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Eravacycline is active against bacterial isolates carrying emergent resistance types

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Abstract

Background: Transferable and mutation-based resistance amongst clinically relevant Gram-negative pathogens remains an ongoing impediment to the development of novel antimicrobial therapies. While there are a number of new agents in development for the treatment of multidrug-resistant and carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* (CRAB), coverage gaps due to resistance persist. Eravacycline was screened against a number of Enterobacteriaceae and *Acinetobacter* isolates from the CDC antimicrobial resistance bank and clinical sources and analyzed for activity against various resistance types. Eravacycline maintained potency against isolates carrying RNA methylase genes, genes conferring fosfomycin resistance, tetracycline efflux pump genes, and porin mutations, and those isolates resistant to ceftazidime-avibactam (Caz-Avi).

Materials/methods: Susceptibility testing by broth microdilution minimal inhibitory concentration (MIC) assays was done using CLSI methodology. Isolates screened were from the CDC Antimicrobial Resistance Bank panels and the laboratory of Dr. Patrice Nordmann. Isolate resistance information is as provided by CDC whole genome sequencing data (RNA methylase, porin mutation, fosfomycin resistance) or PCR and sequencing performed for this study.

Results: The eravacycline $MIC_{50/90}$ values for total *Enterobacteriaceae*, total *Acinetobacter*, and resistance subgroups are presented in the table. Overall eravacycline maintained an MIC_{50} range of 0.25-0.5 and MIC_{90} range of 1-2 µg/ml for all groups screened.

Conclusions: Eravacycline retained potency *in vitro* against a number of emergent resistance types.

Resistance Group (n)	Eravacycline $MIC_{50/90}$ (Range) in µg/ml	Resistance Group (n)	Eravacycline $MIC_{50/90}$ (Range) in µg/ml
Total Enterobacteriaceae (176)	0.25/2 (0.016 – 8)	Total *Acinetobacter* (55)	0.5/1 (0.031 – 2)
Enterobacteriaceae RNA methylase (26)	0.5/2 (0.031 – 4)	*Acinetobacter* RNA methylase (21)	0.5/1 (0.25 – 2)
Enterobacteriaceae *fosA* (22)	0.25/1 (0.031 – 1)	*Acinetobacter* Caz-Avi Resistant (54)	0.5/1 (0.031 – 2)
Enterobacteriaceae metallo-beta-lactamase positive (41)	0.25/2 (0.031 – 4)	*Acinetobacter* Tetracycline Efflux Pump (28)	0.5/1 (0.031 – 2)
Enterobacteriaceae ΔompK35/36 (33)	0.5/2 (0.031 – 4)		
Enterobacteriaceae Caz-Avi Resistant (47)	0.25/2 (0.031 – 4)		
Enterobacteriaceae Tetracycline Efflux Pump (59)	0.5/2 (0.031 – 8)		

Background

Transferable and mutation-based resistance amongst clinically relevant Gram-negative pathogens remains an ongoing impediment to the development of novel antimicrobial therapies. While there are a number of new agents in development for the treatment of multidrug-resistant and carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* (CRAB), coverage gaps due to resistance persist. Novel antimicrobial agents in the aminoglycoside class have been shown to be hindered in activity by transferable 16S ribosomal RNA methyltransferase genes, which are often colocalized with the bla_{NDM} gene in Enterobacteriaceae[1]. Resistance to ceftazidime-avibactam has been reported in a number of species due to mutations in the bla_{KPC} gene or due to porin mutations[2,3]. Porin loss has similarly been associated with reduced activity of the meropenem-vaborbactam combination due to reduced uptake and competition for transport[4]. Increasing resistance to current therapies has renewed interest in older antimicrobial agents such as fosfomycin, however plasmid-mediated fosfomycin resistance genes remain a concern for the increased use of this drug[5].

To better understand how these transferable resistance elements may impact the novel fluorocycline antibiotic eravacycline, it was screened against a number of multidrug resistant Enterobacteriaceae and *Acinetobacter* isolates and analyzed for activity against various resistance types. Eravacycline maintained potency against isolates carrying rRNA methyltransferase genes, transferable fosfomycin resistance, tetracycline efflux pump genes, metallo-beta-lactamase genes, and porin mutations, as well as isolates classified by the CDC as resistant to ceftazidime-avibactam through various known and as yet undefined mechanisms.

Methods

- Susceptibility testing by broth microdilution minimal inhibitory concentration (MIC) assays was done using CLSI methodology[6,7]. Isolates screened were selected from the CDC Antimicrobial Resistance Bank panels and the laboratory of Dr. Patrice Nordmann (University of Fribourg). CDC panel isolates for this study were combined from the Enterobacteriaceae Carbapenem Breakpoint, Gram Negative Carbapenemase Detection, Enterobacteriaceae Carbapenemase Diversity, *Acinetobacter baumannii*, Novel Antibiotic Resistance, Ceftolozane/Tazobactam, Ceftazidime/Avibactam, Enteric Pathogen Diversity, and Imipenem/Relebactam panels. Due to the limited potency of tetracycline antibiotics against isolates of *P. aeruginosa*, those strains were excluded from analysis.
- Isolate genotypic resistance information was analyzed as provided by CDC either as whole genome sequencing or targeted sequencing information, with additional tetracycline resistance typing done by PCR and sequencing performed at Tetraphase for this study as previously described[8,9]. Comparator antibiotics were purchased through commercial sources (Sigma Aldrich, BioVision, or MedChem Express) and results checked against published QC ranges for ATCC control strains[7].

Results

Table 1. Eravacycline activity against total and resistant subgroups of Enterobacteriaceae

	Enterobacteriaceae							
MIC50/90 range	Total (221)	RNA Methylase (30)	fosA (23)	ΔompK35/36 (33)	Caz-Avi Resistant (47)	Metallo-beta-lactamase+ (47)	NDM+ (37)	Tetracycline Resistance (71)
Eravacycline	0.25/1 0.016 - 8	0.5/2 0.031 -4	0.25/1 0.031 - 1	0.5/2 0.031 - 4	0.25/2 0.031 - 4	0.5/2 0.031 - 4	0.5/2 0.031 - 4	0.25/1 0.016 - 8
Tigecycline	0.25/2 0.016 - 8	0.5/4 0.063 - 4	0.5/1 0.063 - 2	0.5/2 0.031 - 4	0.25/4 0.063 - 4	0.5/4 0.063 - 4	0.5/4 0.063 - 4	0.25/2 0.016 - 8
Minocycline	8/>32 0.25 - >32	8/>32 1 - >32	8/32 1 - >32	8/>32 1 - >32	8/>32 0.5 - >32	16/>32 0.5 - >32	16/>32 0.5 - >32	8/>32 0.5 - >32
Meropenem	4/>32 ≤0.016 - >32	>32 0.031 - >32	32/>32 0.031 - >32	8/>32 0.063 - >32	>32 ≤0.016 - >32	>32 2 - >32	>32 4 - >32	1/>32 ≤0.016 - >32
Meropenem/Vaborbactam	0.063/>32 ≤0.016 - >32	32/>32 0.031 - >32	0.5/>32 0.031 - >32	1/>32 ≤0.016 - >32	32/>32 ≤0.016 - >32	>32 1 - >32	>32 2 - >32	0.063/>32 ≤0.016 - >32
Ceftazidime/Avibactam	1/>32 0.063 - >32	>32 1 - >32	2/>32 0.125 - >32	2/>32 0.5 - >32	>32 16 - >32	>32 >32	>32 >32	1/>32 0.25 - >32
Gentamicin	4/>32 0.125 - >32	>32 4 - >32	16/>32 0.25 - >32	>32 0.25 - >32	>32 0.5 - >32	>32 0.5 - >32	>32 0.5 - >32	32/>32 0.5 - >32
Levofloxacin	16/>32 0.031 - >32	32/>32 0.063 - >32	8/>32 0.031 - >32	32/>32 0.125 - >32	32/>32 0.5 - >32	32/>32 0.031 - >32	32/>32 0.063 - >32	16/>32 0.031 - >32
Colistin	0.25/>32 0.063 - >32	0.25/16 0.063 - >32	0.25/8 0.063 - >32	0.25/>32 0.063 - >32	0.25/32 0.125 - >32	0.25/16 0.125 - >32	0.25/32 0.125 - >32	0.25/32 0.063 - >32

Values are given in µg/ml. Where the MIC_{50} and MIC_{90} or high and low range values are equivalent, a single value is presented.

Enterobacteriaceae include *E. coli* (63), and isolates from various species of *Klebsiella* (73), *Enterobacter* (30), *Proteus* (7), *Citrobacter* (7), *Salmonella* (13), *Shigella* (7), *Serratia* (12), *Morganella* (6), *Providencia* (2), *Kluyvera* (1). Resistance classifications were adapted from whole genome sequencing and resistance data provided by the CDC Antimicrobial Resistance Bank where available with supplemental and confirmatory PCR and sequencing performed by Tetraphase Pharmaceticals. Metallo-beta-lactamase producing strains included the bla_{NDM} (36), bla_{IMP} (4) and bla_{VIM} (6) families, while rRNA methylase genes included *armA* (19), *rmtB* (2), *rmtC* (8), *rmtF* (2), and *rmtG* (1) genes with some isolates harboring more than one gene. Tetracycline resistance genes included tetracycline specific efflux pumps *tet*(A), *tet*(B), *tet*(C), and *tet*(J) identified alone or in tandem by PCR. Multiple resistance types were frequently identified within single isolates; 21 of 30 strains reported positive for rRNA methylase genes were also positive for metallo-beta-lactamase enzymes, 19 of these 30 were resistant to ceftazidime-avibactam, 13 of 30 screened positive for a tetracycline specific efflux pump, and 9 of 30 had reported mutations associated with *ompK*35 or *ompK*36. Likewise, 19 isolates screened positive for both a metallo-betalactamase and tetracycline efflux pump, 14 carried an *ompK*35/36 mutation and a tetracycline efflux gene, and four carried both *fosA* and a tetracycline efflux pump.

Table 2. Eravacycline activity against total and resistant subgroups of *A. baumannii*

	Acinetobacter			
MIC50/90 range	Total (55)	RNA Methylase (21)	Caz-Avi Resistant (54)	Tetracycline Resistance (28)
Eravacycline	0.5/1 0.031 - 2	0.5/1 0.25 - 2	0.5/1 0.031 - 2	0.5/1 0.031 - 2
Tigecycline	2/4 0.031 - 2	2 0.5 - 4	2/4 0.125 - 8	2/4 0.125 - 8
Minocycline	8/16 0.016 - 32	16 1 - 16	8/16 0.016 - 32	8/16 1 - 32
Meropenem	>32 2 - >32	>32 2 - >32	>32 2 - >32	>32 4 - >32
Meropenem/Vaborbactam	>32 2 - >32	>32 2 - >32	>32 2 - >32	>32 4 - >32
Ceftazidime/Avibactam	>32 8 - >32	>32 32 - >32	>32 16 - >32	>32 32 - >32
Gentamicin	>32 8 - >32	>32 >32	>32 8 - >32	>32 8 - >32
Levofloxacin	16/>32 4 - >32	16/>32 4 - >32	16/>32 4 - >32	16/>32 4 - >32
Colistin	0.5/4 0.063 - >32	0.5/4 0.125 - >32	0.5/4 0.125 - >32	0.5/2 0.125 - >32

Values are given in µg/ml. Where the MIC_{50} and MIC_{90} or high and low range values are equivalent a single value is presented.

Resistance classifications were adapted as previously described. The rRNA methylase genes reported present in *A. baumannii* were strictly *armA*, while tetracycline specific efflux pumps were mainly representated by the *tet*(B) gene.

- Eravacycline $MIC_{50/90}$ and range values for total Enterobacteriaceae were 0.25/1 µg/ml and 0.016 – 8 µg/ml respectively, and remained within a dilution of these values across all resistance types screened in this analysis, with the exception of the fosfomycin resistant isolates where eravacycline MICs were less than 1 µg/ml for all isolates.
- Eravacycline $MIC_{50/90}$ and range values for total *A. baumannii* were 0.5/1 µg/ml and 0.031 – 2 µg/ml respectively, with the $MIC_{50/90}$ remaining unchanged across each subset.

Conclusions

Eravacycline retained a high degree of *in vitro* potency against isolates carrying a number of transferable resistance mechanisms, including those that have the potential to reduce efficacy of recently approved antimicrobial agents. Eravacycline represents a promising candidate for the treatment of infections caused by multidrug-resistant and carbapenem-resistant Gram negative organisms. Having recently completed two positive phase three trials in the treatment of complicated intra-abdominal infections, eravacycline is currently under review with the EMA and FDA for future clinical use in this indication.

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