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The Antibacterial Activity of Cefpodoxime and the Novel β-lactamase Inhibitor ETX1317 Against Recent Clinical Isolates of β-lactamase-producing *Enterobacteriaceae*

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Abstract

Background
ETX1317 is a novel, diazabicyclooctenone inhibitor of serine β-lactamases that restores β-lactam activity against multidrug-resistant *Enterobacteriaceae*. ETX0282, the oral prodrug of ETX1317, is currently under investigation in combination with cefpodoxime proxetil (CPDP), which is hydrolyzed *in vivo* to release cefpodoxime (CPD). We sought to determine the relative potency of CPD-ETX1317 against a collection of recent, geographically diverse *Enterobacteriaceae* which was enriched for extended spectrum β-lactamase (ESBL) and carbapenemase-producing isolates.

Methods
911 *Enterobacteriaceae* collected during 2013, 2014 and 2015 by IHMA from geographically diverse medical centers in the United States, Europe, Latin America and the Asia-Pacific region were chosen for testing, 70% of which had been previously shown to contain ESBL or carbapenemase genes. Bacterial species included *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Proteus* spp. and *Enterobacter* spp. An additional set of colistin-resistant *mcr-1*+ and CAZ-AVI-resistant *Enterobacteriaceae* were also tested. Susceptibility testing was performed according to CLSI guidelines, and data analysis was performed using CLSI breakpoint criteria.

Results
The MIC_{90} of CPD against this global, diverse, recent collection of 911, β-lactamase-enriched *Enterobacteriaceae* improved by over 1000-fold (from >32 mg/L to 0.06 mg/L) upon the addition of 4 mg/L ETX1317. This degree of potency was consistent across all bacterial species and serine β-lactamase classes tested, including isolates expressing ESBL, AmpC-overexpressing, KPC and OXA-48-like enzymes. It was also maintained against colistin-resistant *mcr-1*+ and CAZ-AVI-resistant *Enterobacteriaceae*. The combination is less active against class B metallo-β-lactamase producing strains.

Conclusions
ETX1317 potently restores the activity of CPD against ESBL-producing, carbapenem-resistant, colistin-resistant and/or CAZ-AVI-resistant *Enterobacteriaceae*. The active combination product of CPD-ETX1317, which results from effective *in vivo* hydrolysis of CPDP-ETX0282, represents the first, new oral β-lactam/β-lactamase inhibitor combination as a therapeutic option for the treatment of resistant Gram-negative uropathogens in decades.

Introduction

Currently, there are few antibiotics with an oral formulation in active clinical development for urinary tract infections (UTIs). 95% of UTIs are caused by *Enterobacteriaceae*. 75% of these are due to *Escherichia coli* but *Klebsiella, Enterobacter, Citrobacter* and *Proteus* spp. are also important UTI pathogens[1]. Emergence of multi-drug resistant (MDR) bacteria, including fluoroquinolone-resistant, AmpC β-lactamase-, ESBL- and carbapenemase-producing strains of *Enterobacteriaceae*, has complicated treatment of patients with these infections. Resistance to existing oral therapies for UTI is forcing physicians to unnecessarily admit patients and administer lengthy IV treatment resulting in excessive healthcare expenses. Many physicians identify the lack of a potent, oral Gram-negative agent as one of the field's biggest unmet needs[2]. In response to this challenge, Entasis Therapeutics is developing an oral Gram-negative drug targeting MDR urinary tract infections, including carbapenem-resistant *Enterobacteriaceae* (CRE). The agent is a combination of cefpodoxime-proxetil plus the diazabicyclo-octenone prodrug, ETX0282. This combination is metabolized *in vivo* to cefpodoxime (CPD) and ETX1317. The intended use for this product is a as first line treatment for cystitis and pyelonephritis in outpatient settings or as an oral step-down therapy in hospital settings, resulting in significant reduction of healthcare costs.

In vitro activity of ETX1317 vs. comparator BLIs

β-lactamase inhibition (IC_{50}, μM)

	Class A				Class C		Class D	
BLI	CTX-M-15	SHV-5	KPC-2	TEM-1	AmpC*	P99	OXA-24/40	OXA-48
Avibactam	0.009	0.23	0.18	6.9	0.52	0.12	32	0.88
ETX2514	0.001	0.004	0.002	0.001	0.006	0.001	0.28	0.005
ETX1317	0.002	0.036	0.043	0.003	0.16	0.024	0.54	0.077

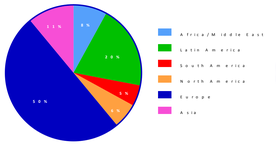
**P. aeruginosa* ortholog

Methods:
As detailed in Durand-Réville *et al*. (2017)[3], reaction progress curves at 490 nm were measured for hydrolysis of 100 μM nitrocefin by each enzyme at a range of inhibitor concentrations in 0.1 M sodium phosphate (pH 7.0), 10 mM $NaHCO_3$, and 0.005% Triton X-100 at ambient temperature. The set of curves were fit globally to a 2nd-order kinetic model of enzyme inactivation. The 60-min time points on the set of best-fit curves were used to calculate the IC_{50} by nonlinear regression to the Hill equation: % inhibition = $100[I]^n/(IC_{50} + [I]^n)$, where [I] is the inhibitor concentration and n is the Hill coefficient.

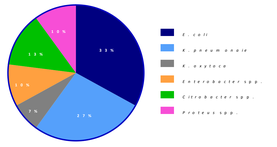
Enterobacteriaceae Susceptibility: Study Design

Organisms:
All IHMA isolates were collected from geographically diverse medical centers located in North America, Latin America, South America, Europe, Middle East, Africa and the Asia-Pacific region during 2013, 2014 and 2015. Isolates were enriched for ESBL* genotypes and consisted of approximately one-third *E. coli*, one-third *Klebsiella* spp., 10% each *Enterobacter*, *Citrobacter* and *Proteus* spp. *mcr-1+ E. coli* strains were a kind gift from L. Poirel and P. Nordmann (University of Fribourg, Switzerland). CAZ-AVI resistant isolates highlighted with an asterisk were generated internally; those highlighted with a double asterisk were purchased from JMI and are described in Aitken *et al*. (2016)[4].

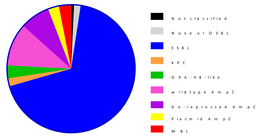
Breakdown by Region



Breakdown by bacterial species



Breakdown by β-lactamase content



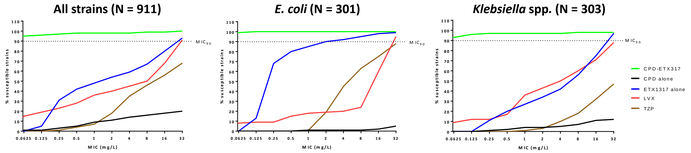
Methods:
Broth microdilution susceptibility testing was conducted according to CLSI guidelines[5] using cation-adjusted Mueller-Hinton broth. Where indicated, CPD-ETX1317 = MIC of cefpodoxime in the presence of 4 mg/L ETX1317; CAZ-AVI = MIC of ceftazidime in the presence of 4 mg/L avibactam; TZP = MIC of piperacillin in the presence of 4 mg/L tazobactam.

Cumulative activity vs. 911 ESBL-enriched *Enterobacteriaceae* from 2013-2015

Drug	%S*	Number (cumulative %) of isolates inhibited at MIC (mg/L)										
		≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
CPD	10.8	0 0%	5 0.5%	6 1%	14 3%	19 5%	34 9%	20 11%	27 14%	17 16%	18 18%	18 20%	733 100%
ETX1317	NA	1 0%	0 0%	46 5%	234 31%	98 42%	54 48%	59 54%	44 59%	78 67%	114 80%	115 93%	68 100%
CPD-ETX1317	98.4	835 85%	28 92%	15 95%	10 96%	5 97%	2 98%	1 98%	0 98%	4 99%	1 99%	2 99%	8 100%
LVX	40	NT	141 15%	35 19%	29 23%	46 28%	77 36%	39 42.8%	40 40%	48 45%	163 50%	207 68%	86 100%
TZP	55.3	NT	NT	0 0%	8 0.9%	26 4%	30 7%	96 18%	161 35%	99 46%	92 56%	101 68%	295 100%

CPD = cefpodoxime; FOS = fosfomycin; LVX = levofloxacin; TZP = piperacillin-tazobactam; NA = applicable; NT = not tested; MIC_{90}s are highlighted with red squares.
*based on 2016 CLSI breakpoint criteria. CPD-ETX1317 breakpoint is based on the CLSI 2016 CPD breakpoint of ≤2.

All strains (N = 911) | *E. coli* (N = 301) | *Klebsiella* spp. (N = 303)



Activity of CPD-ETX1317 vs. bacterial species

Bacterial Species	All	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*Citrobacter* spp.	*E. aerogenes*	*E. cloacae*	*Proteus* spp.
N	911	301	253	53	120	40	51	93
MIC_{50} (mg/L)	≤0.015	≤0.015	≤0.015	≤0.015	0.06	≤0.015	≤0.015	≤0.015
MIC_{90} (mg/L)	0.03	≤0.015	0.03	0.125	0.06	0.06	0.25	0.12

Activity of CPD-ETX1317 vs. β-lactamase class

β-lactamase class	All	Not classified	None or OSBL	ESBL	KPC	OXA-48-like	wildtype AmpC	De-repressed AmpC	Plasmid AmpC	MBL
N	911	7	15	621	18	30	98	69	25	28
MIC_{50} (mg/L)	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015
MIC_{90} (mg/L)	0.03	0.03	0.03	0.03	0.03	0.06	0.03	0.06	0.06	>32

Activity of CPD-ETX1317 vs. colistin-resistant (*mcr-1*+) isolates

Strain(s)	Species	Genotype	MIC (mg/L)			
			ETX1317	CPD	CPD–ETX1317	COL
10 isolates	*E. coli*	*mcr-1+*	0.125 – 4	0.5 - >32	≤0.03	4-8

CPD = cefpodoxime; CPD-ETX1317 = cefpodoxime + ETX1317 at 4 mg/L; COL = colistin

Acknowledgements
We would like to thank R. McLaughlin and S. Moussa for whole genome sequencing of the *Enterobacteriaceae* isolates.

Activity vs. 35 sequenced KPC+ and/or MBL+ *Enterobacteriaceae*

Strain ID	Species	encoded *bla* genes	MIC (mg/L)			
			IPM	CAZ-AVI	CPD-ETX1317	ETX1317
ARC3528*†	*E. cloacae*	AmpC [Δ308-313(SKVALA)]	0.25	32	0.125	4
ARC4165*†	*C. freundii*	CMY-2; AmpC [N366Y]; TEM-1; SHV-5	2	32	≤0.03	4
ARC6479*‡	*K. oxytoca*	NDM-1	16	>32	32	32
ARC6478*‡	*K. pneumoniae*	NDM-1; KPC-17	>32	>32	>32	16
ARC6481*‡	*K. pneumoniae*	NDM-1	>32	>32	0.5	16
ARC3518	*C. freundii*	KPC-2; TEM-1; OXA-1; AmpC	4	4	0.5	0.25
ARC3883	*C. freundii*	CMY-48; KPC-2	8	1	≤0.03	8
ARC5383	*C. freundii*	CMY-like; VIM-1; TEM-1	4	>32	≤0.03	0.25
ARC3532	*E. cloacae*	OXA-9; KPC-3; TEM-1; AmpC	32	4	≤0.03	0.25
ARC4520	*E. cloacae*	VIM-1; TEM-1; AmpC	8	>32	≤0.03	1
ARC5127	*E. cloacae*	IMP-1; OXA-48; OXA-10; SHV-5; TEM-1; AmpC	8	>32	≤0.03	0.5
ARC5130	*E. cloacae*	NDM-1; SHV-5; TEM-1; OXA-1; CTX-M-15; AmpC	>32	>32	≤0.03	0.5
ARC5342	*E. cloacae*	SHV-12; VIM-1; AmpC	16	>32	≤0.03	0.13
ARC3898	*E. coli*	NDM-1; OXA-2; OXA-1; CTX-M-15; TEM-1; CMY-4; AmpC	16	>32	≤0.03	0.5
ARC3902	*E. coli*	NDM-6; CTX-M-15; OXA-2; AmpC; TEM-1	>32	>32	≤0.03	2
ARC5139	*E. coli*	VIM-1; TEM-1; AmpC	2	32	≤0.03	0.25
ARC6074	*E. coli*	AmpC; KPC-3	4	0.25	≤0.03	2
ARC5389	*K. oxytoca*	PER-2; OXY-1-1; KPC-2	16	32	0.25	32
ARC3777	*K. pneumoniae*	KPC-2; TEM-1; SHV-11	16	0.5	≤0.03	1
ARC4378	*K. pneumoniae*	TEM-1; KPC-2; SHV-11	16	1	≤0.03	8
ARC4451	*K. pneumoniae*	SHV-11; KPC-3	>32	0.5	≤0.03	2
ARC4490	*K. pneumoniae*	SHV-11; CTX-M15; KPC-2; OXA-1	8	1	≤0.03	16
ARC4770	*K. pneumoniae*	NDM-1; OXA-1; TEM-1; CTX-M-15; SHV-11	32	>32	≤0.03	0.5
ARC5118	*K. pneumoniae*	KPC-3; TEM-1; SHV-5	>32	4	0.125	32
ARC5378	*K. pneumoniae*	KPC-3; TEM-1; SHV-11	>32	2	0.06	32
ARC6089	*K. pneumoniae*	TEM-1; KPC-2; SHV-11 like	>32	2	0.13	32
ARC6092	*K. pneumoniae*	OXA-1; KPC-2; CTX-M-15; SHV-76	4	0.25	≤0.03	8
ARC6094	*K. pneumoniae*	OXA-1; OKP-B; CTX-M-15; TEM-1	0.5	0.25	≤0.03	16
ARC6095	*K. pneumoniae*	TEM-1; KPC-3; SHV-11	16	1	≤0.03	8
ARC6099	*K. pneumoniae*	KPC-2; SHV-11	8	0.5	0.06	16
ARC6100	*K. pneumoniae*	TEM-1; KPC-2; SHV-11	1	0.25	≤0.03	8
ARC6109	*K. pneumoniae*	TEM-1; KPC-2; SHV-11	0.13	0.5	≤0.03	1
ARC6110	*K. pneumoniae*	OXA-1; TEM-1; CTX-M-15; SHV-28; KPC-2	0.13	0.5	≤0.03	8
ARC6483	*K. pneumoniae*	TEM-1; KPC-2; SHV-12	>32	1	≤0.03	8
ARC6484	*K. pneumoniae*	OXA-9; CARB-2; FOX-5; KPC-2; CTX-M-15; SHV-28; TEM-95-like	32	4	0.25	4

IPM = imipenem, CAZ-AVI = ceftazidime+ avibactam at 4 mg/L; CPD-ETX1317 = cefpodoxime + ETX1317 at 4 mg/L
CAZ-AVI-resistant isolates (either internal or as reported in literature†); KPC genes are highlighted in purple and MBL genes are highlighted in red

Conclusions

- ETX0282 is an oral prodrug which is hydrolyzed *in vivo* to release ETX1317, a novel diazabicyclooctenone inhibitor of serine β-lactamases that has more potent activity against Ambler classes A and C enzymes and a broader spectrum of activity against class D enzymes than avibactam
- ETX0282 is being developed in combination with cefpodoxime proxetil, which is hydrolyzed *in vivo* to release cefpodoxime
- The MIC_{90} of cefpodoxime against 911 recent, globally diverse ESBL-enriched *Enterobacteriaceae* from 2013-2015 was reduced from >32 to 0.06 mg/L in the presence of ETX1317 held at 4 mg/L .
- This potency was maintained across bacterial species and all β-lactamase classes associated with *Enterobacteriaceae* except for Class B MBLs.
- ETX1317 potently restores the activity of cefpodoxime against ESBL-producing, carbapenem-resistant, colistin-resistant and/or CAZ-AVI-resistant *Enterobacteriaceae*
- CPDP-ETX0282, represents the first, new oral β-lactam/β-lactamase inhibitor combination as a therapeutic option for the treatment of resistant Gram-negative uropathogens in decades

References

1. Foxman, B. 2014. *Infect. Dis. Clin. North Amer*. 28: 1-13. **2.** Zowawi, H.M. *et al*. 2015. *Nat. Rev. Urol.* 12:570-84. **3.** Durand-Reville, T. et *al. Nature Microbiol.* 2017 *In press.* **4.** Aitken, S., *et al.* 2016. *Clin. Infect. Dis*. 63(7):954-958. **5.** CLSI M07-A10. 2015.

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