Activity of Gepotidacin Tested Against Molecularly Characterized *Escherichia coli* Isolates Resistant to Commonly Used Oral Therapies for UTI in the US (2019–2020)

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Introduction

- Gepotidacin is a novel, bactericidal, first-in-class triazaacenaphthylene antibiotic that inhibits bacterial DNA replication by a distinct mechanism of action, which confers activity against most strains of target pathogens, such as *Escherichia coli*, *Staphylococcus saprophyticus*, and *Neisseria gonorrhoeae*, including those resistant to current antibiotics.
- This study evaluates the epidemiology of community-acquired UTI *E. coli* included in the Gepotidacin Global Surveillance Study as part of the SENTRY Antimicrobial Surveillance Program.
- In addition, the activity of gepotidacin and comparators were investigated against various subsets, including those with characterized β-lactam resistance mechanisms.

Materials and Methods

Bacterial organisms

- A total of 1,993 *E. coli* collected from 45 US sites located in 9 Census Regions were included as part of the Gepotidacin Global Surveillance Program during 2019–2020.

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, USA) and contained cation-adjusted Mueller-Hinton broth. Agar dilution plates were used for testing fosfomycin and included glucose-6-phosphate (25 mg/L).
- *E. coli* with MIC results ≥2 mg/L for aztreonam and/or ceftazidime and/or ceftriaxone were defined as presumptive ESBL producers and subjected to genome sequencing followed by β-lactamase gene screening and epidemiology typing (MLST, O:H, and *fimH*).

Screening of β-lactamase genes

- Selected isolates had total genomic DNA extracted and sequenced on MiSeq Sequencer platforms at JMI Laboratories.
- FASTQ format sequencing files for each sample set were assembled independently using *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 β-lactamase genes.

Epidemiology typing

- Isolates that met the criteria for the screening of ESBL genes were subjected to MLST and O:H typing. Isolates associated with ST131 were also subjected to *fimH* typing.

Results

- A total of 84.4% (1,682/1,993) *E. coli* isolates did not meet the criteria for screening of β-lactamase genes (ESBL negative) (Table 1). Among these isolates, 15.9% (267/1,682) were not susceptible to the fluoroquinolones ciprofloxacin and/or levofloxacin, whereas 26.2% (440/1,682) were not susceptible to trimethoprim-sulfamethoxazole (SXT).
- A presumptive ESBL phenotype was noted in 15.6% (311/1,993) of *E. coli*, with the highest rates observed in the Middle Atlantic (49.7%), East South Central (21.3%), and Pacific (19.6%) regions. Other regions had rates of 6.2–15.9% (Figure 1).
- The *E. coli* isolates with a presumptive ESBL phenotype were mostly not susceptible to the fluoroquinolones (78.8%), SXT (61.7%), amoxicillin-clavulanate (52.9%), and the oral cephalosporins (99.7%) (Table 2).
- Most ESBL isolates carried CTX-M alleles alone (81.4%; 253/311), whereas 9.6% (30/311) had plasmid AmpC genes, including 1 strain from Washington state that carried both bla_{CMY-2} and bla_{DHA-1}.
- Four *E. coli* isolates carried combinations of CTX-M and CMY-encoding genes, and 1 strain from Texas had an NDM-5 gene. A β-lactamase gene could not be detected in 21 (6.8%) of the 311 screened isolates.
- Approximately half (56.3%; 175/311) of the presumptively ESBL isolates belonged to CC131, of which 70.9% (124/175) were O25b:H4 and carried *fimH*30.
- Gepotidacin inhibited 98.8% and 96.1% of non-ESBL and ESBL isolates, respectively, at ≤4 mg/L, as well as all but 1 ESBL isolate belonging to the CC131 lineage (Table 1).
- Several antimicrobial agents tested showed activity against *E. coli* isolates that did not meet the criteria for β-lactamase gene screening; however, ampicillin, amoxicillin-clavulanate, ciprofloxacin, and SXT had suboptimal (i.e. <90% susceptible) susceptibility rates (55.7–86.5% susceptible) (Table 2).
- Gepotidacin ($MIC_{50/90}$, 2/4 mg/L) and fosfomycin ($MIC_{50/90}$, 0.5/2 mg/L; 98.4–98.9% susceptible) showed the lowest MIC results against the presumptive ESBL and CTX-M producers as well as the CC131 and non-CC131 subsets. Nitrofurantoin (90.3–95.6% susceptible) was also active *in vitro* against these subsets, despite MIC results ($MIC_{50/90}$, 16/32 mg/L) at least 8-fold higher than gepotidacin and fosfomycin (Table 2).

- **High rates of *E. coli* that were not susceptible to commonly used oral agents (e.g., FQs and SXT) were observed, plus a presumptive ESBL phenotype further compromised the activity of oral agents, including oral cephalosporins.**
- **Gepotidacin had potent and stable *in vitro* activity against various subsets, including the resistant CC131 O25b:H4 clone.**
- **These data support the further clinical development of gepotidacin as a treatment option for uUTI caused by *E. coli*, including resistant isolates against which other oral treatment options are limited.**

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

E. coli phenotype/genotype (No. isolates)	Number (cumulative %) of isolates inhibited by gepotidacin at MIC (mg/L) of:										MIC (mg/L)	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	50%	90%
Non-ESBL (1,682)	1 (0.1)	4 (0.3)	14 (1.1)	76 (5.6)	548 (38.2)	910 (92.3)	109 (98.8)	19 (99.9)	1 (100)		2	2
FQ-NS (267)		2 (0.7)	12 (5.2)	35 (18.4)	88 (51.3)	96 (87.3)	24 (96.3)	9 (99.6)	1 (100)		1	4
SXT-NS (440)		2 (0.5)	5 (1.6)	35 (9.5)	162 (46.4)	195 (90.7)	29 (97.3)	11 (99.8)	1 (100)		2	2
ESBL (311)	2 (0.6)	2 (1.3)	3 (2.3)	28 (11.3)	103 (44.4)	124 (84.2)	37 (96.1)	7 (98.4)	4 (99.7)	1 (100)	2	4
FQ-NS (245)	2 (0.8)	2 (1.6)	3 (2.9)	20 (11.0)	85 (45.7)	94 (84.1)	29 (95.9)	5 (98.0)	4 (99.6)	1 (100)	2	4
SXT-NS (192)		3 (1.6)	3 (3.1)	17 (12.0)	65 (45.8)	74 (84.4)	20 (94.8)	7 (98.4)	2 (99.5)	1 (100)	2	4
CTX-M[a] (253)		2 (1.6)	3 (2.8)	26 (13.0)	91 (49.0)	96 (87.0)	26 (97.2)	5 (99.2)	1 (99.6)	1 (100.0)	2	4
CMY/DHA[b] (30)					6 (20.0)	15 (70.0)	7 (93.3)	1 (96.7)	1 (100.0)		2	4
CC131[c] (175)		1 (0.6)	0 (0.6)	16 (9.7)	62 (45.1)	76 (88.6)	19 (99.4)	1 (100.0)			2	4
O25b:H4/*fimH*30 (124)				8 (6.5)	42 (40.3)	58 (87.1)	15 (99.2)	1 (100.0)			2	4
O16:H5/*fimH*41 (26)				8 (30.8)	10 (69.2)	7 (96.2)	1 (100.0)				2	2
Non-CC131 (136)	2 (1.5)	1 (2.2)	3 (4.4)	12 (13.2)	41 (43.4)	48 (78.7)	18 (91.9)	6 (96.3)	4 (99.3)	1 (100.0)	2	4

ESBL, extended-spectrum -β-lactamase; FQ, fluoroquinolones (not susceptible to ciprofloxacin and/or levofloxacin); SXT, trimethoprim-sulfamethoxazole; NS, not susceptible; CC, clonal complex.
[a] Includes 149 $bla_{CTX-M-15}$, 69 $bla_{CTX-M-27}$, 24 $bla_{CTX-M-14}$, and 11 isolates with other bla_{CTX-M} alleles.
[b] Includes 22 bla_{CMY}, 7 bla_{DHA}, and 1 isolate with both bla_{CMY} and bla_{DHA} alleles.
[c] Includes clonal complex 131, which is represented by ST131 (164 isolates), ST131-like (5), ST2279 (3), and ST8671 (3).

Figure 1. Proportions of presumptive ESBL-producing *E. coli* causing UTI in the 9 US Census Regions. The proportion of these isolates belonging to the CC131 clone are also shown.

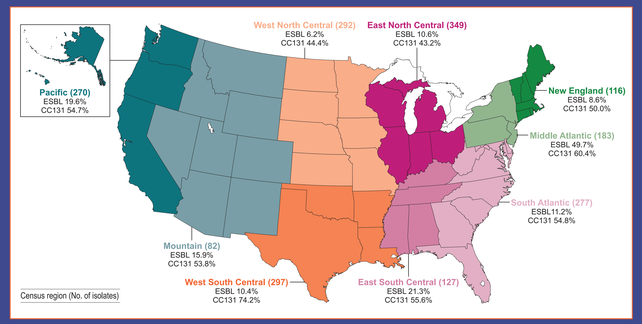

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Table 2. Activity of gepotidacin and comparator agents against various subsets of *E. coli*

Antimicrobial agent	MIC (mg/L)			CLSI[a]		
	50%	90%	Range	%S	%I	%R
Non-ESBL (1,682)						
Gepotidacin	2	2	0.06 to 16			
Ampicillin	8	>64	≤1 to >64	55.7	0.8	43.5
A/C	4	16	0.5 to >32	86.5	11.1	2.4
Cefazolin	2	8	≤0.5 to >32	96.5 [b]		3.5
Ciprofloxacin	0.015	>4	≤0.002 to >4	84.5	1.7	13.9
Nitrofurantoin	16	32	≤2 to >128	98.3	0.9	0.8
SXT	≤0.12	>4	≤0.12 to >4	73.8		26.2
Fosfomycin	0.5	1	0.25 to >256	99.8	0.2	0.1
Aztreonam	≤0.06	0.12	≤0.06 to 1	100.0	0.0	0.0
Ceftriaxone	0.12	0.25	≤0.03 to 1	100.0	0.0	0.0
Ceftazidime	0.12	0.5	0.03 to 1	100.0	0.0	0.0
ESBL (311)						
Gepotidacin	2	4	≤0.03 to 32			
Ampicillin	>64	>64	8 to >64	0.3	0.0	99.7
A/C	16	32	2 to >32	47.1	31.0	21.9
Cefazolin	>32	>32	16 to >32	0.3 [b]		99.7
Ciprofloxacin	>4	>4	0.004 to >4	21.0	3.9	75.2
Nitrofurantoin	16	32	≤2 to >128	92.6	3.5	3.9
SXT	>4	>4	≤0.12 to >4	38.3		61.7
Fosfomycin	0.5	2	0.25 to >256	98.7	0.0	1.3
Aztreonam	>16	>16	0.12 to >16	18.3	17.0	64.6
Ceftriaxone	>8	>8	0.12 to >8	6.8	1.3	92.0
Ceftazidime	16	>32	0.25 to >32	28.7	15.2	56.1
CTX-M (253)[c]						
Gepotidacin	2	4	≤0.03 to 32			
Ampicillin	>64	>64	>64	0.0	0.0	100.0
A/C	8	16	2 to >32	56.3	37.3	6.3
Cefazolin	>32	>32	32 to >32	0.0 [b]		100.0
Ciprofloxacin	>4	>4	0.004 to >4	12.7	3.2	84.1
Nitrofurantoin	16	32	≤2 to >128	92.5	3.6	4.0
SXT	>4	>4	≤0.12 to >4	34.8		65.2
Fosfomycin	0.5	2	0.25 to >256	98.4	0.0	1.6
Aztreonam	>16	>16	0.5 to >16	10.3	16.2	73.5
Ceftriaxone	>8	>8	>8	0.0	0.0	100.0
Ceftazidime	16	>32	0.25 to >32	27.7	16.6	55.7
CC131 (175)[c]						
Gepotidacin	2	4	0.12 to 8			
Ampicillin	>64	>64	>64	0.0	0.0	100.0
A/C	16	16	4 to >32	48.3	42.5	9.2
Cefazolin	>32	>32	32 to >32	0.0 [b]		100.0
Ciprofloxacin	>4	>4	0.015 to >4	4.6	2.9	92.5
Nitrofurantoin	16	32	≤2 to 128	90.3	4.0	5.7
SXT	>4	>4	≤0.12 to >4	36.0		64.0
Fosfomycin	0.5	2	0.25 to >256	98.9	0.0	1.1
Aztreonam	>16	>16	0.25 to >16	8.0	18.3	73.7
Ceftriaxone	>8	>8	0.12 to >8	0.6	1.1	98.3
Ceftazidime	16	>32	0.5 to >32	21.7	16.6	61.7

ESBL, extended spectrum-β-lactamase; A/C, amoxicillin-clavulanate (2:1); SXT, trimethoprim-sulfamethoxazole
[a] Criteria as published by CLSI (2022).
[b] Using breakpoints as a surrogate test to predict susceptibility results to oral cephalosporins for treating uncomplicated UTI.
[c] See footnotes on Table 1 for additional information.

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