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The Novel β-lactamase Inhibitor ETX1317 Effectively Restores the Activity of Cefpodoxime Against Recent Global *Enterobacteriaceae* Isolates from Urinary Tract Infections

ENTASIS THERAPEUTICS Powered by CARB-X

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Abstract

Background

The treatment of urinary tract infections (UTIs) has been complicated by the emergence of multi-drug resistant, β-lactamase-expressing pathogens. ETX0282 is an oral prodrug which is hydrolyzed *in vivo* to release ETX1317, a novel diazabicyclooctenone β-lactamase inhibitor active against serine β-lactamases. ETX0282 is currently in clinical development in combination with cefpodoxime proxetil, a clinically approved antibiotic which is hydrolyzed *in vivo* to release cefpodoxime (CPD). We sought to determine the *in vitro* antibacterial activity of CPD-ETX1317 and comparator compounds against a collection of geographically diverse *Enterobacteriaceae* UTI isolates collected in 2017.

Methods

A total of 1,875 *Enterobacteriaceae* isolates from UTI infections were tested. Organisms were collected as part of the SENTRY Antimicrobial Surveillance Program during 2017. Isolates were collected in 117 medical centers located in 27 countries from North American, European, Latin American and Asia-Pacific regions. Susceptibility testing was performed according to CLSI guidelines, and data analysis was performed using CLSI and EUCAST breakpoint criteria. Cefpodoxime and ETX1317 were tested individually and in combination at a 1:2 ratio.

Results

Against this collection of 1,875 global, recent *Enterobacteriaceae* UTI isolates, the cefpodoxime MIC_{50} and MIC_{90} values of 0.5 and >16 mg/L were improved to 0.06 and 0.12 mg/L in the presence of ETX1317. The cefpodoxime-ETX1317 combination was equally active against isolates from North America (n=813), Europe (n=653), Latin America (n=202) and Asia-Pacific (n=207) and was unaffected by resistance phenotypes. Cefpodoxime-ETX1317 potency was generally consistent across bacterial species. Cefpodoxime-ETX1317 showed similar potency against the *Enterobacter cloacae* species complex (n=82), *Serratia* spp. (n=25) and *Providencia* spp. (n=18) with MIC_{90} values of 0.25 mg/L. The MIC_{90} value of CPD-ETX1317 for *Morganella morganii* (n=29) was 0.5 mg/L.

Conclusions

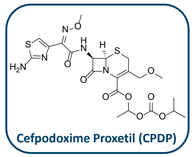
The combination of cefpodoxime and ETX1317 demonstrated potent antibacterial activity against recent, geographically diverse UTI isolates. These data support the continued development of the oral combination of ETX0282 and cefpodoxime proxetil for the treatment of antibiotic-resistant *Enterobacteriaceae* infections.

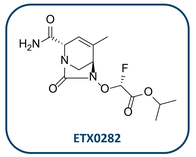
Introduction

95% of UTIs are caused by *Enterobacteriaceae*[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 complicated UTI (cUTI) infections, including those caused by carbapenem-resistant *Enterobacteriaceae* (CRE). The agent is a combination of cefpodoxime-proxetil plus the diazabicyclooctenone oral prodrug, ETX0282, which are each metabolized *in vivo* to cefpodoxime (CPD) and ETX1317. The intended use for this product is the treatment of cUTI, including pyelonephritis, in outpatient settings or as oral step-down therapy in hospital settings.

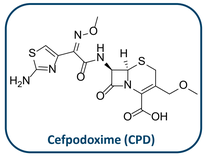
A Broad-spectrum Oral β-lactam/β-lactamase Inhibitor Combination

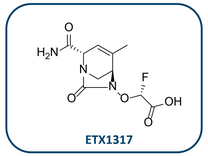
ETX0282-cefpodoxime proxetil is an oral prodrug combination.





The oral combination is hydrolyzed *in vivo* to release the active moieties cefpodoxime-ETX1317.



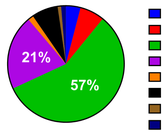


ETX1317 is a β-lactamase inhibitor that inhibits class A, C and many class D β-lactamases. ETX1317 also has intrinsic antibacterial activity against some *Enterobacteriaceae* species.

Study Design: *Enterobacteriaceae* Susceptibility

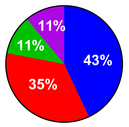
Organisms: 1,875 Enterobacteriaceae isolates from UTI infections were collected in 2017 as part of the SENTRY Antimicrobial Surveillance Program.

Breakdown by bacterial species



- *Citrobacter* spp.
- *Enterobacter* spp.
- *Escherichia coli*
- *Klebsiella* spp.
- *Morganella morganii*
- *Proteus* spp.
- *Providencia* spp.
- *Serratia* spp.

Breakdown by region



- North America
- Europe
- Latin America
- Asia-Pacific

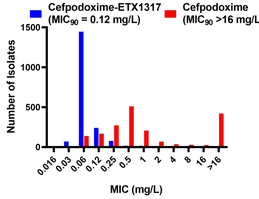
Methods: Broth microdilution susceptibility testing was conducted at JMI Laboratories according to CLSI guidelines[3,4]. CPD-ETX1317 MICs were performed by titrating cefpodoxime with ETX1317 in a 1:2 ratio. Select isolates were subjected to whole genome sequencing using an Illumina MiSeq instrument and genomic analysis was performed with CLCBio Genomics Workbench v9.5.

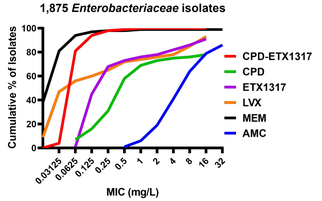
MIC Distribution and Cumulative Activity vs. 1,875 *Enterobacteriaceae* UTI Isolates

	Number (cumulative %) of isolates inhibited at MIC (mg/L)											
Antimicrobial	**≤ 0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**> †**
CPD-ETX1317 (1:2)	2	71	1,445	**240**	77	19	15	5	0	1		
	0.1%	3.9%	81%	**93.8%**	97.9%	98.9%	99.7%	99.9%	99.9%	100%		
CPD			138	169	273	509	206	68	36	30	25	**421**
			7.4%	16.4%	30.9%	58.1%	69.1%	72.7%	74.6%	76.2%	77.5%	**100%**
ETX1317			13	828	443	93	43	49	60	91	**88**	167
			0.7%	44.9%	68.5%	73.4%	75.7%	78.3%	81.5%	86.4%	**91.1%**	100%
LVX	168	711	171	76	85	132	48	25	46	131	**160**	122
	9%	47%	56%	60%	65%	72%	74%	76%	78%	85%	**93%**	100%
SXT						1185	26	26	24	16		**598**
						63%	65%	66%	67%	68%		**100%**

CPD = cefpodoxime; LVX = levofloxacin; SXT = trimethoprim-sulfamethoxazole. MIC_{90}s are highlighted with blue squares.
†Above the top concentration tested.

ETX1317 Restores Activity of Cefpodoxime against 1,875 UTI isolates





Antimicrobial Agent	N	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S (CLSI)	%S (EUCAST)
Cefpodoxime-ETX1317	1,875	≤0.015 - 8	0.06	0.12		
Cefpodoxime (CPD)	1,875	≤0.06 - >16	0.5	>16	72.7	69.1
ETX1317	1,875	≤0.06 - >16	0.25	16		
Amoxicillin-clavulanic acid (AMC)	1,394	0.5 - >32	8	>32	63.6	63.6
Levofloxacin (LVX)	1,875	≤0.008 - >16	0.06	16	75.5	71.6
Nitrofurantoin	1,394	≤2 - >64	32	>64	61.1	
Trimethoprim-sulfamethoxazole	1,875	≤0.5 - >8	≤0.5	>8	66.0	66.0
Ceftazidime-avibactam	1,875	≤0.06 - >16	≤0.06	0.25	99.5	99.5
Meropenem (MEM)	1,875	≤0.015 - >32	0.03	0.06	98.5	98.8

- CPD has a MIC_{90} of >16 mg/L, which is improved by >128-fold to 0.12 mg/L in the presence of ETX1317.
- CPD-ETX1317 was the most potent oral agent tested against this set of 1,875 UTI isolates of *Enterobacteriaceae*.
- CPD-ETX1317 had similar activity compared to IV antibiotics used to treat drug-resistant UTIs, suggesting it may be a suitable step-down therapy.

Activity of Cefpodoxime-ETX1317 (1:2) by Bacterial Species

Bacterial Species	N	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Escherichia coli	1,072	≤0.015 - 1	0.06	0.12
Klebsiella pneumoniae	353	0.03 - 8	0.06	0.12
Klebsiella oxytoca	34	0.06 – 0.25	0.06	0.12
Citrobacter koseri	35	0.12 – 0.25	0.06	0.12
***Citrobacter freundii* spp. complex**	41	0.03 – 0.5	0.06	0.12
Klebsiella (Enterobacter) aerogenes	46	0.03 – 0.25	0.06	0.12
***Enterobacter cloacae* spp. complex**	82	0.06 - 1	0.06	0.25
Morganella morganii	29	0.06 - 1	0.25	0.5
***Proteus* spp.**	137	0.03 – 0.25	0.06	0.06
***Providencia* spp.**	18	≤0.015 – 0.5	0.06	0.25
***Serratia* spp.**	25	0.12 - 1	0.25	0.25
Other *Enterobacteriaceae*	3	0.06 – 0.06	--	--
All isolates	**1,875**	**≤0.015 - 8**	**0.06**	**0.12**

CPD-ETX1317 activity is stable across species of *Enterobacteriaceae*.

Activity of Cefpodoxime-ETX1317 (1:2) by Geographical Region

Region	N	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
North America	813	≤0.015 - 2	0.06	0.12
Europe	653	0.03 - 2	0.06	0.12
Asia / South Pacific	207	0.03 - 8	0.06	0.12
Latin America	202	0.03 - 2	0.06	0.12
ALL	**1,875**	**≤0.015 - 8**	**0.06**	**0.12**

CPD-ETX1317 activity is stable across all the geographical regions tested.

Isolates with Lower Susceptibility to Cefpodoxime-ETX1317

Strain ID	Species	Country	Sequence type (Oxford)	Whole Genome Sequencing Results	MIC (mg/L)					
					CPD-ETX1317	CPD	MEM	CAZ-AVI	LVX	TMX
ARC6930	*K. pneumoniae*	Philippines	ST147	TEM-1; CTX-M-15; SHV-12; **NDM-7**; RamR [W89*]; RamA [D26fs]; AcrA [F356S]; OmpK35 [K3fs]	8	>16	>32	>16	8	>8
ARC6931	*K. pneumoniae*	USA	ST258	TEM-1; OXA-9; SHV-11; KPC-2; RamR [E132*]; OmpK35 [G41fs]	2	>16	>32	0.5	>16	>8
ARC6932	*K. pneumoniae*	USA	ST258	TEM-1; OXA-9; SHV-11; KPC-3; RamR [E132*]; OmpK35 [G41fs]	2	>16	>32	2	16	>8
ARC6933	*K. pneumoniae*	Poland	ST147	OXA-1; CTX-M-15; SHV-11; OmpK35 [K3fs]; OmpK36 [W125*]	2	>16	8	0.5	>16	4
ARC6934	*K. pneumoniae*	Brazil	ST11	TEM-1; OXA-2; CTX-M-2; SHV-11; KPC-2; OmpK35 [G114fs]	2	>16	>32	2	16	4
ARC6936	*K. pneumoniae*	Brazil	ST11	TEM-1; OXA-2; CTX-M-2; SHV-11; KPC-2; OmpK35 [G114fs]	2	>16	>32	1	>16	>8

- All isolates with MIC values of 2 mg/L or greater were subjected to whole genome sequencing (n=6).
- All six isolates were *K. pneumoniae* and were resistant to meropenem, levofloxacin and trimethoprim-sulfamethoxazole. One of the six isolates was resistant to ceftazidime-avibactam (CAZ-AVI).
- The isolate with the highest Cefpdoxime-ETX1317 MIC (8 mg/L), which was also resistant to CAZ-AVI, was found to encode the metallo-β-lactamase NDM-7. This result corresponds to the lack of metallo-β-lactamase inhibition by ETX1317 and avibactam.
- Four of the six isolates encoded for a combination of a KPC carbapenemase paired with a frameshift mutation in one of the major outer membrane porins, OmpK35.

Conclusions

- ETX0282 is an oral prodrug which is hydrolyzed *in vivo* to release ETX1317, a novel diazabicyclooctenone inhibitor of serine β-lactamases with activity against Ambler classes A and C enzymes and many class D enzymes.
- ETX0282 is being developed in combination with cefpodoxime proxetil, which is hydrolyzed *in vivo* to release cefpodoxime.
- The MIC_{90} of cefpodoxime against 1,875 *Enterobacteriaceae* UTI isolates from 2017 was reduced from >16 to 0.12 mg/L when titrated in a 1:2 ratio with ETX1317.
- This potency was maintained across bacterial species and geographical regions.
- Less susceptible isolates encoded for a metallo-β-lactamase or truncated outer membrane porins, usually combined with a KPC carbapenemase.
- CPDP-ETX0282, represents the first in class oral β-lactam/β-lactamase inhibitor combination as a potential therapeutic option for the treatment of resistant Gram-negative uropathogens in decades.

Disclosures

S. McLeod, S. Moussa, J. Mueller, R. Tommasi and A. Miller are employees of Entasis Therapeutics.
M. Huband is an employee of JMI Laboratories.

References

1. Foxman, B. (2014) *Infect. Dis. Clin. North Amer.* 28: 1-13. **2.** Zowawi, H.M. *et al.* (2015) *Nat. Rev. Urol.* 12: 570-584 **3.** CLSI M07-A10. 2015. **4.** CLSI M100, 28th ed. 2018.

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