In Vitro Analysis of AmpC β-lactamase Induction by Tebipenem in *Enterobacterales* and *Pseudomonas aeruginosa*

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Introduction

- Urinary tract infections (UTIs) are among the most common bacterial infections and the great majority of these infections are caused by *Escherichia coli*, followed by *Klebsiella pneumoniae* and *Proteus mirabilis.*
- Tebipenem is an orally bioavailable carbapenem in clinical development for treating complicated UTI and acute pyelonephritis in the US.
- Tebipenem possesses broad-spectrum activity against isolates producing penicillinases, narrow- and extended-spectrum β-lactamases, and intrinsic and plasmid encoded AmpC β-lactamases (see posters 1057, 1226, and 1254).
- The cell-wall degradation caused by β-lactams results in peptide accumulation. These peptides bind to AmpR, which negatively regulates AmpC production. The decrease of AmpR increases transcription of *ampC*.
- It has been shown that some β-lactam agents, such as the aminopenicillins, amoxicillin-clavulanate, narrow-spectrum (i.e., first-generation) cephalosporins, and the cephamycins increase the production of the intrinsic AmpC in Gram-negative bacteria after *in vivo* and *in vitro* exposure.
  - AmpC producers such as *Enterobacter cloacae* complex, *Citrobacter freundii*, and *Serratia marcescens* can easily hydrolyze these agents even at basal AmpC expression levels, what makes them intrinsically resistant to these inducers.
  - Piperacillin-tazobactam, aztreonam, and expanded-spectrum (i.e., third- and fourth-generation) cephalosporins are weak inducers of AmpC hyperproduction, and these can be hydrolyzed if enough enzyme gets produced. The exception would be for cefepime, which can withstand hydrolysis.
  - Among carbapenem agents, imipenem is considered a strong AmpC inducer, although carbapenems are not AmpC substrates.
- This study investigates the induction properties of tebipenem over the AmpC encoding gene in Gram-negative organisms.

Materials and Methods

Bacterial organisms

- A total of 8 *Enterobacterales* species and 1 *Pseudomonas aeruginosa* isolate were selected for the AmpC induction experiments for tebipenem, imipenem, ertapenem, and ceftazidime. These isolates demonstrated a general susceptible phenotype toward broad-spectrum β-lactam agents and represented isolates with stable and baseline production of AmpC (Table 1).
- A second set of 36 *Enterobacterales* and 32 *P. aeruginosa* clinical isolates with proven overexpression of AmpC by qRT-PCR (i.e. expression >10-fold higher than a susceptible control) were tested for susceptibility as well.
- These isolates were recovered from patients with documented infections during 2010–2019 (55.8% from 2015–2019) and sent to a central monitoring laboratory (JMI Laboratories, North Liberty, IA, USA) as part of the SENTRY Antimicrobial Surveillance Program.
- Bacterial identification was confirmed by standard algorithms supported by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.
- Frozen-form broth microdilution panels were manufactured by JMI Laboratories and contained cation-adjusted Mueller-Hinton broth.
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.

Induction experiments of AmpC

- Baseline MIC values were determined by broth microdilution method, as described above, for 9 Gram-negative isolates with basal expression of *ampC*. These isolates were cultured overnight on blood agar plates. A standardized inoculum made from fresh overnight plate cultures was added to flasks containing 50 mL of Luria-Bertani (LB) broth and incubated by shaking at 37°C to OD600 of 0.3.
- Upon reaching the exponential growth phase, each organism culture was split into multiple flasks with each testing drug (tebipenem, ceftazidime, ertapenem, and imipenem) added to various final concentrations (0x, 0.25x, 1x, 4x, and 16x) of the respective baseline MIC. These cultures were incubated under shaking conditions. A 40 mL aliquot of bacterial cultures was sampled at time 0 and 2 hours after drug exposures. Each sample aliquot was harvested by centrifugation and the supernatant was discarded.
- Protein crude extract preparations were made in 200 µL of BugBuster® (Novagen, Darmstadt, Germany) per manufacturer instructions and cell debris was removed by centrifugation. Protein concentrations were determined in each crude extract by standard methodologies.
- AmpC induction was measured by the intensity of β-lactamase hydrolytic activity against 0.1 mM nitrocefin in 0.1 M phosphate buffer at pH 7.0 using spectrophotometry at 482 nm (Δ absorbance/minute/mg of protein). The drug exposure conditions that caused a >4-fold increase in hydrolysis activity compared to the baseline were subjected to post-induction experiments. Briefly, cells were grown under the same conditions as before and tested for susceptibility by broth microdilution.

Results

- In general, tebipenem and imipenem increased production of AmpC among all *Enterobacterales*, except for *C. koseri* and *S. marcescens* (Table 1).
- Exposure to ertapenem and ceftazidime did not seem to affect production of AmpC among the *Enterobacterales* species tested (Table 1).
- Tebipenem, imipenem, and ceftazidime increased the production of AmpC in *P. aeruginosa* after exposure. The same effect over *P. aeruginosa* was not observed with ertapenem.
- Overall, the bacterial cells with confirmed increased presence of AmpC after drug exposure did not show increased MIC (i.e., >4-fold) to antimicrobial agents when compared to baseline values (Table 2).
- When tested against the second set of *Enterobacterales* overproducing AmpC (>10-fold higher than a susceptible control) according to qRT-PCR experiments, tebipenem ($MIC_{50/90}$, 0.03/0.25 µg/mL) inhibited all isolates at ≤1 µg/mL (Table 3).
  - Tebipenem and meropenem ($MIC_{50/90}$, 0.03/0.12 µg/mL) MIC_{50} and MIC_{90} results obtained against this *Enterobacterales* collection were similar.
  - These MIC results for tebipenem and meropenem were at least 2- to 4-fold lower than those for ertapenem ($MIC_{50/90}$, 0.12/2 µg/mL) and imipenem ($MIC_{50/90}$, 0.25/0.5 µg/mL).
- Tebipenem showed MIC_{50} and MIC_{90} results of 4 and 4 µg/mL, respectively, against *P. aeruginosa* isolates that overproduced AmpC (Table 3).

Conclusions

- The induction experiments performed showed that exposure to tebipenem promoted increased production of AmpC in *Enterobacterales*. However, imipenem seemed to be, in general, an AmpC inducer stronger than tebipenem.
  - The AmpC induction phenomenon seemed to be species dependent for both tebipenem and imipenem, since negative or inconsistent results were obtained for *C. koseri* and *S. marcescens.*
- AmpC induction among *Enterobacterales* was not observed for the comparator agents ertapenem and ceftazidime.
- In general, exposure to imipenem, followed by ceftazidime and tebipenem, promoted increased production of AmpC in *P. aeruginosa*.
- *Enterobacterales* or *P. aeruginosa* cells showing increased production of AmpC after drug exposure did not display increased MIC when compared to the respective baseline counterpart.
  - This observation may suggest that the production of AmpC induced in these isolates was not elevated enough to cause a shift in MIC.
- Finally, tebipenem showed potent activity against *Enterobacterales* with confirmed overproduction of AmpC. The tebipenem antimicrobial potency was similar to that observed for meropenem, both of which were greater than those noted for imipenem and ertapenem.

Acknowledgements

This research was supported by a contract by Spero Therapeutics, Inc. and funded in whole or in part with Federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority, under Contract No. HHS0100201800015C.

Table 1. Fold induction of *ampC* expression among Gram-negative clinical isolates after exposure to tebipenem or comparator agents

Species	Baseline MIC (µg/mL) and fold increase in AmpC activity by exposure concentration[a]																			
	Tebipenem					Imipenem					Ertapenem					Ceftazidime				
	MIC	0.25X	1X	4X	16X	MIC	0.25X	1X	4X	16X	MIC	0.25X	1X	4X	16X	MIC	0.25X	1X	4X	16X
Citrobacter freundii	0.015	3.9	5.1	5.7	6.1	1	7.3	5.1	5.6	1.9	0.015	0.7	0.8	0.8	0.8	0.5	0.4	0.8	0.4	0.3
Citrobacter koseri	0.015	2.1	1.1	1.0	3.3	0.06	1.7	1.4	1.3	0.2	0.008	1.0	1.0	0.8	0.8	0.12	0.8	0.7	0.4	0.5
Enterobacter cloacae	0.015	3.0	7.4	4.7	0.7	0.12	2.7	9.0	22.9	27.6	0.015	1.8	1.8	1.8	1.9	0.25	2.4	0.8	0.2	–0.2
Klebsiella aerogenes	0.03	1.6	5.3	7.2	0.4	0.25	2.3	12.2	14.8	–1.2	0.015	0.7	0.0	0.0	0.2	0.12	0.4	–0.8	–3.1	–0.5
Morganella morganii	0.12	8.0	5.6	4.8	5.5	2	5.6	10.6	10.4	4.0	0.03	0.5	0.6	0.7	0.6	0.12	0.7	0.5	0.7	0.9
Providencia rettgeri	0.12	26.1	34.0	25.9	10.4	1	10.6	22.6	38.6	32.4	0.015	–1.9	–3.6	0.0	–0.4	0.03	–5.2	–2.1	–2.4	–2.8
Providencia stuartii	0.06	12.5	40.5	5.6	12.9	0.5	12.2	11.9	7.3	3.0	0.015	0.4	1.0	0.9	0.7	0.12	–0.2	–0.1	–0.1	–1.2
Serratia marcescens	0.03	0.7	0.3	–0.5	–0.4	0.12	1.5	2.3	4.9	1.0	0.015	1.4	1.2	0.8	1.2	0.06	0.3	1.0	0.8	0.7
Pseudomonas aeruginosa	1	82.2	–1.9	–1.7	–19.7	0.5	363.1	13.6	–25.5	1.3	2	–1.8	–8.5	2.4	–22.6	2	5.7	24.2	53.2	125.7

[a] The fold increase was measured as the ΔAbs/min/mg protein of nitrocefin from cultures exposed to drug/ΔAbs/min/mg protein of nitrocefin from a culture without exposure to drug. White cells represent those values with a >4-fold increase in hydrolytic activity.

Table 2. Effects on MIC values associated with Gram-negative isolates with induced *ampC* expression after exposure to tebipenem or comparator agents. Only strains with highest AmpC expression for each inducer are shown.

Species exposed/agent (xMIC concentration)	Fold induction[a]	MIC (µg/mL)															
		TBP	ETP	IMI	CAZ	A-C	ATM	FAZ	FEP	CRO	FUR	LEX	CIP	MEM	PIP-TAZ	TET	TIG
C. freundii																	
Baseline MIC	NA	0.015	0.015	1	0.25	32	0.12	>32	0.06	0.25	2	>256	≤0.03	0.03	2	1	0.25
Tebipenem (16x)	6.1	0.015	0.015	≤0.12	0.25	32	0.12	>32	0.03	0.25	4	256	≤0.03	≤0.015	2	1	0.25
Imipenem (0.25x)	7.3	0.03	0.015	1	0.25	32	0.5	>32	0.03	0.25	2	>256	≤0.03	0.03	2		1
E. cloacae																	
Baseline MIC	NA	0.015	≤0.008	0.25	0.5	16	0.06	>32	0.06	0.25	8	64	≤0.03	≤0.015	2	2	0.5
Tebipenem (1x)	7.4	0.008	≤0.008	≤0.12	0.25	16	0.12	>32	0.06	0.25	8	64	≤0.03	≤0.015	2	2	0.5
Imipenem (16x)	27.6	0.008	≤0.008	≤0.12	0.25	32	0.06	>32	0.03	0.25	8	128	≤0.03	≤0.015	2	2	0.5
K. aerogenes																	
Baseline MIC	NA	0.03	0.015	0.5	0.12	32	≤0.03	4	0.06	≤0.06	4	16	0.06	0.03	2	1	0.5
Tebipenem (4x)	7.2	0.015	0.015	0.25	0.25	16	0.06	2	0.03	≤0.06	2	16	≤0.03	0.03	2	2	0.25
Imipenem (4x)	14.8	0.03	0.015	0.25	0.12	16	0.12	4	0.03	0.12	4	16	≤0.03	0.03	2	2	0.5
M. morganii																	
Baseline MIC	NA	0.12	0.03	4	0.12	>32	≤0.03	>32	0.03	≤0.06	32	>256	≤0.03	0.12	0.25	1	0.5
Tebipenem (0.25x)	8.0	0.12	0.03	2	0.25	>32	≤0.03	>32	0.03	≤0.06	32	>256	≤0.03	0.06	0.25	1	0.5
Imipenem (1x)	10.6	0.12	0.03	2	1	>32	0.06	>32	0.03	0.12	32	>256	≤0.03	0.06	0.25	≤0.5	0.5
P. rettgeri																	
Baseline MIC	NA	0.12	0.015	1	0.03	32	≤0.03	>32	0.015	≤0.06	≤0.5	128	≤0.03	0.12	0.12	2	2
Tebipenem (1x)	34.0	0.12	0.015	1	0.06	32	≤0.03	>32	0.015	≤0.06	≤0.5	128	≤0.03	0.06	0.12	1	1
Imipenem (4x)	38.6	0.12	0.015	1	0.12	32	≤0.03	>32	0.015	≤0.06	≤0.5	64	≤0.03	0.06	0.12	1	1
P. stuartii																	
Baseline MIC	NA	0.06	0.015	1	0.12	16	≤0.03	>32	0.03	≤0.06	2	16	0.06	0.06	1	>16	2
Tebipenem (1x)	40.5	0.06	0.015	0.5	0.25	8	≤0.03	8	0.03	≤0.06	4	8	≤0.03	0.06	1	>16	4
Imipenem (0.25x)	12.2	0.06	0.015	1	0.5	32	≤0.03	16	0.06	≤0.06	8	8	≤0.03	0.06	1	>16	2
S. marcescens																	
Baseline MIC		0.03	≤0.008	0.25	0.06	4	≤0.03	>32	0.015	≤0.06	8	32	0.06	≤0.015	0.25	>16	1
Imipenem (4x)	4.9	0.015	≤0.008	≤0.12	0.03	4	≤0.03	>32	0.03	≤0.06	8	32	0.12	≤0.015	0.25	>16	1
P. aeruginosa																	
Baseline MIC	NA	2	2	1	1	>32	4	>32	1	>8	>64	>256	0.06	0.25	4	16	8
Tebipenem (0.25x)	82.2	2	2	1	1	>32	4	>32	1	>8	>64	>256	0.12	0.25	4	16	8
Imipenem (0.25x)	363.1	1	2	1	2	>32	4	>32	1	>8	>64	>256	0.12	0.25	4	16	8
Ceftazidime (16x)	125.7	2	2	0.5	1	>32	8	>32	1	>8	>64	>256	0.06	0.25	4	16	8

Drug abbreviations as follows: tebipenem (TBP), ertapenem (ETP), imipenem (IMI), ceftazidime (CAZ), amoxicillin-clavulanic acid (A-C), aztreonam (ATM), cefazolin (FAZ), cefepime (FEP), ceftriaxone (CRO), cefuroxime (FUR), cephalexin (LEX), ciprofloxacin (CIP), meropenem (MEM), piperacillin-tazobactam (PIP-TAZ), tetracycline (TET), and tigecycline (TIG). Highlighted cells indicate an MIC increase of >4-fold compared to the baseline MIC.
[a] The fold increase was measured as the ΔAbs/min/mg protein from cultures exposed to drug/ΔAbs/min/mg protein from a culture without exposure to drug. Induction not applicable to isolate prior to antimicrobial exposures (baseline).

Table 3. Antimicrobial activity of tebipenem and comparator agents when tested against *Enterobacterales* and *P. aeruginosa* clinical isolates with overexpression of AmpC

Antimicrobial agent	MIC (µg/mL)			CLSI[a]		
	MIC_{50}	MIC_{90}	MIC range	%S	%I	%R
Enterobacterales (36)						
Tebipenem	0.03	0.25	0.015 to 1	NA	NA	NA
Ertapenem	0.12	2	0.015 to >2	80.6	8.3	11.1
Imipenem	0.25	0.5	≤0.12 to 1	100.0	0.0	0.0
Meropenem	0.03	0.12	≤0.015 to 1	100.0	0.0	0.0
Amoxicillin-clavulanic acid	>32	>32	8 to >32	2.8	11.1	86.1
Aztreonam	>16	>16	2 to >16	31.4	14.3	54.3
Cefepime	0.5	128	0.03 to >256	68.6	8.6	22.9
Ceftazidime	32	>32	1 to >32	28.6	11.4	60.0
Ceftriaxone	>8	>8	0.25 to >8	22.2	13.9	63.9
Cefuroxime	>64	>64	16 to >64	0.0 [b] 0.0 [c]	5.6 5.6	94.4 94.4
Ciprofloxacin	0.12	>16	≤0.03 to >16	55.6	2.8	41.7
Piperacillin-tazobactam	32	>128	4 to >128	47.2	19.4	33.3
Tetracycline	4	>16	1 to >16	50.0	5.6	44.4
Tigecycline	0.25	1	≤0.06 to 4	94.4 [d]	5.6	0.0
P. aeruginosa (32)						
Tebipenem	4	4	2 to 8	NA	NA	NA
Ertapenem	>2	>2	2 to >2	NA	NA	NA
Imipenem	1	2	0.5 to 8	96.9	0.0	3.1
Meropenem	0.5	1	0.12 to 2	100.0	0.0	0.0
Aztreonam	16	>16	4 to >16	18.8	31.2	50.0
Cefepime	16	32	4 to >256	46.9	40.6	12.5
Ceftazidime	32	>32	4 to >32	15.6	12.5	71.9
Ceftriaxone	>8	>8	>8 to >8	NA	NA	NA
Ciprofloxacin	0.12	16	0.06 to >16	68.8	6.2	25.0
Piperacillin-tazobactam	128	>128	8 to >128	6.2	40.6	53.1

The *Enterobacterales* group includes: *Citrobacter freundii* species complex (1), *Enterobacter cloacae* species complex (11), *Escherichia coli* (18), *Serratia marcescens* (6).
[a] Criteria as published by CLSI (2020); NA, not available.
[b] Using oral breakpoints.
[c] Using parenteral breakpoints.
[d] Using breakpoints as per the FDA guidelines.

References

CLSI. M07Ed11. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: Eleventh Edition. Wayne, PA, Clinical and Laboratory Standards Institute, 2018.

CLSI. M100Ed30. Performance standards for antimicrobial susceptibility testing: 30th Informational Supplement. Wayne, PA, Clinical and Laboratory Standards Institute, 2020.

Lindberg F, Lindquist S, Normark S (1988). Genetic basis of induction and overproduction of chromosomal class I β-lactamase in nonfastidious Gram-negative bacilli. *Rev Infect Dis* 10: 782–5.

Livermore DM, Jamrozy D, Mushtaq S, Nichols WW, Young K, Woodford N (2017). AmpC β-lactamase induction by avibactam and relebactam, *Journal of Antimicrobial Chemotherapy*, 72: 3342–3348.

Mushtaq S, Livermore DM (2010). AmpC induction by ceftaroline. *J Antimicrob Chemother*. 65: 586-588.

Tamma PD, Doi Y, Bonomo RA, Johnson JK, Simner PJ, Antibacterial Resistance Leadership Group (2019). A primer on AmpC β-lactamases: Necessary knowledge for an increasingly multidrug-resistant world. *Clin Infect Dis*. 69: 1446-1455.

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