In vitro Activity of Gepotidacin against *Escherichia coli* Causing Urinary Tract Infections in the United States, Including Molecularly Characterized, Fluoroquinolone-Resistant Subsets

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Introduction

- Gepotidacin (GSK2140944) is a novel, triazaacenaphthylene, bacterial type IIA topoisomerase inhibitor in Phase 3 clinical trials for the treatment of gonorrhea and uncomplicated urinary tract infection (UTI).
- This new agent inhibits DNA gyrase and type IV topoisomerase by a mechanism of action that is different from that of fluoroquinolones.
- This study characterized fluoroquinolone-not susceptible *Escherichia coli* causing UTI collected from patients with UTIs for a gepotidacin UTI global surveillance study as part of the SENTRY Antimicrobial Surveillance Program and evaluated the *in vitro* activity of gepotidacin and comparators against various phenotypic and genotypic subsets.

Materials and Methods

Bacterial organisms

- A total of 1,035 *E. coli* collected from 38 US sites located in 9 Census regions were included as part of the Gepotidacin Global UTI Surveillance Program for 2019.
- Only isolates responsible for UTI were included; these isolates were cultured from urine samples (85.3%) or urine catheters (14.7%).
- 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.
- 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 mecillinam and fosfomycin and the latter included glucose-6-phosphate (25 µg/mL).
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.
- *E. coli* with MIC results ≥0.5 µg/mL for ciprofloxacin and/or ≥1 µg/mL for levofloxacin (not susceptible to either one or both agents based on CLSI 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 construction protocol (Illumina, San Diego, CA, USA) following the manufacturer's instructions and were sequenced on MiSeq 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.

Gepotidacin demonstrated potent activity against fluoroquinolone-susceptible and -not susceptible subsets of *E. coli* causing UTI in the US.

In general, gepotidacin inhibited between 90.2% and 100% of QRDR subsets at ≤4 mg/L and MIC values did not seem to be affected by any combinations of QRDR mutations observed in this study.

Higher MIC results were noted for gepotidacin when tested against the rare isolates carrying *qnrB/S* genes.

These data support the clinical development of gepotidacin as a potential treatment option for uUTI caused by fluoroquinolone-susceptible and -not susceptible subsets of *E. coli*.

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 (µg/mL) of:									MIC (µg/mL)	
	0.12	0.25	0.5	1	2	4	8	16	32	50%	90%
FQ-susceptible (758)	1 (0.1)	0 (0.1)	17 (2.4)	208 (29.8)	459 (90.4)	68 (99.3)	5 (100.0)			2	2
FQ-not susceptible (277)		10 (3.6)	28 (13.7)	96 (48.4)	105 (86.3)	28 (96.4)	6 (98.6)	3 (99.6)	1 (100.0)	2	4
GyrA (S83L) (8)				3 (37.5)	5 (100.0)					—	—
GyrA (S83L and D87N) (1)						1 (100.0)				—	—
GyrA (S83L) ParC (S80I, S80R, or E84G) (18)[a]			3 (16.7)	13 (88.9)	1 (94.4)	0 (94.4)	0 (94.4)	1 (100.0)		1	2
GyrA (S83L and D87N/Y) ParC (S80I) (41)[b]		1 (2.4)	4 (12.2)	17 (53.7)	11 (80.5)	4 (90.2)	3 (97.6)	0 (97.6)	1 (100.0)	1	4
GyrA (S83L and D87N) ParC (S80I) ParE (L416F) (57)[c]		7 (12.3)	12 (33.3)	30 (86.0)	7 (98.2)	1 (100.0)				1	2
GyrA (S83L and D87N) ParC (S80I and E84A/G/K/V) (146)[d]		2 (1.4)	9 (7.5)	33 (30.1)	81 (85.6)	21 (100.0)				2	4
GyrA (S83L and D87N) ParC (S80I and E84V) ParE (D420N) (2)						1 (50.0)	1 (100.0)			—	—
WT for QRDR (4)[e]							2 (50.0)	2 (100.0)		—	—
Qnr (12)[f]			1 (8.3)	0 (8.3)	2 (25.0)	2 (41.7)	3 (66.7)	3 (91.7)	1 (100.0)	8	16

FQ, fluoroquinolone; 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; QRDR, quinolone resistance-determining region.
[a] Includes 1 isolate each with QnrB19 (gepotidacin MIC 2 µg/mL) and QnrS1 (gepotidacin MIC 16 µg/mL).
[b] Includes 1 isolate each with QnrB6 (gepotidacin MIC 8 µg/mL) and QnrS1 (gepotidacin MIC 32 µg/mL).
[c] Includes 1 isolate each with QnrB4 (gepotidacin MIC 2 µg/mL) and QnrS1 (gepotidacin MIC 0.5 µg/mL).
[d] Includes 2 isolates each with QnrB19 (gepotidacin MIC 4 µg/mL for both).
[e] Includes 1 isolate with QnrB6 and 3 isolates with QnrS1 and WT sequences for QRDR in GyrA, GyrB, ParC, and ParE.
[f] Includes QnrB4 (1), QnrB6 (2), QnrB19 (3), and QnrS1 (6) with or without mutations in QRDR.

Figure 1. Proportions of *E. coli* causing UTI and showing a not susceptible phenotype to either one or both fluoroquinolones within each of the 9 US Census regions

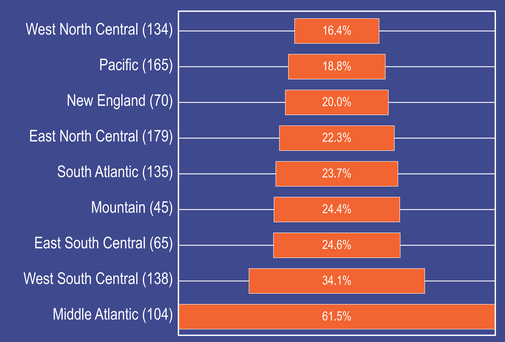

Table 2. Activity of gepotidacin and comparator agents against various subsets of *E. coli*

Antimicrobial agent	MIC (µg/mL)			CLSI[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Fluoroquinolone-susceptible (758)						
Gepotidacin	2	2	0.12 to 8			
Ampicillin	4	>64	≤1 to >64	57.3	0.3	42.5
A/C	4	16	0.5 to >32	86.5	9.5	4.0
Cefazolin	2	16	1 to >32	93.3 [b] 93.3 [c]		6.7 6.7
Ciprofloxacin	0.008	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	32	≤2 to >128	98.8	0.5	0.7
Trimethoprim-sulfamethoxazole	≤0.12	>16	≤0.12 to >16	75.8		24.2
Trimethoprim	0.5	>8	0.03 to >8	75.3		24.7
Fosfomycin	0.5	1	0.25 to >256	99.9	0.0	0.1
Mecillinam	0.5	8	0.06 to >32	92.7	2.1	5.1
Fluoroquinolone-not susceptible (277)						
Gepotidacin	2	4	0.25 to 32			
Ampicillin	>64	>64	≤1 to >64	15.5	0.0	84.5
Amoxicillin/clavulanate	8	16	1 to >32	59.6	30.7	9.7
Cefazolin	>32	>32	≤0.5 to >32	45.1 [b] 45.1 [c]		54.9 54.9
Ciprofloxacin	>4	>4	0.25 to >4	0.4	7.2	92.4
Levofloxacin	8	32	0.25 to >32	6.9	2.9	90.3
Nitrofurantoin	16	32	≤2 to 128	94.2	3.6	2.2
Trimethoprim-sulfamethoxazole	>16	>16	≤0.12 to >16	37.0		63.0
Trimethoprim	>8	>8	0.06 to >8	36.5		63.5
Fosfomycin	0.5	2	0.25 to >256	98.2	0.4	1.4
Mecillinam	1	8	0.12 to >32	92.8	1.4	5.8
Plasmid-encoded genes (12)						
Gepotidacin	8	16	0.5 to 32			
Ampicillin	>64	>64	2 to >64	8.3	0.0	91.7
Amoxicillin/clavulanate	8	32	2 to 32	58.3	25.0	16.7
Cefazolin	>32	>32	1 to >32	25.0 [b] 25.0 [c]		75.0 75.0
Ciprofloxacin	>4	>4	0.5 to >4	0.0	16.7	83.3
Levofloxacin	16	>32	0.5 to >32	25.0	0.0	75.0
Nitrofurantoin	16	16	8 to 128	91.7	0.0	8.3
Trimethoprim-sulfamethoxazole	>16	>16	≤0.12 to >16	25.0		75.0
Trimethoprim	>8	>8	0.06 to >8	25.0		75.0
Fosfomycin	0.5	4	0.25 to 32	100.0	0.0	0.0
Mecillinam	2	32	0.25 to >32	83.3	0.0	16.7

[a] Criteria as published by CLSI M100 (2021).
[b] Using breakpoints as a surrogate test to predict susceptibility results to oral cephalosporins for treating uncomplicated UTI.
[c] Using breakpoints for parenteral therapy of uncomplicated UTI. Based on dosage regimen of 1 gram every 12 hours.

Results

- A total of 26.8% (277/1,035) *E. coli* isolates were not susceptible to either ciprofloxacin or levofloxacin or both (Tables 1 and 2). In general, this phenotype was noted in between 16.4% and 24.6% of *E. coli* from most US Census regions. However, the West South Central (34.1%) and Middle Atlantic (61.5%) regions had the highest rates of *E. coli* not susceptible to fluoroquinolones (Figure 1).
- In general, a total of eight QRDR genotype patterns were observed among isolates not susceptible to fluoroquinolones (Table 1).
  - Among these, most isolates (52.7%; 146/277) had double mutations in GyrA and ParC, followed by isolates (20.6%; 57/277) with double mutations in GyrA and single mutations in ParC and ParE (Table 1).
  - The third most common genotype was represented by isolates (14.8%; 41/277) with double mutations in GyrA and a single mutation in ParC (Table 1).
- Gepotidacin had MIC_{50} and MIC_{90} values of 2 µg/mL when tested against isolates susceptible to both ciprofloxacin and levofloxacin (Tables 1 and 2). Other agents, such as oral cephalosporins, nitrofurantoin, fosfomycin, and mecillinam were active (between 92.7% and 99.9% susceptible) against this fluoroquinolone-susceptible subset.
  - Amoxicillin-clavulanate (86.5% susceptible) and trimethoprim-sulfamethoxazole (75.8% susceptible) had more limited activity against fluoroquinolone-susceptible isolates (Table 2).
- Overall, gepotidacin had MIC_{50} values of 1 µg/mL or 2 µg/mL and MIC_{90} values of 2 µg/mL or 4 µg/mL when tested against phenotypic or genotypic subsets of isolates not susceptible to fluoroquinolones and containing various combinations of QRDR mutations (with or without plasmid-encoded resistance genes) (Table 1).
  - Among 277 *E. coli* isolates not susceptible to fluoroquinolones, four (1.4%) had wildtype sequences for QRDR and carried *qnrB6* (1 isolate) or *qnrS1* (3 isolates). These isolates showed a gepotidacin MIC of 8 µg/mL or 16 µg/mL, respectively (Table 1).
  - A total of 12 (4.3%) isolates carried *qnrB* (6) or *qnrS* (6) with or without mutations in QRDR, and gepotidacin ($MIC_{50/90}$, 8/16 mg/L) had MIC results of 0.5–32 mg/L against this subset (Table 1).
- Against the fluoroquinolone-not susceptible subset fosfomycin (98.2% susceptible), nitrofurantoin (94.2% susceptible), and mecillinam (92.8% susceptible) were active, whereas amoxicillin-clavulanate (59.6% susceptible) and trimethoprim-sulfamethoxazole (37.0% susceptible) had limited activity (Table 2).

Acknowledgements

This study at JMI Laboratories was supported by GlaxoSmithKline. JMI Laboratories received compensation fees for services in relation to preparing the 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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