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In Vitro-In Vivo Discordance with β-lactams against Metallo-β-lactamase-Producing Enterobacterales: Implications for Susceptibility Testing

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ABSTRACT (revised)

Background: Using murine models of infection, we previously reported the potent in vivo activity of carbapenems against metallo-β-lactamase-producing Enterobacterales despite the observed resistance in vitro. In the current study, we examined the in vivo activity of cefepime human-simulated regimen against metallo-β-lactamase-producing Enterobacterales in a murine thigh infection model.

Methods: A population of clinical isolates and isogenic engineered metallo-β-lactamase-producing Enterobacterales transformants expressing metallo-β-lactamases but no detectable cefepime-hydrolyzing serine β-lactamases were utilized. KPC-producing isolates were included as positive controls. Cefepime, piperacillin-tazobactam and meropenem MICs were determined using broth microdilution in conventional cation-adjusted Mueller Hinton and EDTA-supplemented (zinc-limited) broth. In vivo efficacy of a cefepime human-simulated regimen (2 g q8h as 2 h infusion) was determined in the neutropenic murine thigh infection model against the test strains. Efficacy was measured as the change in log10cfu/thigh at 24 h compared with 0 h controls.

Results: Metallo-β-lactamase-producing Enterobacterales strains were found to be cefepime, piperacillin-tazobactam and meropenem non-susceptible in conventional broth. Supplementation with EDTA at a concentration of 300 mg/L resulted in multi-fold reduction in the MICs and restoration of susceptibility. In accordance with the MICs generated in zinc-limited broth, administration of cefepime human-simulated regimen was associated with substantial bacterial reductions among mice infected with metallo-β-lactamase-producing Enterobacterales. Absence of MIC reduction in zinc-limited broth and lack of efficacy among mice infected with KPC-producing isolates were observed.

Conclusions: For metallo-β-lactamase-producing Enterobacterales, susceptibility testing with Mueller-Hinton Broth, a zinc-rich testing medium is flawed since it does not recapitulate the host environment in which zinc concentrations are low.

INTRODUCTION

- A series of assessments of outcomes of β-lactam agents against metallo-β-lactamase-producing Enterobacterales infections in murine models have provided evidence that *in vitro-in vivo* discordance exists provided that the agents are dosed to attain clinically-achievable exposures (1-5).
- The results of these investigations revealed a major flaw in the currently utilized *in vitro* susceptibility testing methodologies; while the zinc levels in the conventional culture media such as the cation adjusted Mueller Hinton Broth (CAMHB) utilized in broth microdilution varied among different manufacturers, they were generally higher than the physiologic unbound zinc levels particularly at infection sites (1)
- The impact of the discordance in zinc levels is significant for metallo-β-lactamase-producing organisms because zinc is important for protein folding as well as bicyclic β-lactam ring hydrolysis (6).

OBJECTIVES

- To examined the efficacy of a clinically-achievable exposure of cefepime (FEP), a fourth-generation cephalosporin, against a broad variety of metallo-β-lactamase-producing Enterobacterales using a neutropenic murine thigh infection model.

MATERIALS & METHODS

Antimicrobial Test Agents

- Cefepime vials (2 g, Sagent Pharmaceuticals, Inc) used for *in vivo* testing.
- Cefepime HCl (Sigma-Aldrich, batch LRAB8503), piperacillin (Sigma-Aldrich, batch 098M4886V), tazobactam (Tecoland Corp., batch J1104B) and meropenem (Sigma-Aldrich, batch LRAB7853) analytical grade standards were used for *in vitro* testing.

Neutropenic Murine Thigh Infection Model

- Female ICR mice were rendered neutropenic by cyclophosphamide; uranyl nitrate was given to induce renal impairment.
- Thighs were inoculated with 0.1 mL of 10^7 CFU/ml bacterial suspensions.

FEP Human-Simulated Regimen (HSR)

- A previously established FEP HSR, providing an exposure comparable to that observed in humans following a dose of 2g q8h as 2h infusion.
- The percentages of dosing interval during which the unbound FEP concentrations remained above a threshold plasma concentration (%$fT>C_T$) achieved at C_Ts ≥64 and ≤8 mg/L were ≤8% and ≥83%, respectively, in humans and mice receiving the selected HSR (7).

Bacteria and In vitro Susceptibility

- Clinical Enterobacterales isolates expressing various metallo-β-lactamases (n=21) but not expressing any serine carbapenemases, ESBLs or extended-spectrum cephalosporinases.
- Clinical isolates were acquired from and molecularly characterized by the FDA-CDC Antimicrobial Resistance Isolate Bank (Atlanta, GA, USA), selected from Hartford Hospital CAIRD isolates repository or IHMA, Inc. (Schaumburg, IL)
- Clinical Enterobacterales expressing serine carbapenemases (n=3, KPC-2, KPC-3) were utilized as positive controls.
- A reference strain *Klebsiella pneumoniae* ATCC® 10031™ to which bla_{NDM-1}, bla_{NDM-4}, bla_{VIM-1}, bla_{IMP-1} or bla_{KPC-2} genes were introduced as well as the parental strain were also examined.
- FEP, piperacilin/tazobactam (TZP), and meropenem (MEM) MICs were determined in triplicate using broth microdilution in CAMHB as outlined by the CLSI and in CAMHB supplemented with EDTA (300 mg/L) as previously shown to provide a zinc-limited environment (1).

In Vivo Efficacy of Human-Simulated Exposures

- Efficacy of FEP HSR R was assessed against the aforementioned Enterobacterales strains.
- Efficacy was measured as the change in log_{10}CFU/thigh at 24h compared with 0h controls.

RESULTS

Table 1. β-lactamase gene content of the isolates and modal MICs determined in CAMHB and CAMHB+EDTA 300 mg/L. Isolates appear in an ascending order of the FEP MICs in CAMHB+EDTA 300 mg/L.

Isolate ID	Organism	Known β-lactamases	MIC (mg/L) in CAMHB			MIC (mg/L) in CAMHB+EDTA 300 mg/L		
			FEP	TZP	MEM	FEP	TZP	MEM
Clinical metallo-β-lactamase-producing isolates								
CK 49	*Citrobacter koseri*	NDM-5	>256	>256	>64	≤0.031	0.5	≤0.063
KP 885	*Klebsiella pneumoniae*	NDM-7	256	>256	>64	≤0.031	1	≤0.063
ECL 100	*Enterobacter cloacae*	NDM-1	64	>256	64	0.063	16	0.25
EC 483	*Escherichia coli*	VIM-1	>256	>256	32	0.063	1	≤0.063
EC 746	*Escherichia coli*	TEM-OSBL(b); NDM-5	256	>256	64	0.063	1	≤0.063
KP 573	*Klebsiella pneumoniae*	VIM-1	>256	>256	64	0.063	2	≤0.063
ECL 172	*Enterobacter cloacae*	NDM-7	128	>256	>64	0.125	4	0.125
EC 482	*Escherichia coli*	VIM-1	>256	>256	16	0.125	2	≤0.063
ECL 179	*Enterobacter cloacae*	TEM-OSBL(b); NDM-5	256	>256	>64	0.125	2	≤0.063
PM 29	*Proteus mirabilis*	NDM-1	128	>256	64	0.125	≤0.25	0.125
ECL 99	*Enterobacter cloacae*	VIM-1	256	>256	32	0.25	2	≤0.063
KP 955	*Klebsiella pneumoniae*	SHV-OSBL(b); TEM-OSBL(b); NDM-19	>256	>256	>64	0.25	2	0.125
CF 50	*Citrobacter freundii*	NDM-7	256	>256	>64	0.5	4	≤0.063
KP 749	*Klebsiella pneumoniae*	IMP-26	256	64	64	0.5	16	0.125
SM 95	*Serratia marcescens*	IMP-1	>256	>256	64	0.5	0.5	≤0.063
KP 750	*Klebsiella pneumoniae*	VIM-1	>256	>256	>64	1	64	1
PS 79	*Providencia stuartii*	NDM-5	256	>256	>64	1	4	0.5
EC 694	*Escherichia coli*	NDM-5	>256	>256	>64	2	32	≤0.063
KP 747	*Klebsiella pneumoniae*	VIM-26	>256	>256	>64	2	1	0.125
EC 491	*Escherichia coli*	NDM	>256	>256	64	4	64	≤0.063
EC 608	*Escherichia coli*	NDM-1	>256	>256	>64	8	64	≤0.063
Clinical KPC-producing isolates								
KP 651	*Klebsiella pneumoniae*	KPC-2; TEM-1D	>256	>256	64	>32	>256	64
KP 652	*Klebsiella pneumoniae*	KPC-3; OXA-9; TEM-1B	>256	>256	64	>32	>256	64
KP 752	*Klebsiella pneumoniae*	KPC-2, VIM-1	>256	>256	>64	>32	>256	>64
Isogenic strains								
ATCC 10031	*Klebsiella pneumoniae*	Parent Strain	≤0.25	≤0.25	≤0.063	≤0.031	≤0.25	≤0.063
ATCC 10031+ KPC-2	*Klebsiella pneumoniae*	KPC-2	64	>256	16	32	>256	16
ATCC 10031+ NDM-1	*Klebsiella pneumoniae*	NDM-1	64	>256	>64	≤0.031	≤0.25	≤0.063
ATCC 10031+ NDM-4	*Klebsiella pneumoniae*	NDM-4	128	>256	>64	≤0.031	≤0.25	≤0.063
ATCC 10031+ IMP-1	*Klebsiella pneumoniae*	IMP-1	64	>256	16	≤0.031	≤0.25	≤0.063
ATCC 10031+ VIM-1	*Klebsiella pneumoniae*	VIM-1	>256	>256	64	≤0.031	≤0.25	≤0.063

Figure 1. Comparative efficacy of FEP HSR vs. 21 clinical metallo-β-lactamase-producing Enterobacterales isolates. Isolates appear in an ascending order of the FEP MICs in CAMHB+EDTA 300 mg/L. Data are means ± standard deviations.

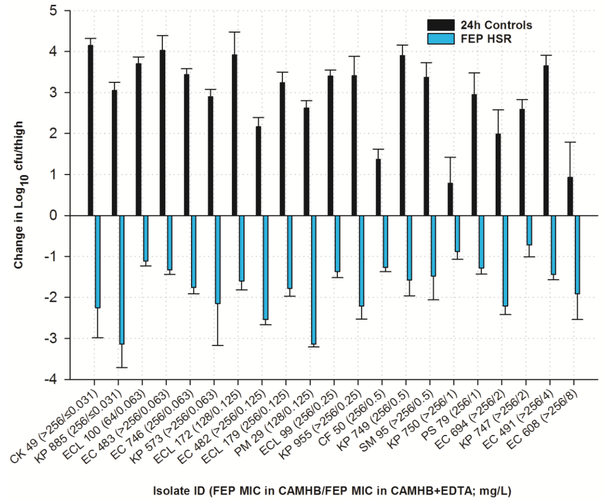


Figure 2. Comparative efficacy of FEP HSR vs. 3 clinical KPC-producing Enterobacterales isolates.
Data are means ± standard deviations.

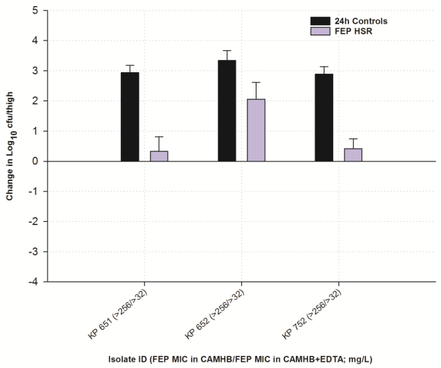
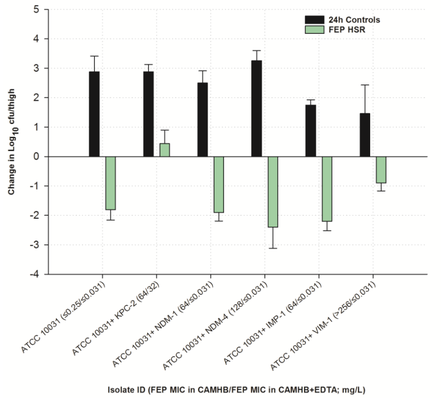


Figure 3. Comparative efficacy of FEP HSR vs. carbapenemase-producing Enterobacterales transformants with isogenic background and the parental strain. Data are means ± standard deviations.



CONCLUSIONS

- Despite the β-lactam hydrolytic capability observed in CAMHB, when broth was treated with EDTA for cation sequestration, all metallo-β-lactamase-producing Enterobacterales strains showed reduction in the MICs of FEP, MEM and, to varying degrees, TZP, while lack of MIC reduction was observed among KPC-producers.
- The MIC reduction among metallo-β-lactamase-producing Enterobacterales strains was attributed to zinc-sequestration and the impairment of metallo-β-lactamase hydrolytic activity under low zinc conditions.
- The marked *in vivo* activity of FEP against the metallo-β-lactamase-producers did not correspond with the elevated MICs generated in CAMHB as the FEP plasma levels achieved were substantially lower than the observed MICs.
- MICs determined in zinc-limited media appear to provide more accurate prediction of the susceptibility of the metallo-β-lactamase-producing Enterobacterales to β-lactams. These results have the potential to refine our current susceptibility testing for these strains.

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