with QRDR mutations were absent of PMQR genes, and all were inhibited by gepotidacin at MIC of ≤16 mg/L.
- In contrast, 20 (80%) FQ-NS isolates with wildtype QRDR carried *qnr*. The other 5 isolates overexpressed OqxAB, whereas expression of AcrAB remained at basal levels.
  - Gepotidacin $MIC_{50/90}$ values of 16/64 mg/L were observed against the 25 FQ-NS *K. pneumoniae* with wildtype QRDR (Tables 1 and 2).
- In general, comparator agents had limited activity (<73% susceptible) when tested against FQ-NS *K. pneumoniae* and resistant subsets, except for mecillinam (92% susceptible) tested against the wildtype QRDR subset (Table 2).

Table 2: **Activity of gepotidacin and comparator agents against FQ-S and FQ-NS *E. coli* and *K. pneumoniae***

Phenotype/genotype (No)	MIC_{50}/MIC_{90} in mg/L (% susceptible by EUCAST)*						
	GEP	AMC	CFZ	CIP	MEC	SXT	NIT
E. coli (310)	1/4 (-)	4/16 (81.3)	2/>32 (74.8)	0.015/>4 (73.9)	0.25/4 (93.5)	≤0.12/>4 (70.6)	16/32 (98.4)
FQ-S (229)	1/4 (-)	4/16 (87.8)	1/16 (86.5)	0.008/0.12 (100)	0.25/4 (93.9)	≤0.12/>4 (77.7)	16/32 (99.6)
FQ-NS (81)	1/4 (-)	8/32 (63.0)	32/>32 (42.0)	>4/>4 (0.0)	0.5/4 (92.6)	2/>4 (50.6)	16/32 (95.1)
Non-WT QRDR[a] (76)	1/4 (-)	8/32 (61.8)	32/>32 (42.1)	>4/>4 (0.0)	0.5/4 (92.1)	0.5/>4 (50.0)	16/32 (94.7)
Non-WT QRDR and no PMQR[b] (54)	1/2 (-)	4/16 (79.6)	4/>32 (53.7)	>4/>4 (0.0)	0.5/4 (90.7)	4/>4 (48.1)	16/32 (94.4)
WT QRDR[c] (5)	-/- (-)	-/- (80.0)	-/- (40.0)	-/- (0.0)	-/- (100)	-/- (60.0)	-/- (100)
K. pneumoniae (154)	4/16 (-)	4/32 (67.3)	2/>32 (61.7)	0.03/>4 (66.9)	0.5/32 (86.4)	0.25/>4 (63.6)	64/>128 (-)
FQ-S (103)	4/8 (-)	2/16 (86.3)	1/>32 (82.5)	0.015/0.25 (100)	0.5/4 (93.2)	≤0.12/>4 (81.6)	64/>128 (-)
FQ-NS (51)	8/32 (-)	16/>32 (29.4)	>32/>32 (19.6)	4/>4 (0.0)	4/>32 (72.5)	>4/>4 (27.5)	128/>128 (-)
Non-WT QRDR[d] (26)	4/32 (-)	16/>32 (11.5)	>32/>32 (7.7)	>4/>4 (0.0)	8/>32 (53.8)	>4/>4 (15.4)	>128/>128 (-)
Non-WT QRDR and no PMQR[e] (8)	4/- (-)	16/- (37.5)	>32/- (25.0)	>4/- (0.0)	4/- (50.0)	4/- (37.5)	>128/- (-)
WT QRDR[f] (25)	16/64 (-)	16/32 (48.0)	>32/>32 (32.0)	1/2 (0.0)	2/8 (92.0)	>4/>4 (40.0)	64/>128 (-)

FQ-S, fluoroquinolone susceptible; FQ-NS, fluoroquinolone not susceptible isolates with MIC ≥0.5 mg/L for ciprofloxacin and/or ≥1 mg/L for levofloxacin; QRDR, quinolone resistance determining region; GEP, gepotidacin; AMC, amoxicillin-clavulanate; CFZ, cefazolin; CIP, ciprofloxacin; MEC, mecillinam; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; *EUCAST breakpoints applied, except for amoxicillin-clavulanate, which was tested at 2/1 ratio and interpreted according to CLSI; "-", breakpoints not available, or $MIC_{50/90}$ values not calculated due to small number of isolates.
[a] Includes all isolates with mutation in the QRDR of GyrA (DNA gyrase subunit A), ParC (DNA topoisomerase IV subunit A), and/or ParE (DNA topoisomerase IV subunit E). A total of 28.9% (22/76) of these isolates carried PMQR genes (18 *aac*(6')-*Ib-cr*; 2 *qnrB1*; 1 *qnrS1*; and 1 *qepA*).
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[f] Includes isolates with wildtype QRDR, and 80.0% (20/25) of these isolates carried PMQR genes. The remaining 20.0% (5/20) of isolates without PMQR genes, overexpressed OqxAB (24- to 178-fold).

Conclusions

- Gepotidacin showed activity against FQ-S and FQ-NS *E. coli* and *K. pneumoniae* causing UTI in patients in European countries, Israel and Turkiye.
  - The gepotidacin activity was retained particularly against FQ-NS isolates carrying QRDR mutations, where standard oral antibiotics showed limited activity.
- Gepotidacin MIC results were not substantially affected by QRDR mutations. However, highest MIC values were observed against FQ-NS isolates with PMQR genes and/or overexpression of efflux-pump genes.
- These data support the use of gepotidacin for the treatment of uUTI caused by *E. coli* and *K. pneumoniae* in Europe, Israel and Turkiye.

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