Poster T-38

The Novel β-lactamase Inhibitor Enmetazobactam is More Potent than Tazobactam against ESBL-producing *Enterobacteriaceae*

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Abstract

Background: New carbapenem-sparing therapies are needed for infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. Cefepime (FEP) combined with the novel ESBL inhibitor enmetazobactam (EMT, formerly AAI101) is in phase 3 development for the treatment of adult patients with cUTI/AP. In this study, the in vitro activity of EMT relative to tazobactam (TZB) was compared against ESBL-producing- and FEP-resistant *Enterobacteriaceae*. **Methods:** Broth microdilution MIC assays were performed following CLSI guidelines. Isolates tested were *Escherichia coli* isogenic strains (15) expressing ESBLs (CTX-M, SHV, TEM); clinical isolates (CI) of *E. coli* (109) and *Klebsiella pneumoniae* (102) expressing diverse ESBLs with or without AmpC and/or OXA-48; and CI of *Enterobacteriaceae* (41) resistant to FEP (MIC ≥16 µg/ml) but susceptible to meropenem (MIC ≤1 µg/ml). **Results:** Against the 15 isogenic strains of ESBL-producing *E. coli,* addition of 4 or 8 µg/ml of EMT reduced the MIC_{90} value of FEP 128-fold from 16 to 0.12 µg/ml (Table). The MIC_{90} of piperacillin-EMT (8 µg/ml) was ≥32-fold lower than piperacillin-TZB (256 µg/ml). Against the ESBL-producing *E. coli* and *K. pneumoniae* CI, MIC_{90} values for FEP-EMT were ≥1,024-fold (0.12 µg/ml) and ≥128-fold (1 µg/ml) lower, respectively, than the FEP MIC_{90} value alone (>64 µg/ml) for both species. FEP-TZB was less potent against the ESBL-producing *K. pneumoniae* CI, with an MIC_{90} of 8 µg/ml. Against the FEP-resistant *Enterobacteriaceae*, FEP-EMT exhibited an MIC_{90} of 1 µg/ml. FEP-EMT exhibited similar potency as meropenem against all organism groups. **Conclusions:** EMT exhibits more potent inhibitory activity than TZB against ESBL-producing *Enterobacteriaceae*. Continued development of FEP-EMT as empiric therapy in settings where ESBLs with or without AmpC and/or OXA-48 are prevalent is warranted.

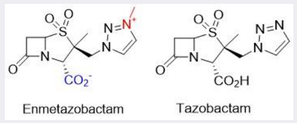
Table. Cumulative % MIC distributions for the tested ESBL-producing or cefepime-resistant organism groups of *Enterobacteriaceae*

Organism group (n)/ antibacterial agent[a]	Cumulative % of isolates at MIC [µg/ml]														MIC_{50}	MIC_{90}
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
E. coli, ESBL-producing isogenic strains (15)																
Cefepime			13.3	20.0	20.0	33.3	53.3	80.0	93.3	100					4	16
Cefepime-enmetazobactam[4]	86.7	100													≤0.06	0.12
Cefepime-enmetazobactam[8]	80.0	100													≤0.06	0.12
Piperacillin-tazobactam[4]						33.3	46.7	46.7	53.3	73.3	73.3	80.0	86.7	100	16	>256
Piperacillin-enmetazobactam[4]						46.7	86.7	93.3	100						4	8
Meropenem	100														≤0.06	≤0.06
E. coli, ESBL-producing clinical isolates (109)[b]																
Cefepime		0.9	0.9	3.7	6.4	13.8	23.9	36.7	52.3	67.0	81.7	100[c]			16	>64
Cefepime-enmetazobactam[8]	69.7	90.8	98.2	98.2	99.1	99.1	99.1	99.1	99.1	100					0.06	0.12
Piperacillin-tazobactam[4]				0.9	8.3	40.4	63.3	75.2	82.6	88.1	92.7	94.5	100[c]		4	64
Meropenem	97.2	98.2	98.2	99.1	99.1	99.1	99.1	100							0.03	0.03
K. pneumoniae, ESBL-producing clinical isolates (102)[b]																
Cefepime					2.0	3.9	6.9	16.7	30.4	42.2	52.9	100[c]			64	>64
Cefepime-enmetazobactam[8]	42.2	66.7	77.5	89.2	92.2	93.1	98.0	100							0.12	1
Cefepime-tazobactam[8]	32.4	49.0	60.8	68.6	76.5	85.3	88.2	92.2	95.1	96.1	97.1	100[c]			0.25	8
Piperacillin-tazobactam[4]					1.0	7.8	15.7	28.4	44.1	53.9	56.9	64.7	100[c]		32	>128
Meropenem	72.5	80.4	81.4	86.3	91.2	92.2	95.1	95.1	100[c]						0.03	1
Enterobacteriaceae, cefepime-resistant, meropenem-susceptible clinical isolates (41)[d]																
Cefepime									29.3	61.0	68.3	73.2	100[c]		32	>128
Cefepime-enmetazobactam[8]	51.2	68.3	70.7	85.4	95.1	97.6	97.6	97.6	100						0.06	1
Piperacillin-tazobactam[4]					7.3	19.5	29.3	41.5	58.5	65.9	68.3	70.7	100[c]		16	>128
Meropenem	75.6	78.0	82.9	90.2	100										0.03	0.5

[a] Values in square brackets represent the concentration of enmetazobactam or tazobactam used in combination (i.e. 4 or 8 µg/ml).
[b] Isolates obtained from 2014 to 2105 in a global surveillance program. Meropenem-resistant *K. pneumoniae* isolates are OXA-48 genotype except for one isolate.
[c] Values in italics are MICs for isolates that exceeded the highest concentration tested for either cefepime (i.e. >64 µg/ml or >128 µg/ml), piperacillin-tazobactam (i.e. >128 µg/ml), cefepime-tazobactam (i.e. >64 µg/ml) or meropenem (i.e. >8 µg/ml).
[d] Isolates obtained in 2016 from a global surveillance program and comprised 1 *E. aerogenes*, 6 *E. cloacae* spp. complex, 16 *E. coli*, 15 *K. pneumoniae*, 2 *P. mirabilis*, and 1 *P. stuartii*.

Background

- The World Health Organization has listed ESBL-producing *Enterobacteriaceae* as a priority for development of new therapies (1).
- Use of carbapenems, a "last resort" class of β-lactams, to treat serious infections caused by ESBL-producing *Enterobacteriaceae* promotes the emergence and dissemination of carbapenem-resistant pathogens (2,3).
- Development of new "carbapenem-sparing" options as empiric therapy for ESBL-producing *Enterobacteriaceae* is critical in limiting carbapenem resistance development in Gram-negative pathogens (4, 5).
- Enmetazobactam is a novel ESBL inhibitor belonging to the penicillanic acid sulfone class that exerts potent inhibitory activity against CTX-M, TEM, SHV, and other class A β-lactamases by a mechanism that differs from tazobactam (6, 7).
- The combination of cefepime-enmetazobactam is intended as an empiric, carbapenem-sparing option in settings with a high incidence of ESBL-producing *Enterobacteriaceae*.
- The safety and efficacy of cefepime 2 g-enmetazobactam 0.5 g and piperacillin 4 g-tazobactam 0.5 g administered every 8 h is currently being investigated in a randomized, double-blind, non-inferiority Phase 3 study in adults with cUTI or AP (8).
- This study investigated the in vitro activity of cefepime-enmetazobactam against ESBL-producing isogenic strains and clinical isolates and cefepime-resistant clinical isolates of *Enterobacteriaceae.*



Methods

Isogenic strains. A panel of 15 isogenic *E. coli* DH10B recombinant strains expressing diverse single ESBL genes on plasmid pBC SK(+/-) was assembled to assess the in vitro activity of cefepime-enmetazobactam and comparator agents.

Clinical isolates. Clinical isolates obtained from hospitalized patients were collected by IHMA Europe Sàrl (Monthey, Switzerland) or JMI Laboratories (North Liberty, IA). Organisms were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry or next-generation sequencing.

Susceptibility testing. Minimal inhibitory concentrations (MIC) for the tested antimicrobial agents were determined by broth microdilution assay according to Clinical Laboratory Standards Institute (CLSI) guidelines. Quality control strains *E. coli* ATCC 25922, *E. coli* ATCC 35218 (TEM-1 producer), *K. pneumoniae* ATCC 700603 (SHV-18 producer), and *P. aeruginosa* ATCC 27853 (inducible PDC producer) were used to ensure appropriate assay performance.

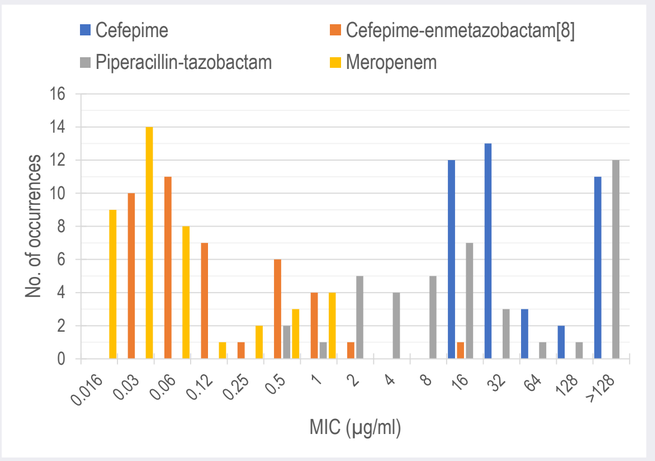
Results

Table 1. Cefepime-enmetazobactam exhibits potent antibacterial activity against a diversity of ESBL-producing isogenic strains (n=15) of *E. coli*

Expressed ESBL isoform	MIC (µg/ml)					
	FEP	FEP-EMT (4 µg/ml)[1]	FEP-EMT (8 µg/ml)	PIP-TAZ (4 µg/ml)	PIP-EMT (4 µg/ml)	MEM
E. coli DH10B[2]	≤0.06[3]	≤0.06	≤0.06	2	2	≤0.06
SHV-2	4	≤0.06	0.12	32	4	≤0.06
SHV-5	8	≤0.06	≤0.06	256	4	≤0.06
SHV-7	8	≤0.06	≤0.06	32	4	≤0.06
SHV-8	2	≤0.06	≤0.06	2	2	≤0.06
SHV-30	2	0.12	0.12	32	2	≤0.06
SHV-102	16	0.12	≤0.06	> 256	8	≤0.06
SHV-106	4	≤0.06	≤0.06	16	2	≤0.06
SHV-120	0.25	≤0.06	≤0.06	> 256	16	≤0.06
SHV-129	16	≤0.06	≤0.06	128	4	≤0.06
SHV-141	0.25	≤0.06	≤0.06	2	2	≤0.06
SHV-154	8	≤0.06	≤0.06	4	4	≤0.06
TEM-10	4	≤0.06	≤0.06	4	4	≤0.06
TEM-26	0.5	≤0.06	≤0.06	2	2	≤0.06
CTX-M-14	8	≤0.06	0.12	2	2	≤0.06
CTX-M-15	32	≤0.06	≤0.06	2	2	≤0.06

Abbreviations: FEP, cefepime; EMT, enmetazobactam; PIP, piperacillin; MEM, meropenem
[1] Values in brackets represent the fixed concentration of enmetazobactam or tazobactam used in combination.
[2] All ESBL isoforms were expressed in the parental strain *E. coli* DH10B.
(Table 1 adapted from reference 6)

Figure 1. Cefepime-enmetazobactam (fixed at 8 µg/ml) is more potent than piperacillin-tazobactam against cefepime-resistant, meropenem-susceptible clinical isolates (n=41) of *Enterobacteriaceae*



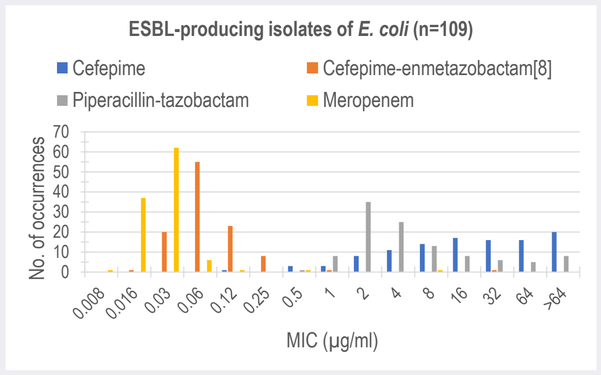
(Figure 1 adapted from reference 9)

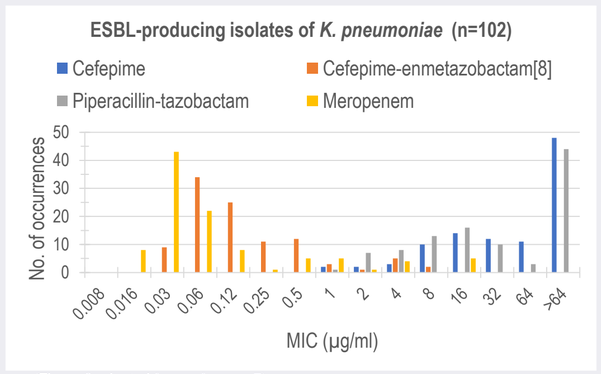
Table 2. Cefepime-enmetazobactam exhibits potent antibacterial activity against clinical isolates of ESBL-producing *E. coli* and *K. pneumoniae*

Organism group/antibacterial agent	MIC (µg/ml)			% Susceptibility	
	MIC_{50}	MIC_{90}	Range	CLSI	EUCAST
Non-ESBL genotype (n=1,233)					
Cefepime	0.06	0.12	0.016 to >64	99.6	99.2
Cefepime-enmetazobactam[8]	0.06	0.12	0.016 to 1	*(100)*[1]	*(100)*[1]
Piperacillin-tazobactam	2	8	0.12 to >128	95.1	93
Meropenem	0.03	0.03	0.008 to 0.5	100	100
Ceftolozane-tazobactam	0.25	0.5	0.06 to >32	99.5	98.6
Ceftazidime	0.25	0.5	0.03 to >64	98.3	96.6
Ceftazidime-avibactam	0.12	0.25	≤0.016 to 1	100	100
ESBL genotype (n=211)					
Cefepime	32	>64	0.12 to >64	27	4.3
Cefepime-enmetazobactam[8]	0.06	0.5	0.016 to 32	*(99.5)*[1]	*(95.7)*[1]
Piperacillin-tazobactam	8	>128	0.5 to >128	64	52.6
Meropenem	0.03	0.25	0.008 to >8	95.3	95.7
Ceftolozane-tazobactam	0.5	16	0.12 to >32	73.9	68.2
Ceftazidime	32	>64	0.25 to >64	16.1	2.8
Ceftazidime-avibactam	0.25	1	≤0.016 to 2	100	100

[1] Breakpoints for cefepime-enmetazobactam have not been established. Values in italics represent the percent susceptibilities when applying cefepime breakpoints (CLSI, susceptible, dose-dependent ≤8 µg/ml; EUCAST, ≤ 1 µg/ml).
(Table 2 adapted from reference 7)

Figure 2. Cefepime-enmetazobactam (fixed at 8 µg/ml) exhibits similar potency to meropenem against clinical isolates of ESBL-producing *E. coli* and *K. pneumoniae*





(Figure 2 adapted from reference 7)

Conclusions

- Enmetazobactam restores the *in vitro* activity of cefepime against ESBL-producing clinical isolates and isogenic strains of *E. coli* and *K. pneumoniae* expressing a diversity of β-lactamases.
- Overall, cefepime-enmetazobactam exhibits comparable antibacterial activity to meropenem and outperforms piperacillin-tazobactam against the ESBL-producing clinical isolates of *E. coli* and *K. pneumoniae.*
- Cefepime-enmetazobactam is intended as an empiric, 'workhorse, carbapenem-sparing option' for Gram-negative infections in settings where ESBL-producing *Enterobacteriaceae* are endemic.

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