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Eravacycline is Potent Against Third Generation Cephalosporin- and Carbapenem-Resistant *Enterobacteriaceae*, Carbapenem-Resistant *Acinetobacter baumannii*, and Has Isolate-Specific Bactericidal Activity

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Abstract

Background: Eravacycline (ERV) is a novel fluorocycline antibiotic in phase 3 clinical trials for complicated intra-abdominal (cIAI) and urinary tract (cUTI) infections. To evaluate its potential effectiveness against diverse clinical Gram-negative (GN) isolates, ERV was tested in minimal inhibitory concentration (MIC) assays against third-generation cephalosporin-resistant (3GC-R) *Enterobacteriaceae* and carbapenem-resistant (CP-R) *Enterobacteriaceae* and *A. baumannii* (AB), and in time-kill assays against panels of AB, *E. coli* (EC) and *K. pneumoniae* (KP), including CP-R and ESBL$^+$ isolates. **Methods:** MIC assays against panels of 3GC-R and CP-R isolates, confirmed by genotype and/or phenotype, were performed as per CLSI guidelines. Time-kill assays were done in duplicate at 2, 4, and 8X MICs essentially as per CLSI guidelines with the following modifications: starting cultures of ~1 x 10^5 – 1 x 10^6 colony forming units (CFU)/mL in 5 mL were shaken at 300 rpm at 35ºC in 50 mL conical tubes. Cultures were sampled over 24 hrs, serially diluted, and plated on tryptic soy agar for CFU counts. Per organism, 12 to 13 clinical isolates were tested in time-kill assays; isolates were from diverse sources including respiratory, UTI, IAI, bloodstream, and wound infections. For KP, 3 isolates were CP-R, one contained a KPC gene; 11 isolates were 3GC-R and contained ESBL *bla*$_{CTXM}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes. Ten AB isolates were CP-R. For EC, 8 isolates were 3GC-R and contained ESBL *bla*$_{CTXM}$, *bla*$_{OXA}$, *bla*$_{SHV}$ and *bla*$_{CMY}$ genes. **Results:** ERV had MIC$_{50/90}$ values of 0.5/2, 0.5/2, 0.5/2 and 2/4 µg/mL, against isolates of CP-R AB (n=76), KP (n=83), *Enterobacter cloacae* (ECl; n=25) and *Proteus mirabilis* (PM; n=68), respectively, and 0.25/0.5, 0.5/2, 0.5/2, and 1 /4 µg/mL against 3GC-R EC (n=133), KP (n=204), ECl (n=122), PM (n=20), respectively. ERV was bactericidal, independent of resistance genotype or phenotype, against 5/12 EC, 5/13 KP, and 8/12 AB isolates. **Conclusions:** ERV shows promising *in vitro* potency against CP-R and ESBL$^+$ isolates of *Enterobacteriaceae* and AB. The bactericidal activity *in vitro* of ERV may translate to enhanced efficacy against certain infections *in vivo,* especially those caused by difficult-to-treat drug-resistant GN pathogens.

Background

Eravacycline (TP-434) has been shown to be effective against a majority of Gram-negative multidrug-resistant (MDR) pathogens in preclinical studies[1]. Eravacycline is being developed as a broad spectrum intravenous (IV) antibiotic with potential for oral step-down for empiric treatment of severe and life-threatening bacterial infections. It has the potential to be used as a once-daily IV monotherapy capable of treating MDR Gram-negative pathogens and its efficacy was demonstrated in a recent phase 2 trial for the treatment of complicated intra-abdominal infections[2,3]. Eravacycline also offers potent, broad spectrum coverage of other serious and MDR Gram-positive, anaerobic, and atypical pathogens. Tetraphase is continuing to evaluate eravacycline's differentiated profile in two phase 3 studies to assess its use in the treatment of complicated intra-abdominal infections (cIAIs) and complicated urinary tract infections (cUTIs). The current study aims to characterize the potency of eravacycline against carbapenem-resistant *Enterobacteriaceae* (CRE) and bactericidality against panels of clinical isolates of *A. baumannii, K. pneumoniae,* and *E. coli.*

Methods and References

Susceptibility and bactericidality testing: Isolates were obtained from ATCC (Manassas, VA), IHMA (Chicago, IL), Eurofins (Chantilly, VA), or Walter Reed Hospital (Bethesda, MD). Susceptibility testing was performed according to CLSI methodology[4]. Time-kill assays were performed as described by CLSI guidelines[5], with the following modifications: five milliliter cultures inoculated to a final starting density of ~1 x 10^5 – 1 x 10^6 colony forming units (CFU) /mL were shaken vigorously (300 rpm) at 35ºC in 50 mL polypropylene conical tubes. Cultures were sampled at various time points, serially diluted in sterile saline, and plated on tryptic soy agar. A bactericidal response was defined by a 3-log reduction from starting colony forming units (CFUs; approximately 1 x 10^6 CFU/mL). In Table 3, ≥2.8 $\log_{10}$ reductions are noted in green and bacteriostatic responses (≤1 $\log_{10}$ CFU increase from starting CFUs) are noted in blue.

References

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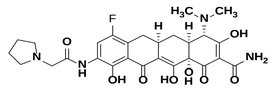
Results

Table 1. Eravacycline is potent against carbapenem-resistant *Enterobacteriaceae* (CRE) and isolates resistant to 3rd generation cephalosporins

Organism		ERV[1,2]	CP	TIG	3rd GC	FQ	AG
Acinetobacter baumannii CP-R	MIC$_{50/90}$	0.5/2	>16/>32	2/8	>32/>32	>4/16	>8/>32
	Range	(≤0.0156-4)	(8->32)	(0.125-8)	(>16->32)	(2->32)	(≤0.25->32)
	n	76	102	102	76	76	76
Enterobacter cloacae 3rd GC-R	MIC$_{50/90}$	0.5/2	0.5/4	1/4	>32/>64	0.25/>4	0.5/16
	Range	(0.03-4)	(≤0.0156->32)	(0.06-8)	(0.25->64)	(0.008->32)	(≤0.25->32)
	n	122	122	122	119	122	122
Enterobacter cloacae CP-R	MIC$_{50/90}$	0.5/2	4/32	1/4	>32/>64	0.5/>4	1/>32
	Range	(0.25-4)	(≤0.0156->32)	(0.125-4)	(0.25->64)	(0.0312->32)	(≤0.25->32)
	n	25	25	25	22	25	25
Escherichia coli 3rd GC-R	MIC$_{50/90}$	0.25/0.5	0.06/0.5	0.25/0.5	>32/>64	>4/32	4/>32
	Range	(≤0.0156-1)	(≤1->32)	(0.0625->8)	(0.5->64)	(≤0.25->32)	(≤0.25->32)
	n	133	133	133	133	133	133
Klebsiella pneumoniae 3rd GC-R	MIC$_{50/90}$	0.5/2	1/32	1/4	>32/64	>4/>32	>8/>32
	Range	(0.0312-16)	(≤1->32)	(0.12-16)	(2->64)	(<=0.25->64)	(≤0.25->64)
	n	204	204	204	204	204	204
Klebsiella pneumoniae CP-R	MIC$_{50/90}$	0.5/2	>8/>32	1/2	>32/>32	>4/>32	8/>32
	Range	(0.125-16)	(0.0625->32)	(0.25-16)	(2->64)	(0.0625->64)	(0.25->64)
	n	83	83	83	83	83	83
Proteus mirabilis 3rd GC-R	MIC$_{50/90}$	1/4	4/8	4/8	8/>32	>2/8	>8/16
	Range	(0.5-8)	(0.25-8)	(1-16)	(0.125->64)	(≤0.25-16)	(0.5->64)
	n	20	20	20	20	20	20
Proteus mirabilis CP-R	MIC$_{50/90}$	2/4	4/16	4/8	≤0.0313/4	2/>4	2/16
	Range	(0.5-16)	(0.0625->32)	(1-16)	(≤0.015-32)	(0.015->64)	(≤0.25->64)
	n	68	68	68	68	68	68

[1] MIC$_{50/90}$ and range values are in µg/mL.
[2] ERV, eravacycline; CP, carbapenem (meropenem or imipenem); TIG, tigecycline; 3rd GC, 3rd generation cephalosporin (cefotaxime or ceftazidime); FQ, fluoroquinolone (levofloxacin or ciprofloxacin); AG, aminoglycoside (gentamicin). For *Enterobacteriaceae,* 3rd GC-resistant (3rd GC-R) isolates were defined as ceftazidime MIC ≥16 µg/ml, cefotaxime MIC ≥4 µg/ml, or ceftriaxone, MIC ≥4 µg/ml; CP-resistant (CP-R) isolates were defined as imipenem MIC ≥4 µg/ml, meropenem MIC ≥4 µg/ml, or ertapenem, MIC ≥2 µg/ml. For *A. baumannii,* CP-R was defined as imipenem MIC ≥8 µg/ml or meropenem MIC ≥8 µg/ml.

Figure 1. Structure of Eravacycline



Conclusions

- **Eravacycline is a novel fluorocycline antibiotic with potent activity against difficult-to-treat Gram-negative pathogens resistant to carbapenems and 3rd generation cephalosporins.**
- **Eravacycline shows bactericidal activity *in vitro* against some isolates of *A. baumannii, K. pneumoniae,* and *E. coli,* which may translate to enhanced efficacy against certain infections *in vivo.***

Table 2. Susceptibility profiles of isolates tested in bactericidal assays

A. baumannii

Antibiotic	AB248	AB250	AB565	AB566	AB567	AB928	AB931	AB932	AB937	AB947	AB951	AB959
	MIC (µg/mL)											
Eravacycline	0.5	2	0.5	0.5	0.0156	0.5	1	0.125	2	2	0.125	1
Meropenem	32	32	16	32	>32	16	8	0.25	4	>32	>32	1
Tigecycline	1	2	2	2	0.125	2	2	0.5	2	4	0.25	2
Known Resistance Genotype	*tet*(A)	*tet*(B)	(*bla* status ND)	(*bla* status ND)	(*bla* status ND)	*tet*(B)	(*bla* status ND)	(*bla* status ND)	*tet*(B)	*tet*(B)	*tet*(B)	*tet*(B)
Infection Site	NA	NA	NA	NA	NA	tracheal	tracheal	UTI	sputum	w ound	sputum	w ound

ND, not determined; NA, not available

K. pneumoniae

Antibiotic	KP109	KP153	KP451	KP770	KP810	KP811	KP1054	KP1055	KP1056	KP1057	KP1058	KP1074	KP1075
	MIC (µg/mL)												
Eravacycline	0.25	1	0.25	0.5	0.5	0.25	1	0.25	0.25	0.25	0.25	0.25	1
Meropenem	0.063	0.031	≤0.0156	>32	8	0.0312	0.0625	0.0625	0.0312	0.0312	0.0312	0.125	4
Tigecycline	0.25	1	0.25	1	0.5	0.25	1	0.25	0.25	0.5	0.25	0.25	2
Known Resistance Genotype	*bla*$_{SHV}$	*tet*(A), *bla*$_{CTX-M-15/132}$, *bla*$_{OXA-1}$, *bla*$_{SHV}$	*bla*$_{SHV}$	*bla*$_{KPC}$, *bla*$_{SHV}$	*tet*(A), (*bla* status ND)	(*bla* status ND)	*tet*(A), *bla*$_{SHV-28}$, *bla*$_{CTX-M15}$	*bla*$_{SHV-28}$, *bla*$_{CTX-M15}$	*bla*$_{SHV-5}$, *bla*$_{CTX-M15}$	*tet*(A), *bla*$_{CTX-M15}$	*tet*(A), *bla*$_{CTX-M2}$	*bla*$_{SHV-12}$, *bla*$_{CTX-M2}$	*tet*(A), *bla*$_{CTX-M2}$
Infection Site	NA	NA	UTI	blood	sputum	urine	gall bladder	peritoneal	peritoneal	peritoneal	peritoneal	appendix	GI

ND, not determined; NA, not available

E. coli

Antibiotic	EC133	EC360	EC588	EC590	EC777	EC786	EC802	EC806	EC1024	EC1029	EC1033	EC1041
	MIC (µg/mL)											
Eravacycline	0.0312	0.5	0.125	0.25	0.25	0.125	0.25	0.125	0.5	0.0625	0.125	0.5
Meropenem	≤0.0156	≤0.0156	0.0312	0.0312	≤0.0156	≤0.0156	≤0.0156	0.0312	0.0625	0.125	0.0625	0.0625
Tigecycline	0.125	0.5	0.125	0.25	0.25	0.125	0.25	0.125	0.5	0.25	0.5	0.5
Known Resistance Genotype	*tet*(B), *tet*(D), *bla*$_{SHV}$, *bla*$_{CTXM}$	*tet*(A), *bla*$_{OXA}$, *bla*$_{CTXM-15/13-5/13}$	(*tet* and *bla* status ND)	*tet*(M), (*bla* status ND)	(*tet* and *bla* status ND)	(*tet* and *bla* status ND)	*tet*(A), (*bla* status ND)	(*bla* status ND)	*tet*(A), *bla*$_{SHV-12}$, *bla*$_{CTX-M2}$	*tet*(A), *bla*$_{CTXM55}$, *bla*$_{CTXM14}$	*tet*(A), *bla*$_{CTXM15}$, *bla*$_{CMY-6}$	*bla*$_{CTXM2-6}$
Infection Site	NA	UTI	NA	NA	UTI	UTI	UTI	UTI	peritoneal	appendix	peritoneal	peritoneal

ND, not determined; NA, not available

Table 3. Bactericidal activity of ERV against *A. baumannii, K. pneumoniae* and *E. coli* clinical isolates

A. baumannii

$\log_{10}$ CFU change at 24 hours

Antibiotic	AB248	AB250	AB565	AB566	AB567	AB928	AB931	AB932	AB937	AB947	AB951	AB959
No drug	2.79	2.39	3.39	2.78	2.68	2.78	3.73	3.66	2.87	3.24	2.55	2.66
ERV 2X MIC	2.80	-1.02	2.55	2.11	2.56	2.57	-2.28	NA	-3.45	-0.49	2.42	2.03
ERV 4X MIC	0.74	0.16	-2.55	-0.88	2.94	2.07	-3.77	0.61	-1.29	-3.30	2.47	-2.74
ERV 8X MIC	-2.50	-4.05	-4.23	-3.75	-0.62	-3.05	-3.77	-3.19	-1.38	-3.82	-2.30	-2.74

K. pneumoniae

$\log_{10}$ CFU change at 24 hours

Antibiotic	KP109	KP153	KP451	KP770	KP810	KP811	KP1054	KP1055	KP1056	KP1057	KP1058	KP1074	KP1075
No drug	3.20	4.34	3.31	2.08	2.84	3.33	3.01	3.24	3.19	2.90	3.12	2.88	3.00
ERV 2X MIC	-3.70	-1.47	3.28	2.97	2.85	3.24	1.05	3.24	3.18	-1.20	3.08	3.04	1.97
ERV 4X MIC	-4.30	-1.54	-0.32	-0.03	2.87	2.78	-4.67	-1.51	-1.77	2.52	2.82	2.89	-1.11
ERV 8X MIC	-4.30	-3.00	-4.10	-0.89	-2.75	-1.61	-3.24	1.05	-2.25	-1.91	-1.18	-1.58	-0.84

E. coli

$\log_{10}$ CFU change at 24 hours

Antibiotic	EC133	EC360	EC588	EC590	EC777	EC786	EC802	EC806	EC1024	EC1029	EC1033	EC1041
No drug	2.91	2.74	3.08	2.83	2.99	3.00	3.32	3.27	3.05	2.95	2.79	2.86
ERV 2X MIC	2.83	-1.43	-1.91	0.11	-0.28	-0.13	-1.11	-3.25	-3.40	2.67	2.79	-0.12
ERV 4X MIC	1.56	-1.15	-1.15	-1.87	-1.08	-0.93	-0.69	-3.25	-4.48	1.77	-1.34	-3.02
ERV 8X MIC	-1.46	-1.47	-1.17	-4.03	-1.14	-2.25	-0.82	-3.28	-4.48	-1.02	-4.70	-2.19

cidal	≥2.8 log drop in CFU at 24 hr
static	≤ 1.0 log increase in CFU at 24 hr