# Current Epidemiology and Activity of Gepotidacin and Comparator Agents Against Escherichia coli Surveillance Isolates Causing Urinary Tract Infections in the United States


RE Mendes[1], TB Doyle[1], SJR Arends[1], D Butler[2], N Scangarella-Oman[2], JM Streit[1], M Castanheira[1]

[1]JMI Laboratories, North Liberty, IA [2]GlaxoSmithKline, Collegeville, PA

**PRESENTED AT:**

WORLD MICROBE FORUM
20-24 JUNE 2021 | ONLINE WORLDWIDE
An ASM & FEMS Collaboration

# INTRODUCTION

Gepotidacin is an investigational bacterial type II topoisomerase inhibitor in Phase 3 clinical trials for the treatment of gonorrhea and uncomplicated urinary tract infections (UTI)

Gepotidacin inhibits bacterial DNA gyrase and topoisomerase IV by a unique mechanism

This study evaluated the epidemiology of *Escherichia coli* causing UTI in patients seen in the United States. In addition, this study investigated the activity of gepotidacin and comparators against various subsets, including those with characterized resistance mechanisms

# MATERIALS AND METHODS

## Bacterial organisms

A total of 1,035 *E. coli* (96.1% community-acquired, where epidemiological data is available) cultured from 38 U.S. sites were included as part of the Gepotidacin Global UTI Surveillance Program during 2019

Isolates were mostly (85.3%) cultured from urine samples, and the remaining isolates were cultured from urethral catheters

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 following the 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, as well as agar dilution pates for testing fosfomycin

Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains

Isolates displaying MIC results of ≥2 μg/mL for ceftazidime, aztreonam and/or ceftriaxone were selected for molecular screening of extended-spectrum β-lactamase (ESBL) genes

## Screening of β-lactamase genes

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

## Epidemiology typing

Multilocus sequence typing (MLST) was performed by extracting a defined set of 7 housekeeping gene fragments (~500 bp)

- Each fragment was compared to known allelic variants for each locus (housekeeping gene) on the MLST website (PubMLST, https://pubmlst.org)

An allele sharing 100% genetic identity with a known variant received a numeric designation

- A 7-number sequence (1 for each housekeeping gene) formed an allelic profile, defined as sequence type (ST)
- Isolates containing alleles that did not match an existing sequence in the MLST database were submitted/deposited for allele and ST assignments

The O:H serotyping and fimH typing were performed using tools available at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/ResFinder)

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

# GEPOTIDACIN DEMONSTRATED POTENT ACTIVITY AGAINST VARIOUS E. COLI SUBSETS, INCLUDING THE MDR ST131 O25B:H4 CLONE

## High rates of ESBL-producing *E. coli* were detected in various U.S. Census regions and likely associated with the expansion and dissemination of the ST131 clone

## This novel oral antibiotic may allow for the treatment of these community-acquired infections

Figure 1 Proportions of ESBL-producing *E. coli* causing UTI in the 9 US Census regions. The proportions of ESBL isolates belonging to ST131 are also shown

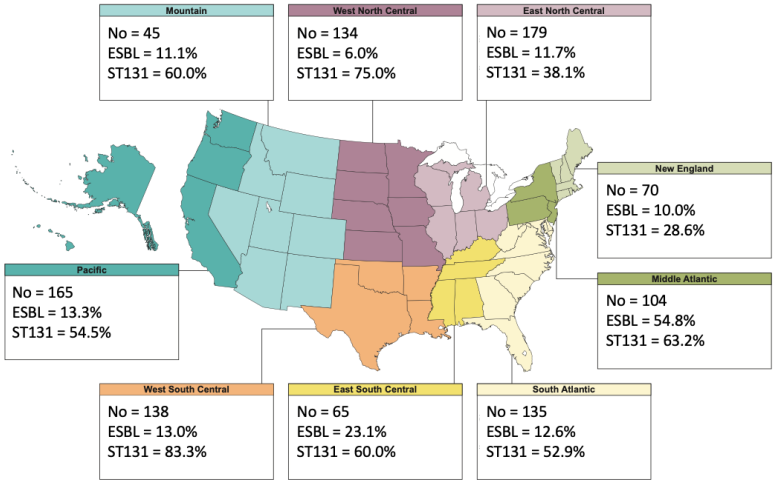


Table 1 MIC distribution of gepotidacin against various subsets of *E. coli*

| | Number and cumulative % of isolates inhibited at gepotidacin MIC (µg/mL) of: | | | | | | | | | MIC (µg/mL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phenotype/Genotype | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 50% | 90% |
| Non-ESBL (865) | 1<br>0.1% | 8<br>1.0% | 31<br>4.6% | 253<br>33.9% | 491<br>90.6% | 73<br>99.1% | 7<br>99.9% | 1<br>100.0% | | 2 | 2 |
| ESBL (170) | | 2<br>1.2% | 14<br>9.4% | 51<br>39.4% | 73<br>82.4% | 23<br>95.9% | 4<br>98.2% | 2<br>99.4% | 1<br>100.0% | 2 | 4 |
| CTX-M[a] (135) | | 2<br>1.5% | 14<br>11.9% | 44<br>44.4% | 57<br>86.7% | 14<br>97.0% | 2<br>98.5% | 1<br>99.3% | 1<br>100.0% | 2 | 4 |
| ST131[b] (100) | | | 7<br>7.0% | 30<br>37.0% | 49<br>86.0% | 13<br>99.0% | 1<br>100.0% | | | 2 | 4 |
| O25b:H4/fimH30[c] (75) | | | 4<br>5.3% | 20<br>32.0% | 40<br>85.3% | 10<br>98.7% | 1<br>100.0% | | | 2 | 4 |
| O16:H5/fimH41[d] (12) | | | 3<br>25.0% | 5<br>66.7% | 4<br>100.0% | | | | | 1 | 2 |
| Non-ST131[e] (70) | | 2<br>2.9% | 7<br>12.9% | 21<br>42.9% | 24<br>77.1% | 10<br>91.4% | 3<br>95.7% | 2<br>98.6% | 1<br>100.0% | 2 | 4 |

ESBL, extended spectrum-b-lactamase.
[a] 77 blaCTX-M-15, 43 blaCTX-M-27, 9 blaCTX-M-14, and 6 isolates each had a distinct blaCTX-M allele.
[b] O antigens detected were: O25 (81 isolates), O16 (13), O153 (1) and non-typeable (5).
[c] 47 blaCTX-M-15, 23 blaCTX-M-27, 3 blaCTX-M-14, and 2 isolates had overexpression of AmpC.
[d] 1 blaCTX-M-15, 9 blaCTX-M-27, and 2 blaCTX-M-14.
[e] 35 ST types.

# RESULTS

A total of 83.6% (865/1,035) *E. coli* were ESBL-negative (Table 1). An ESBL phenotype was noted in 16.4% (170/1,035) of *E. coli*, with the highest rates observed in the Mid-Atlantic (54.8%) and East-South Central (23.1%) regions. Other regions had ESBL phenotype rates of 6.0-13.3% (Figure 1)

Most ESBL *E. coli* isolates carried $bla_{\text{CTX-M}}$ alleles (79.4%; 135/170). Of these ESBL isolates, 58.8% (100/170) belonged to ST131, of which 75.0% (75/100) were O25b:H4 and carried *fimH*30 (Table 1)

O16:H5 (91.7% $bla_{\text{CTX-M-14/27}}$; data not shown) was the second most common O:H type among ST131 (Table 1)

ESBL-negative *E. coli* showed limited susceptibility to ampicillin (55.0% susceptible), amoxicillin-clavulanate (85.6% susceptible), ciprofloxacin (84.0% susceptible) and trimethoprim-sulfamethoxazole (71.0% susceptible) (Table 2)

Nitrofurantoin and fosfomycin also showed activity (>90% susceptible) against the ESBL subsets, whereas other agents had limited activity (Table 2)

In general, gepotidacin had consistent $MIC_{50}$ results of 2 µg/mL and $MIC_{90}$ of 2-4 µg/mL against non-ESBL, ESBL isolates and its subsets, except against isolates representing the O16H:5 subset, against which $MIC_{50/90}$ of 1/2 µg/mL values were observed (Tables 1 and 2)

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

| Antimicrobial agent | MIC (µg/mL) | | | CLSI[a] | | |
|---|---|---|---|---|---|---|
| | 50% | 90% | Range | %S | %I | %R |
| Non-ESBL (865) | | | | | | |
| Gepotidacin | 2 | 2 | 0.12 to 16 | | | |
| Ampicillin | 8 | >64 | ≤1 to >64 | 55 | 0.2 | 44.7 |
| A/C | 4 | 16 | 0.5 to >32 | 85.6 | 11.9 | 2.4 |
| Cefazolin | 2 | 8 | ≤0.5 to >32 | 96.2[b]<br>96.2[c] | | 3.8<br>3.8 |
| Ciprofloxacin | 0.015 | >4 | ≤0.002 to >4 | 84 | 1.7 | 14.2 |
| Nitrofurantoin | 16 | 32 | ≤2 to >128 | 98.6 | 0.8 | 0.6 |
| TMP-SMX | ≤0.12 | >16 | ≤0.12 to >16 | 71 | | 29 |
| Fosfomycin | 0.5 | 1 | 0.25 to >256 | 99.8 | 0.1 | 0.1 |
| Aztreonam | 0.12 | 0.25 | ≤0.03 to 1 | 100 | 0 | 0 |
| Ceftriaxone | ≤0.06 | 0.12 | ≤0.06 to 0.5 | 100 | 0 | 0 |
| Ceftazidime | 0.12 | 0.5 | 0.03 to 1 | 100 | 0 | 0 |
| ESBL (170) | | | | | | |
| Gepotidacin | 2 | 4 | 0.25 to 32 | | | |
| Ampicillin | >64 | >64 | 8 to >64 | 0.6 | 0 | 99.4 |
| A/C | 16 | 32 | 2 to >32 | 47.1 | 31.8 | 21.2 |
| Cefazolin | >32 | >32 | 32 to >32 | 0.0[b]<br>0.0[c] | | 100<br>100 |
| Ciprofloxacin | >4 | >4 | 0.008 to >4 | 18.8 | 2.9 | 78.2 |
| Nitrofurantoin | 16 | 32 | ≤2 to 128 | 92.4 | 4.1 | 3.5 |
| TMP-SMX | >16 | >16 | ≤0.12 to >16 | 37.1 | | 62.9 |
| Fosfomycin | 0.5 | 2 | 0.25 to >256 | 97.6 | 0 | 2.4 |
| Aztreonam | 16 | >16 | 0.12 to >16 | 14.7 | 23.5 | 61.8 |
| Ceftriaxone | >8 | >8 | 0.25 to >8 | 4.7 | 1.8 | 93.5 |
| Ceftazidime | 16 | >32 | 0.5 to >32 | 27.8 | 15.4 | 56.8 |
| CTX-M (135) | | | | | | |
| Gepotidacin | 2 | 4 | 0.25 to 32 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ampicillin | >64 | >64 | >64 to >64 | 0 | 0 | 100 |
| A/C | 8 | 16 | 2 to 32 | 56.3 | 39.3 | 4.4 |
| Cefazolin | >32 | >32 | >32 to >32 | 0.0 [b] | | 100 |
| | | | | 0.0 [c] | | 100 |
| Ciprofloxacin | >4 | >4 | 0.008 to >4 | 9.6 | 2.2 | 88.1 |
| Nitrofurantoin | 16 | 32 | ≤2 to 128 | 92.6 | 4.4 | 3 |
| TMP-SMX | >16 | >16 | ≤0.12 to >16 | 32.6 | | 67.4 |
| Fosfomycin | 0.5 | 2 | 0.25 to >256 | 97 | 0 | 3 |
| Aztreonam | >16 | >16 | 2 to >16 | 8.1 | 21.5 | 70.4 |
| Ceftriaxone | >8 | >8 | >8 to >8 | 0 | 0 | 100 |
| Ceftazidime | 16 | >32 | 0.5 to >32 | 28.9 | 16.3 | 54.8 |
| ST131 (100) | | | | | | |
| Gepotidacin | 2 | 4 | 0.5 to 8 | | | |
| Ampicillin | >64 | >64 | >64 to >64 | 0 | 0 | 100 |
| A/C | 16 | 16 | 4 to >32 | 48 | 43 | 9 |
| Cefazolin | >32 | >32 | >32 to >32 | 0.0 [b] | | 100 |
| | | | | 0.0 [c] | | 100 |
| Ciprofloxacin | >4 | >4 | 0.12 to >4 | 2 | 2 | 96 |
| Nitrofurantoin | 16 | 32 | ≤2 to 128 | 90 | 4 | 6 |
| TMP-SMX | >16 | >16 | ≤0.12 to >16 | 32 | | 68 |
| Fosfomycin | 0.5 | 2 | 0.5 to >256 | 98 | 0 | 2 |
| Aztreonam | >16 | >16 | 2 to >16 | 7 | 25 | 68 |
| Ceftriaxone | >8 | >8 | 2 to >8 | 0 | 2 | 98 |
| Ceftazidime | 16 | >32 | 1 to >32 | 25 | 17 | 58 |
| Non-ST131 | | | | | | |
| Gepotidacin | 2 | 4 | 0.25 to 32 | | | |
| Ampicillin | >64 | >64 | 8 to >64 | 1.4 | 0 | 98.6 |
| A/C | 16 | >32 | 2 to >32 | 45.7 | 15.7 | 38.6 |
| Cefazolin | >32 | >32 | 32 to >32 | 0.0 [b] | | 100 |
| | | | | 0.0 [c] | | 100 |
| Ciprofloxacin | >4 | >4 | 0.008 to >4 | 42.9 | 4.3 | 52.9 |
| Nitrofurantoin | 16 | 32 | 8 to 64 | 95.7 | 4.3 | 0 |
| TMP-SMX | >16 | >16 | ≤0.12 to >16 | 44.3 | | 55.7 |
| Fosfomycin | 0.5 | 2 | 0.25 to >256 | 97.1 | 0 | 2.9 |
| Aztreonam | 16 | >16 | 0.12 to >16 | 25.7 | 21.4 | 52.9 |
| Ceftriaxone | >8 | >8 | 0.25 to >8 | 11.4 | 1.4 | 87.1 |
| Ceftazidime | 16 | >32 | 0.5 to >32 | 31.9 | 13 | 55.1 |

ESBL, extended spectrum-β-lactamase; A/C, amoxicillin-clavulanate (2:1); TMP-SMX, trimethoprim-sulfamethoxazole

[a] Criteria as published by CLSI (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

# CONCLUSIONS

Rates of ESBL-producing *E. coli* clinical isolates causing UTI of up to approximately 55% were detected within the US Census regions

Isolates associated with the ST131 clonal lineage represented the majority of ESBL *E. coli* in 7 out of 9 US Census regions, demonstrating the expansion of this clone as a major factor contributing to rising ESBL rates

Gepotidacin demonstrated potent activity against non-ESBL and ESBL *E. coli*, and various subsets, including the multidrug-resistant (MDR) ST131 O25b:H4 clone

A limited number of oral antimicrobial agents remained active against these subsets of *E. coli*, challenging the emperic treatment of these infections and potentially requiring the use of parenteral drugs

These data warrant the clinical development of gepotidacin as an oral treatment option for UTI caused by *E. coli*, including where other oral treatment options are limited due to resistance

## Acknowledgements

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.

# DISCLOSURES

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

N Scangarella-Oman and D Butler are employees and share holders of GlaxoSmithKline

# REFERENCES

Clinical and Laboratory Standards Institute (2018). M07Ed11. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M07 Eleventh Edition. Wayne, PA, USA.

Clinical and Laboratory Standards Institute (2021). Performance standards for antimicrobial susceptibility testing. M100 31st Edition. Wayne, PA, USA.

Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F (2015). Rapid and easy in silico serotyping of Escherichia coli isolates by use of whole-genome sequencing data. Journal of Clinical Microbiology 53: 2410-2426.

Mendes RE, Jones RN, Woosley LN, Cattoir V, Castanheira M (2019). Application of next-generation sequencing for characterization of surveillance and clinical trial isolates: Analysis of the distribution of b-lactamase resistance genes and lineage background in the United States. Open Forum Infect Dis 6: S69-S78.

Roer L, Tchesnokova V, Allesøe R, Muradova M, Chattopadhyay S, Ahrenfeldt J, Thomsen MCF, Lund O, Hansen F, Hammerum AM, Sokurenko E, Hasman H (2017). Development of a web tool for Escherichia coli subtyping based on fimH alleles. J Clin Microbiol 55: 2538-2543.