Frequency of Spontaneous Resistance for Gepotidacin and Levofloxacin Against a Collection of Gram-Negative and Gram-Positive Organisms via Single-Step Mutational Analysis

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Introduction

Gepotidacin (GSK2140944) is a novel, bactericidal, first in class triazaacenaphthylene antibiotic in clinical development for the treatment of gonorrhea and uncomplicated urinary tract infection (acute cystitis).

Gepotidacin selectively inhibits bacterial DNA replication by a distinct mechanism of action, which confers *in vitro* activity against most strains of target pathogens, such as *E. coli, S. saprophyticus* and *N. gonorrhoeae,* including those resistant to current antibiotics.

The purpose of this study was to evaluate the potential of gepotidacin and levofloxacin to select for spontaneous resistance in certain gram-negative and gram-positive bacterial isolates. Mutants recovered from gepotidacin selection were characterized by whole genome sequencing and single nucleotide polymorphism analysis.

Materials and Methods

The activities of gepotidacin and levofloxacin were evaluated against 3 clinical isolates from each of the following species: *Citrobacter freundii*, *Enterobacter cloacae* species complex, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Providencia rettgeri*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus*.

All isolates were collected in 2019.

The organisms tested included a mixture of wild-type, extended-spectrum, beta-lactamase-producing, and fluoroquinolone-resistant phenotypes.

Baseline agar dilution MIC values were determined in triplicate using Clinical and Laboratory Standards Institute (CLSI) M07Ed11 (2018) methods using Mueller-Hinton agar.

Spontaneous resistance frequencies were determined by plating bacterial suspensions on Mueller-Hinton agar containing gepotidacin or levofloxacin at 4x and 10x their respective MICs. After 48 hrs ambient incubation at 35°C, resulting colonies were passaged twice on drug-free media. Resistant colonies were confirmed (≥4-fold increase in MIC) and assessed for cross resistance to azithromycin, aztreonam, ceftazidime, levofloxacin, linezolid, meropenem, nitrofurantoin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin by broth microdilution MIC testing.

For Whole Genome Sequencing (WGS), gepotidacin resistant mutants and their corresponding parent isolates were sequenced using a combination of short- (Nextera XT™/ Illumina) and long-read (Qiagen Genomic Tip 100 / MinION Nanopore) sequencing methods. Each sample set was assembled independently using *de novo* assembler SPAdes 3.13.0. The WGS of select mutants enabled characterization of the target genes (*gyrA/B*, *parC/E*, and *grlA/B*) as well as single nucleotide polymorphisms (SNPs).

Disclosures

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

No spontaneous resistance to gepotidacin was observed at 10x MIC concentrations.

Mutant frequencies ranged from $6.1x10^{-8}$ to $<2.5x10^{-10}$ at 4x MIC gepotidacin concentrations.

Reduced susceptibility to other antibiotics was seen for select *P. mirabilis*, *K. pneumoniae*, *P. rettgeri* and *E. cloacae* mutants obtained from gepotidacin 4x MIC plates. These were not target-based mutants.

Target-based mutations were only observed among *E. faecalis* mutants from gepotidacin 4x MIC plates.

For Enterobacterales selected on gepotidacin 4x MIC plates, mutations among potential efflux modulating transcriptional regulators were observed.

Table 1 Summary of gepotidacin and levofloxacin mutation frequencies by species

	Range of Mutant frequencies with:			
Organism (n)	Gepotidacin at 4x MIC	Gepotidacin at 10x MIC	Levofloxacin at 4x MIC	Levofloxacin at 10x MIC
C. freundii (3)	$2.7x10^{-8}$ to $<5.0x10^{-9}$	$<5.4x10^{-9}$ to $<8.3x10^{-10}$	$<9.2x10^{-9}$ to $<4.5x10^{-10}$	$<8.3x10^{-10}$ to $<4.5x10^{-10}$
E. cloacae sc (3)	$6.1x10^{-8}$ to $<2.5x10^{-10}$	$<6.5x10^{-10}$ to $<2.5x10^{-10}$	$5.8x10^{-10}$ to $<2.5x10^{-10}$	$<6.5x10^{-10}$ to $<2.5x10^{-10}$
K. aerogenes (3)	$<4.7x10^{-10}$ to $<3.7x10^{-10}$	$<4.7x10^{-10}$ to $<3.7x10^{-10}$	$5.9x10^{-9}$ to $4.0x10^{-10}$	$<4.7x10^{-10}$ to $<3.7x10^{-10}$
K. pneumoniae (3)	$2.7x10^{-8}$ to $<6.6x10^{-10}$	$<5.5x10^{-9}$ to $<5.7x10^{-10}$	$2.8x10^{-8}$ to $<5.0x10^{-10}$	$1.4x10^{-8}$ to $<5.0x10^{-10}$
P. mirabilis (3)	$7.1x10^{-10}$ to $<5.4x10^{-10}$	$<7.1x10^{-10}$ to $<5.4x10^{-10}$	$<7.1x10^{-10}$ to $<5.4x10^{-10}$	$<7.1x10^{-10}$ to $<5.4x10^{-10}$
P. rettgeri (3)	$7.8x10^{-9}$ to $3.5x10^{-9}$	$<5.4x10^{-10}$ to $<4.3x10^{-10}$	$5.2x10^{-9}$ to $<4.6x10^{-10}$	$<5.4x10^{-10}$ to $<4.3x10^{-10}$
E. faecalis (3)	$1.7x10^{-8}$ to $<3.2x10^{-10}$	$<8.3x10^{-9}$ to $<3.2x10^{-10}$	$<8.3x10^{-9}$ to $<3.2x10^{-10}$	$<7.6x10^{-10}$ to $<3.2x10^{-10}$
S. saprophyticus (3)	$<9.2x10^{-10}$ to $<5.9x10^{-10}$	$<9.2x10^{-10}$ to $<5.9x10^{-10}$	$<9.2x10^{-10}$ to $<5.9x10^{-10}$	$<9.2x10^{-10}$ to $<5.9x10^{-10}$

sc, species complex

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Results

Gepotidacin did not select for any mutants on 10x MIC plates for any of the organisms tested, translating to spontaneous resistance frequencies ranging from $<8.3x10^{-9}$ to $<2.5x10^{-10}$ (Table 1). On gepotidacin 4x MIC plates, mutant frequencies ranged from $6.1x10^{-8}$ to $<2.5x10^{-10}$ for all species (Table 1).

Levofloxacin mutant frequencies ranged from $1.4 x10^{-8}$ to $<2.5x10^{-10}$ on 10x MIC plates and $2.8x10^{-8}$ to $<2.5x10^{-10}$ on 4x MIC plates (Table 1).

Reduced susceptibility to levofloxacin (≥4-fold increases in MIC) was observed for 4/10 *E. cloacae* species complex mutants tested, 3/14 *K. pneumoniae* mutants, 1/1 *P. mirabilis* mutant, and 30/31 *P. rettgeri* gepotidacin-selected mutants (data not shown).

WGS revealed target-based mutations (GyrA, $A_{34}V$ and GyrB, $D_{437}V$) among 2 *E. faecalis* mutants (Table 2). No other gepotidacin target-based mutations were observed.

Among the gram-negative isolates sequenced, SNPs within transcriptional regulators (SoxR, TetR/AcrR family, and LysR family) and the *rpoC* gene were detected (Table 2).

Table 2 Single nucleotide polymorphisms detected among select mutants recovered from gepotidacin frequency of resistance studies

Isolate	Species	SNP or location	Gene(s)
135	*E. faecalis*	$D_{437}V$	DNA gyrase subunit B - *gyrB*
136	*E. faecalis*	$A_{34}V$	DNA gyrase subunit A - *gyrA*
162	*C. freundii*	$A_{280}E$	adenylosuccinate lyase - *purB*
		upstream	Endochitinase
		$R_{101}P$	F0F1 ATP synthase subunit alpha - *atpA*
137	*E. cloacae*	upstream	RomA family MBL fold metallo-hydrolase
143	*E. cloacae*	$\Delta T_{36}\ E_{37}\ A_{38}\ G_{39}\ V_{40}\ A_{41}$	TetR/AcrR family transcriptional regulator
210	*K. pneumoniae*	downstream	IS630 family transposase
		$R_{431}P$	DNA-directed RNA polymerase subunit beta - *rpoC*
213	*K. pneumoniae*	downstream	IS630 family transposase
		$K_{334}Q$	DNA-directed RNA polymerase subunit beta - *rpoC*
224	*P. mirabilis*	between	Hypothetical and uncharacterized protein
		$I_{158}F$	MFS transporter
		$R_{20}L$	redox-sensitive transcriptional activator *soxR*
		upstream	TetR/AcrR family transcriptional regulator
		upstream	Hypothetical protein
174	*P. rettgeri*	upstream	response regulator - *uvrY*
		$D_{117}E$, $E_{118}V$, $N_{121}I$	LysR family transcriptional regulator
		upstream	Ubiquinone biosynthesis hydroxylase, *ubiH*
		downstream	type II secretion system F family protein
		downstream	DNA gyrase subunit B - *gyrB*
		upstream	Homoserine trans-succinylase
		R33C	Redox-sensitive transcriptional activator - *soxR*
		upstream	Multidrug resistant protein - *mdtN*
		upstream	Formate dehydrogenase-O major subunit

References

1. Bayliss SC, Hunt VL, Yokoyama M, Thorpe HA and Feil EJ.(2017) The use of Oxford Nanopore native barcoding for complete genome assembly. *Gigascience* 6: 1-6.
2. CLSI. M07ED11 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: eleventh edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2018.
3. CLSI. M100Ed31. Performance standards for antimicrobial susceptibility testing: 31st informational supplement. Wayne, PA, Clinical and Laboratory Standards Institute, 2021.

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