In vitro activity of gepotidacin against *Escherichia coli* causing urinary tract infections between 2019–2021 in Europe, Russia, Israel, and Turkey, including molecularly characterized fluoroquinolone-resistant subsets

RE Mendes[1], JH Kimbrough[1], SJR Arends[1], V Kantro[1], D Torumkuney[2], R Kapoor[3], M Castanheira[1]

[1] JMI Laboratories, North Liberty, IA, USA; [2] GlaxoSmithKline, London, UK; [3] GlaxoSmithKline, Collegeville, PA, USA

Introduction

- Gepotidacin (GSK2140944) is a novel, bactericidal, first-in-class triazaacenaphthylene antibiotic that inhibits bacterial DNA replication by a distinct mechanism of action and binding site,[1, 2] and provides well-balanced inhibition of 2 different Type II topoisomerase enzymes.[3]
- This agent is in Phase 3 clinical trials for the treatment of gonorrhea and uncomplicated urinary tract infection (uUTI).
- Gepotidacin has shown activity against most strains of *Escherichia coli*, including isolates resistant to current clinically available antibiotics.[4, 5] This study evaluated the activity of gepotidacin against *E. coli* clinical isolates causing UTI in Europe, Russia, Israel, and Turkey. This analysis included molecular characterization for fluoroquinolone (FQ) resistance mechanisms.

Materials and Methods

Bacterial organisms

- A total of 1,664 *E. coli* from the Gepotidacin Global UTI Surveillance Program (2019–2021) were included in the study. These isolates originated from 30 medical sites in 15 European countries and 5 sites in Russia and Turkey.
- Only isolates responsible for UTI according to local clinical criteria 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).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution and agar dilution following Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.[6]
- Frozen-form broth microdilution panels were manufactured by JMI Laboratories (North Liberty, Iowa) and contained cation-adjusted Mueller-Hinton broth. Agar dilution plates were used for testing fosfomycin (included glucose-6-phosphate at 25 µg/mL) and mecillinam.[6, 7]
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.[7] Interpretation of MIC results was performed using EUCAST criteria, except for amoxicillin-clavulanate MIC values that were interpreted using CLSI breakpoints.[7, 8]
- *E. coli* with MIC results ≥0.5 mg/L for ciprofloxacin and/or ≥1 mg/L for levofloxacin (not susceptible [NS] to either agent based on CLSI/EUCAST criteria) were selected for screening of fluoroquinolone resistance mechanisms. Isolates were subjected to genome sequencing, followed by screening of plasmid-mediated fluoroquinolone resistance genes and mutations in the quinolone resistance-determining regions (QRDR) of GyrA, GyrB, ParC, and ParE.

Screening of resistance determinants

- 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 fluoroquinolone resistance genes and reference GyrA, GyrB, ParC, and ParE sequences from a susceptible control strain.[9]

- Gepotidacin demonstrated potent activity against both FQ-S and FQ-NS *E. coli* causing UTIs in Europe, Russia, Israel and Turkey. In addition, the gepotidacin MIC results were not affected by QRDR mutations and most of plasmid-mediated FQ-R genes.
- Subsets of isolates with *qnr* genes were associated with higher MIC values for gepotidacin, as well as for other tested antibiotics, including amoxicillin-clavulanate, cefazolin and levofloxacin.
- These data support the development of gepotidacin for the treatment of UTI caused by both FQ-S and FQ-NS *E. coli* isolates.

Results

- A total of 24.9% (415/1,664) *E. coli* met the MIC criteria for screening of FQ-resistance (R) mechanisms (Table 1), and the occurrence of this phenotype was higher among isolates from Eastern European countries (35.1%) than that observed among *E. coli* originating from Western European countries (17.5% of all isolates).
- Most FQ-NS isolates (39.0%; 162/415) had double mutations at GyrA and ParC, followed by isolates (29.9%; 124/415) with double mutations at GyrA and single mutations at ParC (Table 1).
- Among FQ-NS isolates, plasmid-mediated FQ-R genes, such as *qnr* variants, were detected in 11.3% (47/415) of these isolates, whereas *aac*-(6')-*Ib-cr* variants were noted in 17.8% (74/415) of isolates, including 1 strain with both genes (Table 1).
- Gepotidacin had an MIC_{50} of 2 mg/L and an MIC_{90} of 4 mg/L against both FQ-S and FQ-NS isolates (Tables 1 and 2).
- Nitrofurantoin had activity against the FQ-S and FQ-NS subsets (99.7% and 97.1%S, respectively), whereas amoxicillin-clavulanate (84.7% and 62.2% susceptible) and trimethoprim-sulfamethoxazole (79.7% and 44.4% susceptible) had limited activity (Table 2).
- Fosfomycin (91.8–98.4% susceptible) and mecillinam (91.5–98.6% susceptible) were also active (i.e., >90% susceptible) against the various *E. coli* subsets presented here (Table 2).
- Gepotidacin had an MIC_{50} of 1 mg/L or 2 mg/L and an MIC_{90} of 2 mg/L, 4 mg/L, or 8 mg/L against isolates with various QRDR mutations (Table 1).
- Against isolates carrying plasmid-mediated FQ-R genes, gepotidacin had MIC_{50} and MIC_{90} values of 2 mg/L and 4 mg/L against those isolates with *aac*-(6')-*Ib-cr*, whereas MIC_{50} and MIC_{90} values of 8 mg/L and 16 mg/L against the *qnr* subset (Table 1).

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Table 1. Distribution of gepotidacin MICs against phenotypic and genotypic subsets of *E. coli*

Phenotype/Genotype			Number and cumulative % of isolates inhibited at gepotidacin MIC (mg/L) of:							MIC (mg/L)	
			≤0.12	0.25	0.5	1	2	4	8	16	32	50%	90%
FQ-susceptible (1,249)				3 (0.2)	32 (2.8)	392 (34.2)	654 (86.5)	153 (98.8)	13 (99.8)	2 (100.0)		2	4
FQ-not susceptible (415)			3 (0.7)	11 (3.4)	36 (12.0)	136 (44.8)	147 (80.2)	52 (92.8)	17 (96.9)	11 (99.5)	2 (100.0)	2	4
QRDR GyrA	ParC	ParE											
Single[a]	WT	WT			2 (7.1)	9 (39.3)	7 (64.3)	7 (89.3)	3 (100.0)			2	8
Single[a]	Single[b]	WT			5 (41.7)	5 (83.3)	0 (83.3)	2 (100.0)				1	4
Double[c]	Single[c]	WT	2 (1.6)	6 (6.5)	7 (12.1)	43 (46.8)	48 (85.5)	13 (96.0)	3 (98.4)	2 (100.0)		2	4
Double[d]	Single[d]	Single[d]		1 (1.6)	14 (24.6)	34 (80.3)	9 (95.1)	3 (100.0)				1	2
Double[e]	Double[e]	WT	1 (0.6)	3 (2.5)	8 (7.4)	44 (34.6)	79 (83.3)	26 (99.4)	0 (99.4)	1 (100.0)		2	4
Plasmid-mediated resistance													
qnr (47)[f]				1 (2.1)	0 (2.1)	1 (4.3)	4 (12.8)	11 (36.2)	17 (72.3)	11 (95.7)	2 (100.0)	8	16
aac(6')-*Ib-cr* (73)					5 (6.8)	24 (39.7)	29 (79.5)	15 (100.0)				2	4

FQ, fluoroquinolone; QRDR, quinolone resistance determining region; WT, wildtype; GyrA, DNA gyrase subunit A; GyrB, DNA gyrase subunit B; ParC, DNA topoisomerase IV subunit A; ParE, DNA topoisomerase IV subunit B. Mutations in GyrB were not detected.

[a] S83L in GyrA.
[b] S80I, S80R, or E84G.
[c] S83L/D87G or S83L/D87H or S83L/D87N or S83L/D87Y in GyrA and S80I or S80R or S80W or E84K in ParC.
[d] S83L/D87N in GyrA, S80I in ParC, and L416F in ParE.
[e] S83L/D87N or S83L/D87Y in GyrA and S80I/E84A, S80I/E84G, S80I/E84V, or S80I/A90V in ParC.
[f] Represents the following genes: *qnrB* (8), *qnrS* (37), *qnrVC4* (1), and *qnrB38*/*aac*(6')-*Ib-cr* (1) with or without mutations in QRDR.

Table 2. Activity of gepotidacin and comparator agents against various subsets of *E. coli*

Antimicrobial agent	MIC (mg/L)			EUCAST[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Fluoroquinolone-susceptible (1,249)						
Gepotidacin	2	4	0.25 to 16			
Amoxicillin-clavulanate	4	16	0.5 to >32	84.7[b]	10.3	5.0
Ampicillin	4	>64	≤1 to >64	58.1[b]		41.9
Cefazolin	2	16	≤0.5 to >32		83.1[c, d]	16.9
Ciprofloxacin	0.015	0.12	≤0.002 to 0.25	100.0	0.0	0.0
Levofloxacin	0.03	0.25	≤0.015 to 0.5	100.0	0.0	0.0
Nitrofurantoin	16	16	≤2 to >128	99.7[b]		0.3
Trimethoprim-sulfamethoxazole	≤0.12	>4	≤0.12 to >4	79.7	0.8	19.5
Trimethoprim	0.5	>8	0.03 to >8	77.8[b]		22.2
Fosfomycin	0.5	1	≤0.12 to >256	98.4[b]		1.6
Mecillinam	0.5	4	0.03 to >32	93.9[b]		6.1
Fluoroquinolone-not susceptible (415)						
Gepotidacin	2	4	0.06 to 32			
Amoxicillin-clavulanate	8	32	2 to >32	62.2[b]	27.5	10.4
Ampicillin	>64	>64	2 to >64	13.0[b]		87.0
Cefazolin	16	>32	1 to >32		39.8[c,d]	60.2
Ciprofloxacin	>4	>4	0.25 to >4	0.2	12.1	87.7
Levofloxacin	8	32	0.25 to >32	8.0	6.5	85.5
Nitrofurantoin	16	32	≤2 to >128	97.1[b]		2.9
Trimethoprim-sulfamethoxazole	>4	>4	≤0.12 to >4	44.4	1.2	54.3
Trimethoprim	>8	>8	0.03 to >8	42.5[b]		57.5
Fosfomycin	0.5	2	0.25 to >256	94.0[b]		6.0
Mecillinam	1	8	0.06 to >32	93.5[b]		6.5
qnr genes (47)						
Gepotidacin	8	16	0.25 to 32			
Amoxicillin-clavulanate	8	>32	2 to >32	68.1[b]	12.8	19.1
Ampicillin	>64	>64	2 to >64	2.1[b]		97.9
Cefazolin	>32	>32	1 to >32		34.0[c,d]	66.0
Ciprofloxacin	1	>4	0.5 to >4	0.0	42.6	57.4
Levofloxacin	1	>32	0.5 to >32	23.4	29.8	46.8
Nitrofurantoin	16	32	≤2 to 32	100.0[b]		0.0
Trimethoprim-sulfamethoxazole	>4	>4	≤0.12 to >4	40.4	0.0	59.6
Trimethoprim	>8	>8	0.06 to >8	39.1[b]		60.9
Fosfomycin	0.5	1	0.25 to >256	93.6[b]		6.4
Mecillinam	1	8	0.25 to >32	91.5[b]		8.5
aac(6')-*Ib-cr* genes (73)						
Gepotidacin	2	4	0.5 to 4			
Amoxicillin-clavulanate	16	32	8 to >32	8.2[b]	71.2	20.5
Ampicillin	>64	>64	>64 to >64	0.0[b]		100.0
Cefazolin	>32	>32	2 to >32		11.0[c,d]	89.0
Ciprofloxacin	>4	>4	>4 to >4	0.0	0.0	100.0
Levofloxacin	16	32	4 to 32	0.0	0.0	100.0
Nitrofurantoin	16	32	≤2 to >128	95.9[b]		4.1
Trimethoprim-sulfamethoxazole	>4	>4	≤0.12 to >4	36.1	0.0	63.9
Trimethoprim	>8	>8	0.12 to >8	37.0[b]		63.0
Fosfomycin	0.5	8	0.25 to >256	91.8[b]		8.2
Mecillinam	0.5	2	0.12 to >32	98.6[b]		1.4

[a] Criteria as published by EUCAST (2022) as available, except for amoxicillin-clavulanate that used CLSI (2012).
[b] Breakpoints for uUTI treatment via oral administration.
[c] Breakpoints for UTI.
[d] Intermediate isolates can be considered as susceptible if an increased drug concentration can be achieved in the site of infection or by increasing the dosing regimen.

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Contact

Rodrigo E. Mendes, Ph.D.
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, Iowa 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: rodrigo-mendes@jmilabs.com

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