IDWeek 2020

1312

Metallo-β-lactamase-Producing Enterobacterales: Is it Time to Rethink Our Assessment Tools?

Kamilia Abdelraouf and David P. Nicolau

Center for Anti-Infective Research & Development, Hartford Hospital, Hartford, CT

David P. Nicolau, PharmD, FCCP, FIDSA
Center for Anti-Infective Research and Development
Hartford Hospital
80 Seymour Street
Hartford, CT 06102, USA
Telephone: +1 (860) 972-3941
E-mail: david.nicolau@hhchealth.org

ABSTRACT (revised)

Background: We previously reported the potent *in vivo* activity of ceftazidime/avibactam human-simulated regimen (HSR) against metallo-β-lactamase (MBL)-producing Enterobacterales despite the observed resistance *in vitro* and the lack of avibactam MBL-inhibitory activity. Similar to avibactam, relebactam (REL) is a diazabicyclooctane that inhibits serine β-lactamases belonging to Classes A - C but not MBLs. In the current study, we examined the *in vivo* activity of cefepime (FEP)/REL combination HSR against MBL-producing Enterobacterales in a murine thigh infection model.

Methods: Twenty six clinical MBL-producing Enterobacterales isolates expressing VIM, IMP or NDM including 25 isolates co-expressing at least one β-lactamase of Class A or C (KPC, CTX-M, TEM, SHV, ACT, CMY) were utilized. MICs of FEP and FEP/REL combination (at fixed REL concentration of 4 mg/L) were determined using broth microdilution in cation-adjusted Mueller Hinton broth (CAMHB) as well as CAMHB treated with EDTA 300 mg/L (CAMHB-EDTA 300, zinc-limited broth). FEP HSR (2 g q12h as 0.5 h infusion) alone and in combination with REL HSR (250 mg q6h as 0.5 h infusion) were established in the infection model. Thighs of neutropenic ICR mice were inoculated with bacterial suspensions of 10^7 CFU/ml. Two hours later, mice were administered the FEP HSR (6 isolates) or the FEP/REL HSR (26 isolates). Efficacy was measured as the change in $\log_{10}$CFU/thigh at 24 h compared with 0 h controls.

Results: All isolates were FEP resistant and the addition of REL had no impact on the MIC of the isolates when examined in CAMHB. In zinc-limited broth, all isolates that co-expressed serine β-lactamases remained resistant to FEP, while several fold reduction in FEP/REL MICs was observed. In *in vivo* studies, the average bacterial burden at 0 h was 5.78 ± 0.31 $\log_{10}$CFU/thigh. In accordance with the *in vitro* susceptibility in CAMHB, administration of FEP HSR was associated with net bacterial growth ranging from 0.46 ± 0.60 to 2.97 ± 0.53 $\log_{10}$CFU/thigh. In contrast, FEP/REL combination HSR resulted in substantial bacterial reductions among all isolates ranging from -0.45 ± 0.17 to -2.73 ± 0.27 $\log_{10}$CFU/thigh, indicating that REL enhanced the FEP activity *in vivo*.

Conclusions: Despite the powerful β-lactam hydrolytic capability of MBLs *in vitro*, FEP inactivation in the murine model was attributed predominantly to the expression of the serine β-lactamases. The *in vitro*/ *in vivo* discordance in β-lactam/β-lactamase activity against MBL-producing Enterobacterales when the MICs are assessed in conventional media reveals a potential flaw in the currently utilized *in vitro* susceptibility testing methodologies and highlights a challenge encountered during the development of new agents against these isolates.

INTRODUCTION

- The *in vivo* activity of human-simulated exposures of broad spectrum β-lactam agents such ceftazidime/avibactam and carbapenems against MBL-producing Enterobacterales in animal infection models despite the observed resistance *in vitro* has been reported (1-5).
- Given that MBL-producing Enterobacterales utilize zinc to facilitate bicyclic β-lactam ring hydrolysis, the presence of zinc in the conventional culture media such as the cation adjusted Mueller Hinton Broth (CAMHB) utilized in broth microdilution at a higher concentration than the physiologic zinc levels particularly at infection sites could be responsible for the *in vitro*/ *in vivo* discordance.

OBJECTIVES

- To examine the *in vivo* activity of HSR of cefepime (FEP) in combination with relebactam (REL), a diazabicyclooctane that inhibits serine β-lactamases belonging to Classes A – C, against MBL-producing Enterobacterales in a murine neutropenic thigh infection model.
- To assess the *in vitro* susceptibility of the isolates to FEP and FEP/REL in CAMHB and zinc-limited broth and compare the MICs to the observed *in vivo* activities.

MATERIALS & METHODS

Antimicrobial Test Agents

- Cefepime vials (1 g, WG Critical Care, LLC) and cefepime HCl (Batch number LRAB8503, Sigma-Aldrich) were used for *in vivo* and *in vitro* testing, respectively.
- Relebactam (MK-7655, Merck & Co., Inc, lots 002D040, 002D044)

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.

Pharmacokinetic Studies

- Pharmacokinetics of REL in combination with FEP were assessed in the infection model.
- FEP HSR (2 g q12h as 0.5 h infusion) alone and in combination with REL HSR (250 mg q6h as 0.5 h infusion) were established in the infection model.

Bacteria and In vitro Susceptibility

- Twenty six clinical Enterobacterales strains expressing various metallo-β-lactamases (VIM, IMP, NDM) of which 25 strains co-expressed serine carbapenemases, ESBLs or extended-spectrum cephalosporinases.
- FEP and FEP/REL MICs (at REL fixed concentration 4 mg/L) 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

- Efficacies of FEP HSR and/or FEP/REL HSR were assessed against the MBL-producing Enterobacterales.
- Efficacy was measured as the change in $\log_{10}$CFU/thigh at 24h compared with 0h controls.

RESULTS

Table 1. Comparison of FEP exposures achieved in humans (2 g q12h as 0.5h infusion) and mice receiving HSR: a) FEP monotherapy, b) FEP in combination with REL HSR

	%*f*T>MIC for MIC of:						
	4	***8***	***16***	***32***	***64***	***128***	***256***
Human	70	55	40	25	9	1	0
Mouse[a]	67	55	43	25	13	0	0
Mouse[b]	68	59	46	28	11	2	0

Table 2. Comparison of REL exposures achieved in humans (250 mg q6h as 0.5h infusion) and mice receiving HSR (administered in combination with FEP HSR)

	%*f*T>MIC for MIC of:							*f*AUC_{0-24} (mg.h/L)	*f*C_{max} (mg/L)
	0.5	***1***	***2***	***4***	***8***	***16***	***32***		
Human	100	100	70	38	12	0	0	97.2	12.9
Mouse	100	100	74	41	13	0	0	98.6	10.6

Table 3. β-lactamase gene content of the isolates and modal MICs determined in CAMHB and CAMHB+EDTA 300 mg/L. ECL: *Enterobacter cloacae*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*

Isolate ID	Known β-lactamases	MIC (mg/L) in CAMHB		MIC (mg/L) in CAMHB+EDTA 300 mg/L	
		FEP	FEP/REL	FEP	FEP/REL
ECL 130	SHV-5(e); ACT; **IMP-8**	**>32**	**>32**	**>64**	**0.5**
ECL 163	SHV-12; TEM-OSBL; CTX-M-15; ACT-TYPE; **NDM-6**	>32	>64	>64	1
ECL 167	TEM-OSBL; CTX-M-15; ACT-TYPE; **NDM-7**	>32	>64	>64	0.5
ECL 171	TEM-OSBL; ACT-TYPE; **NDM-7**	>32	>64	32	0.25
EC 660	TEM-OSBL(b); CTX-M-15; **NDM-19**	>32	>64	>64	4
EC 662	CTX-M-15; **NDM-4**	>32	>64	>64	4
EC 680	TEM-OSBL(b); CTX-M-15; **NDM-5**	>32	>64	>64	4
EC 681	TEM-OSBL(b); CTX-M-15; **NDM-5**	>32	>64	>64	4
EC 690	TEM-OSBL; CTX-M-15; **NDM-5**	>32	>64	>64	4
EC 692	TEM-OSBL; CTX-M-55; **NDM-5**	>32	>64	>64	2
EC 700	TEM-OSBL(b); CTX-M-15; CMY-2; **NDM-5**	>32	>64	>64	4
KP 655	**VIM-1**, OXA-9, SHV-12, TEM-1A	>32	>64	>64	0.25
KP 667	**NDM-1**, CTX-M-15,OXA-1, TEM-1B	>32	>64	>64	0.125
KP 684	**IMP-4**, OKP-B-2, OXA-1, SFO-1, TEM-1B	>32	>64	>64	0.5
KP 746	SHV-12, **VIM-1**	>32	>32	64	0.25
KP 752	KPC-2, **VIM-1**	>32	>32	>64	0.25
KP 753	SHV-OSBL(u); TEM-OSBL(u); CTX-M-15; **IMP-26**	32	32	>64	≤0.06
KP 755	SHV-12(e); TEM; CTX-M-15; **NDM-7**	>32	>32	>64	0.25
KP 756	SHV-OSBL; CTX-M-27; CMY; **NDM-1**	>32	>32	>64	0.25
KP 863	SHV-OSBL(b); TEM-OSBL(b); CTX-M-15; **NDM-5**	>32	>64	>64	1
KP 877	SHV-OSBL(b); TEM-OSBL(b); CTX-M-15; **NDM-6**	>32	>64	>64	1
KP 880	SHV-12; TEM-OSBL; CTX-M-15; **NDM-7**	>32	>64	>64	0.25
KP 882	SHV-OSBL; TEM-OSBL; CTX-M-15; **NDM-7**	>32	>64	>64	0.5
KP 885	**NDM-7**	>32	>64	≤0.06	≤0.06
KP 889	TEM-OSBL(b); CTX-M-15; **NDM-7**	>32	>64	>64	0.125
KP 895	SHV-OSBL(b); CTX-M-15; **NDM-9**	>32	>64	>64	1

Figure 1. Comparative efficacy of FEP vs. FEP/REL HSRs against 6 clinical MBL-producing Enterobacterales strains co-expressing ESBLs, KPC or or extended-spectrum cephalosporinases (isolates are shaded in blue in Table 3). Data are means ± standard deviations.

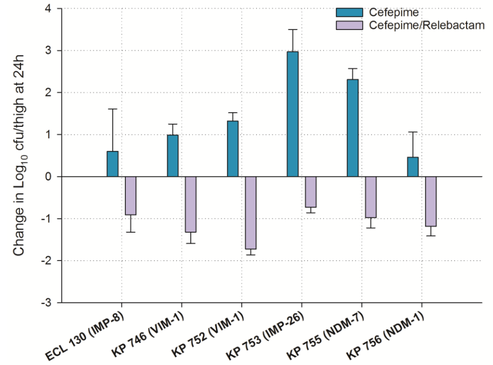
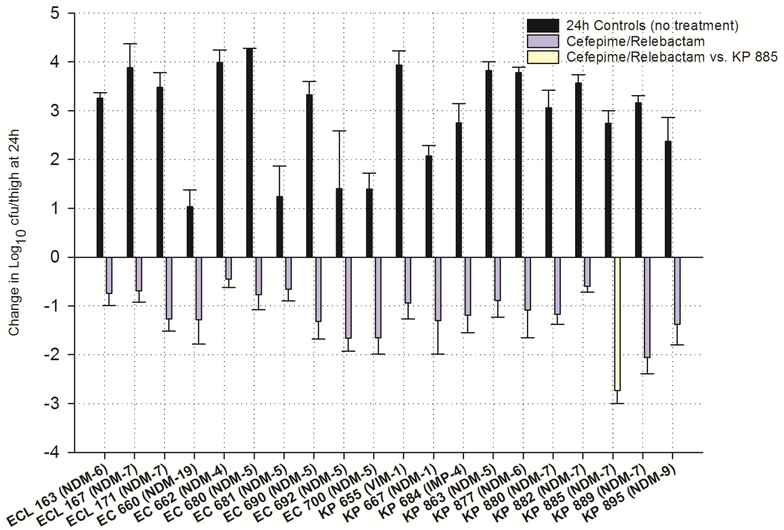


Figure 2. Efficacy of FEP/REL HSR against 20 clinical MBL-producing Enterobacterales strains. Data are means ± standard deviations.



CONCLUSIONS

- *In vitro*/ *in vivo* discordance in FEP/REL activity against MBL-producing Enterobacterales was observed when the MICs were assessed in conventional media (CAMHB).
- The FEP and FEP/REL MICs generated in zinc-limited media better predicted the outcome of FEP and/or FEP/REL treatment in the murine model.
- For MBL-producing Enterobacterales isolates that harbor serine β-lactamase enzymes that have the capability to inactivate FEP, failure of FEP monotherapy in the murine model was attributed predominantly to the expression of the serine β-lactamases.
- The conventional *in vitro* antibiotic susceptibility testing systems in many respects may fail to replicate the physiological factors that exist in the animal models, which can significantly impact the ability of the test to predict the outcome of antibiotic therapy, a challenge frequently encountered during the development of new agents against MBL-producing Enterobacterales.

REFERENCES

1. Asempa TE, Abdelraouf K, Nicolau DP. Metallo-beta-lactamase resistance in Enterobacteriaceae is an artefact of currently utilized antimicrobial susceptibility testing methods. JAC 2020; 75: 997-1005.
2. MacVane SH, Crandon JL, Nichols WW et al. Unexpected in vivo activity of ceftazidime alone and in combination with avibactam against New Delhi metallo-beta-lactamase-producing Enterobacteriaceae in a murine thigh infection model. AAC 2014; 58: 7007-9.
3. Wiskirchen DE, Nordmann P, Crandon JL et al. Efficacy of humanized carbapenem exposures against New Delhi metallo-beta-lactamase (NDM-1)-producing enterobacteriaceae in a murine infection model. AAC 2013; 57: 3936-40.
4. Wiskirchen DE, Nordmann P, Crandon JL et al. In vivo efficacy of human simulated regimens of carbapenems and comparator agents against NDM-1-producing Enterobacteriaceae. AAC 2014; 58: 1671-7.
5. Roujansky A, de Lastours V, Guérin F et al. Analysis of Paradoxical Efficacy of Carbapenems against carbapenemase-producing *Escherichia coli* in a Murine Model of Lethal Peritonitis. AAC 2020: AAC.00853-20.

ACKNOWLEDGEMENTS

We thank our colleagues at the Center for Anti-Infective Research and Development, Hartford, CT, for assistance with the conduct of the study. This study was supported by a grant from Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.