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MINIREVIEW

Activity of β-Lactam Antibiotics against Metallo-β-Lactamase-Producing *Enterobacterales* in Animal Infection Models: a Current State of Affairs

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ABSTRACT Metallo-β-lactamases (MBLs) result in resistance to nearly all β-lactam antimicrobial agents, as determined by currently employed susceptibility testing methods. However, recently reported data demonstrate that variable and supraphysiologic zinc concentrations in conventional susceptibility testing media compared with physiologic (bioactive) zinc concentrations may be mediating discordant *in vitro-in vivo* MBL resistance. While treatment outcomes in patients appear suggestive of this discordance, these limited data are confounded by comorbidities and combination therapy. To that end, the goal of this review is to evaluate the extent of β-lactam activity against MBL-harboring *Enterobacterales* in published animal infection model studies and provide contemporary considerations to facilitate the optimization of current antimicrobials and development of novel therapeutics.

KEYWORDS β-lactams, animal models, metallo-β-lactamase

The emergence and rapid spread of bacterial infections due to carbapenemase-producing organisms pose a global health challenge (1, 2). Using conventional antimicrobial susceptibility testing (AST) methods, metallo-β-lactamases (MBLs) confer high-level β-lactam resistance which severely limits therapeutic options (2–7). In clinical practice, phenotypic profiling as characterized by the MIC is used to guide therapeutic decision-making via susceptibility classification and breakpoints. In addition, MIC values are pivotal in generating pharmacokinetic/pharmacodynamic (PK/PD) indices in both preclinical and clinical studies for optimizing dosing regimens (8, 9). Finally, *in vitro* susceptibility data are also used for epidemiological purposes to track changing resistance patterns within geographic regions or institutions (10–12).

An important step in developing effective therapeutic strategies is to understand the dynamics of microbial resistance in the context of *in vitro* susceptibility, host factors, and clinically relevant antibiotic exposure (13–15). Indeed, based on host factors, modifications to traditional susceptibility testing criteria are required for certain agents, including daptomycin and cefiderocol, to derive clinically meaningful MICs representative of therapeutic success or failure (16–18). Recently, we demonstrated that *in vitro* resistance of MBL-harboring *Enterobacterales* did not correlate with *in vivo* resistance or therapeutic failure after carbapenem treatment. Therein, clinically achievable exposures of meropenem resulted in bacterial reduction among a variety of clinical MBL-harboring *Enterobacterales* in a murine lung and thigh infection model (19). Of note, we also showed that the *in vitro-in vivo* discordance observed with MBLs and meropenem extends to noncarbapenem β-lactams, such as cefepime (20). As such, we concluded that differences between physiologic and supraphysiologic zinc concentrations in humans and Mueller-Hinton broth, respectively, could be mediating discordant zinc-dependent MBL resistance.

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Further insights can be gained from human studies. However, interpretation of current outcome data among patients infected with MBL-producing organisms is challenging, as most studies have small sample sizes and are retrospective in design with investigators having no control over patient characteristics and treatment (21–23). Furthermore, infected patients are critically ill at baseline and typically receive combination antimicrobial therapy or ineffective empiric therapy. In the preclinical research arena, animal infection models have been instrumental in bridging *in vitro* data to human studies. These experimental animal models provide a reliable means of confirming or establishing PK/PD relationships and evaluating antimicrobial efficacy against isolates typically not represented in clinical trials (9, 24). Furthermore, specific magnitudes of bacterial reduction, such as 1-log kill, have been adopted as preclinical surrogates for clinical efficacy (25–27).

As a result of an apparent high level of *in vitro* resistance, clinical use of β-lactam antibiotics and subsequent efficacy data against MBL-harboring organisms are limited. To address this knowledge gap and ascertain the effect, if any, of β-lactam monotherapy, this review leverages data from MBL-infected animal studies, of which the majority were designed to evaluate investigational metallo-β-lactamase inhibitors *in vivo*.

LITERATURE SEARCH

A literature search for studies evaluating antimicrobial efficacy against metallo-β-lactamase-producing isolates using PubMed from January 2000 to December 2020 was conducted. A 2-step search strategy was developed, as follows: one identifying articles on "metallo-β-lactamase," and a second limiting the search to mentions of "*in vivo*," "murine," or "mouse." In addition, a manual search of reference lists from relevant articles was performed.

DATA ABSTRACTION AND ANALYSIS

Study data were entered into a Microsoft Excel spreadsheet, and accuracy was verified by all authors. Type of animal infection model, isolate genotype, *in vitro* susceptibility, dose, and administration route of the β-lactam, β-lactamase-inhibitor (BL-I), metallo-β-lactamase-inhibitor (MBL-I), and endpoint measured (i.e., change in bacterial burden) were extracted and collated. Extracted data were limited to *Enterobacterales* and studies in which bacterial density in the control (vehicle) arm at study endpoint was reported. Given that the primary objective of many studies meeting inclusion criteria was to assess the potential of the BL/MBL-I combination to reverse β-lactam-resistance, bacterial density (i.e., CFU count) results after treatment with the β-lactam monotherapy were rarely reported as a numerical value. In those instances, we abstracted data from graphs.

In studies that reported bacterial density at the time of treatment (0-h control), we determined if microbiological activity of β-lactam monotherapy or BL/MBL-I combination therapy at the study endpoint met the following criteria: (i) $\geq$1-$\log_{10}$ bacterial growth relative to 0-h control, (ii) bacteriostatic (stasis) relative to 0-h control, or (iii) $\geq$1-$\log_{10}$ bacterial reduction relative to 0-h control. In studies that did not report 0-h control bacterial density, we defined microbiological activity at study endpoint as (i) "no difference" if no change in bacterial density relative to the control group at the study endpoint was observed or (ii) "↓CFU" if a reduction in bacterial density relative to the control group at the study endpoint was observed.

ANIMAL MODELS AND STUDY ENDPOINTS

We identified 27 articles that reported *in vivo* studies evaluating β-lactam activity against MBL-producing *Enterobacterales*. Key characteristics of the animal infection model, β-lactam doses administered, and change in bacterial burden are summarized in Table 1 and 2 (19, 20, 28–52). Table 1 highlights studies ($n = 14$) that reported bacterial density counts for 0-h control animals, allowing for a $\geq$1-$\log_{10}$ bacterial reduction

TABLE 1 Activity of β-lactam monotherapy or combination therapy on MBL-bacterial burden in animal studies reporting 0-h control bacterial density required for bacteriostatic and 1-$\log_{10}$ bacterial reduction assessment[a]

Reference	Animal infection model	Isolate (genotype)	Inoculum (CFU/ml)	BL MIC (mg/liter)	BL dose and frequency[b]	MBL-I or BL-I dose and frequency	Dose initiation	Measured endpoint	BL alone activity	BL/MBL-I or BL/BL-I activity
Ooi et al., 2020 (38)	Murine neutropenic thigh model	*E. coli* IR3 (NDM-1)	1.95×10^6	MEM: 16	MEM 50 mg/kg q2h s.c.	Compound-272 100 mg/kg q2h s.c.	1 h postinoculation	9 h thigh	~0.5-log kill	≥1-log kill
					MEM 250 mg/kg q2h s.c.	Compound-272 100 mg/kg q2h s.c.	1 h postinoculation	9 h thigh	≥1-log kill	≥1-log kill
Abdelraouf et al., 2020 (20)	Murine neutropenic thigh model	21 Isolates (NDM, VIM, IMP)	10^7	FEP MIC range: 64 to >256; FEP MIC range[b]: ≤0.03 to 8	FEP 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (19/21 isolates)	
		5 Isolates (wild-type, isogenic-derived and clinical NDM, VIM, IMP)		FEP MIC range: ≤0.25 to >256; FEP MIC range[c]: ≤0.03 to 32	FEP 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (4/5 isolates)	
		3 Isolates (KPC-2, KPC-3)		FEP MIC: >256; FEP MIC range[c]: >32	FEP 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	Stasis (2/3 isolates)	
Das et al., 2020 (46)	Murine neutropenic thigh model	*E. coli* NTBC121 (NDM-1)	NA	MEM: 32	MEM 50 mg/kg q4h s.c.	ANT2681 ~10 mg/kg q4h i.v.	2 h postinoculation	24 h thigh	Stasis	Stasis
					MEM 50 mg/kg q4h s.c.	ANT2681 200 mg/kg q4h i.v.	2 h postinoculation	24 h thigh	Stasis	≥1-log kill
					MEM 75 mg/kg q4h s.c.		2 h postinoculation	24 h thigh	Stasis	
					MEM 100 mg/kg q4h s.c.		2 h postinoculation	24 h thigh	≥1-log kill	
					MEM 200 mg/kg q4h s.c.		2 h postinoculation	24 h thigh	≥1-log kill	
Asempa et al., 2020 (19)	Murine neutropenic lung model	10 Isolates (NDM, VIM, IMP)	10^7	MEM MIC range: 16 to >64; MEM MIC range[c]: ≤0.06 to 0.5	MEM 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h lung	≥1-log kill (10/10 isolates)	
	Murine neutropenic thigh model	24 Isolates (NDM, VIM, IMP)	10^7	MEM MIC range: 8 to >64; MEM MIC range[c]: ≤0.06 to 1	MEM 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (21/24 isolates)	
Moya et al., 2019 (47)	Murine neutropenic thigh model	4 Isolates (NDM, CMY, SHV, TEM, CTX-M-15)	5×10^6	FEP MIC range: 128 to >256	FEP 1,200 mg/kg/day fractionated q2h s.c.	ZID 900 mg/kg/day fractionated q2h s.c.	2 h postinoculation	24 h thigh	≥1-log growth (4/4 isolates)	≥0.5-log kill (4/4 isolates)
					FEP 1,200 mg/kg/day fractionated q2h s.c.	WCK 5153 900 mg/kg/day fractionated q2h s.c.	2 h postinoculation	24 h thigh	≥1-log growth (4/4 isolates)	≥0.5-log kill (4/4 isolates)
				ATM MIC range: >256	ATM 900 mg/kg/day fractionated q2h s.c.	ZID 900 mg/kg/day fractionated q2h s.c.	2 h postinoculation	24 h thigh	≥1-log growth (4/4 isolates)	≥0.5-log kill (4/4 isolates)
					ATM 900 mg/kg/day fractionated q2h s.c.	WCK 5153 900 mg/kg/day fractionated q2h s.c.	2 h postinoculation	24 h thigh	≥1-log growth (4/4 isolates)	≥0.5-log kill (4/4 isolates)
		4 Isolates (NDM, CMY, SHV, TEM, CTX-M-15)	5×10^6	MEM MIC range: ≥128	MEM-cilastatin 225 mg/kg/day fractionated q4h s.c. (HSR)		2 h postinoculation	24 h thigh	≥1-log growth (4/4 isolates)	
Weiss et al., 2019 (48)	Murine neutropenic thigh model	*K. pneumoniae* UNT170-1 (KPC-2)	~10^5 to 10^6	MEM: ≥16	MEM 300 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	≥1-log growth	
				LYS228: 0.5 to 1	LYS228 60 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	Stasis	
					LYS228 1,620 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	≥1-log kill	
		K. pneumoniae UNT184-1 (NDM-1)	~10^5 to 10^6	MEM: ≥16	MEM 300 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	≥1-log kill	
				LYS228: 8 to 16	LYS228 60 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	~0.5-log kill	
					LYS228 1620 mg/kg/day fractionated q4h s.c.[d]		2 h postinoculation	24 h thigh	≥1-log kill	
Everett et al., 2018 (49)	Murine neutropenic thigh model	*E. coli* IR3 (NDM-1)	1.5×10^6	MEM: 32	MEM 50 mg/kg i.v.	ANT431 300 mg/kg i.v.	1, 3, 5, and 7 h postinoculation	9 h thigh	~1-log growth	~0.5-log kill
					MEM 250 mg/kg i.v.	ANT431 300 mg/kg i.v.	1, 3, 5, and 7 h postinoculation	9 h thigh	~0.5-log kill	≥1-log kill

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TABLE 1 (Continued)

Reference	Animal infection model	Isolate (genotype)	Inoculum (CFU/ml)	BL MIC (mg/liter)	BL dose and frequency[b]	MBL-I or BL-I dose and frequency	Dose initiation	Measured endpoint	BL alone activity	BL/MBL-I or BL/BL-I activity
Monogue et al., 2017 (50)	Murine neutropenic lung model	*K. pneumoniae* (NDM, OXA-48, CTX-M)	10^7	CAZ MIC: >64	CAZ 2 g q8h (HSR, s.c.)[d]	AVI 500 mg q8h (HSR, s.c.)[d]	2 h postinoculation	24 h lung		≥1-log kill
				ATM MIC: 64	ATM 2 g q6h (HSR, s.c.)[d]		2 h postinoculation	24 h lung	≥1-log growth	
					CAZ 2 g q8h (HSR, s.c.)[d] + ATM 2 g q6h (HSR, s.c.)[d]	AVI 500 mg q8h (HSR, s.c.)[d]	2 h postinoculation	24 h lung		≥1-log kill
Marshall et al., 2017 (51)	Murine neutropenic thigh model	*K. pneumoniae* 1.41 (NDM-1, CTX-M-15, DHA, SHV, TEM)	5.01 $\log_{10}$	CAZ: >512	CAZ 256 mg/kg q8h s.c.	AVI 64 mg/kg q8h s.c.	2 h postinoculation	24 h thigh	Stasis	≥1-log kill
					CAZ 128 mg/kg q8h s.c.	AVI 32 mg/kg q8h s.c.			≥1-log growth	Stasis
					CAZ 64 mg/kg q8h s.c.	AVI 16 mg/kg q8h s.c.			≥1-log growth	≥1-log growth
					CAZ 32 mg/kg q8h s.c.	AVI 8 mg/kg q8h s.c.				≥1-log growth
				ATM: 128	ATM 256 mg/kg q8h s.c.		2 h postinoculation	24 h thigh	≥1-log growth	
					ATM 128 mg/kg q8h s.c.				≥1-log growth	
					ATM 64 mg/kg q8h s.c.				≥1-log growth	
				CAZ/AVI: 64	CAZ 32 mg/kg q8h s.c. + ATM 32 mg/kg q8h s.c.	AVI 8 mg/kg q8h s.c.	2 h postinoculation	24 h thigh		~0.5-log kill
					CAZ 16 mg/kg q8h s.c. + ATM 16 mg/kg q8h s.c.	AVI 4 mg/kg q8h s.c.				≥1-log growth
					CAZ 8 mg/kg q8h s.c. + ATM 8 mg/kg q8h s.c.	AVI 2 mg/kg q8h s.c.				≥1-log growth
					CAZ 4 mg/kg q8h s.c. + ATM 4 mg/kg q8h s.c.	AVI 1 mg/kg q8h s.c.				≥1-log growth
Ghazi et al., 2015 (52)	Murine neutropenic thigh model	9 Isolates (wild-type, isogenic-derived and clinical VIM)	10^7	MEM MIC range: 0.06 to >512	MEM 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (9/9 isolates)	
				FEP MIC range: 0.06 to >512	FEP 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (3/9 isolates)	
MacVane et al., 2014 (28)	Murine neutropenic thigh model	6 Isolates (wild-type, isogenic-derived and clinical NDM)	10^7	CAZ MIC range: 0.25 to >128	CAZ 2 g q8h (HSR, s.c.)[d]	AVI 500 mg q8h (HSR, s.c.)[d]	2 h postinoculation	24 h thigh	≥1-log kill (2/6 isolates)	≥1-log kill (5/6 isolates)
Crandon et al., 2013 (29)	Murine neutropenic thigh model	14 Isolates (NDM)	10^7	ATM MIC range: 16 to >256	ATM 2 g q6h (HSR, s.c.)[d]	AVI 600 mg q6h (HSR, s.c.)[d]	2 h postinoculation	24 h thigh	≥1-log kill (2/14 isolates)	≥1-log kill (12/14 isolates)
Wiskirchen et al., 2014 (43)	Murine neutropenic thigh model	4 Isolates (wild-type, isogenic-derived and clinical NDM)	10^7	DOR MIC range: 0.03 to 32	DOR 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (4/4 isolates)	
				ETP MIC range: 0.012 to >32	ETP 1 g q24h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (4/4 isolates)	
				CAZ MIC range: 0.25 to >128	CAZ 2 g q8h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (2/4 isolates)	
				ATM MIC range: ≤0.25 to >256	ATM 2 g q6h (HSR, s.c.)[d]		2 h postinoculation	24 h thigh	≥1-log kill (2/4 isolates)	
Daikos et al., 2007 (31)	Murine neutropenic thigh model	*K. pneumoniae*-S (wild-type)	10^7	IMI: 0.125	IMI 30 mg/kg q2h i.p.		2 h postinoculation	24 h thigh	≥1-log kill	
					IMI 60 mg/kg q2h i.p.				≥1-log kill	
		K. pneumoniae-Ia (VIM-1)		IMI: 2	IMI 30 mg/kg q2h i.p.		2 h postinoculation	24 h thigh	Stasis	
					IMI 60 mg/kg q2h i.p.				≥1-log kill	
		K. pneumoniae-Ib (VIM-1)		IMI: 4	IMI 30 mg/kg q2h i.p.		2 h postinoculation	24 h thigh	Stasis	
					IMI 60 mg/kg q2h i.p.				Stasis	
		K. pneumoniae-R (VIM-1)		IMI: 32	IMI 30 mg/kg q2h i.p.		2 h postinoculation	24 h thigh	Stasis	
					IMI 60 mg/kg q2h i.p.				Stasis	

[a]BL, β-lactam; MBL-I, metallo-β-lactamase inhibitor; MEM, meropenem; IMI, imipenem; DOR, doripenem; ETP, ertapenem; FEP, cefepime; CAZ, ceftazidime; AZT, aztreonam; AVI, avibactam; ZID, zidebactam; NAC, nacubactam; HSR, human simulated regimen; cUTI, complicated urinary tract infection; s.c., subcutaneous; i.p., intraperitoneal; i.v., intravenous; NA, not available.

[b]q2h, every 2 hours, q4h, every 4 hours; q6h, every 6 hours; q8h, every 8 hours; q24h, every 24 hours.

[c]MIC determined in zinc-depleted media.

[d]Pretreatment with uranyl nitrate.

TABLE 2 Activity of β-lactam monotherapy or combination therapy on MBL-bacterial burden in animal studies[a]

Reference	Animal infection model	Isolate (genotype)	Inoculum (CFU/ml)	BL MIC (mg/liter)	BL dose and frequency	MBL-I or BL-I dose and frequency	Dose initiation	Measured endpoint	Activity, BL alone	Activity, BL/MBL-I or BL/BL-I
Samuelsen et al., 2020 (32)	Murine neutropenic peritonitis model	*K. pneumoniae* 50752501 (NDM-1)	5×10^6	MEM: 64	MEM 33 mg/kg single dose s.c.	ZN148 10 mg/kg single dose s.c.	MEM: 1.5 h postinoculation; ZN148: 1 h postinoculation	5 h blood 5 h peritoneal fluid	↓CFU ↓CFU	↓CFU ↓CFU
					MEM 33 mg/kg single dose s.c.	ZN148 33 mg/kg single dose s.c.	MEM: 1.5 h postinoculation; ZN148: 1 h postinoculation	5 h blood 5 h peritoneal fluid	↓CFU ↓CFU	↓CFU ↓CFU
					MEM 33 mg/kg single dose s.c.	ZN148 100 mg/kg single dose s.c.	MEM: 1.5 h postinoculation; ZN148: 1 h postinoculation	5 h blood 5 h peritoneal fluid	↓CFU ↓CFU	↓CFU ↓CFU
Chen et al., 2020 (33)	Murine peritonitis model	*E. coli* EC10 (NDM-1)	2×10^7	MEM: 128	MEM 10 mg/kg single dose i.p.	Cisplatin 5 mg/kg single dose i.p.	0.5 h postinoculation	48 h liver 48 h spleen	No difference No difference	↓CFU ↓CFU
Liu et al., 2019 (34)	Murine neutropenic thigh model	*E. coli* B2 (NDM-5)	1.5×10^6	MEM: 32	MEM 16 mg/kg single dose i.p.	PEP4 16 mg/kg single dose i.p.	2 h postinoculation	24 h thigh	No difference	↓CFU
Liu et al., 2019 (35)	Murine thigh model	*E. coli* ZC-YN3 (NDM-1)	5×10^6	MEM: 16	MEM 10 mg/kg s.c.	Pterostilbene 80 mg/kg s.c.	NA postinoculation, 3 doses at q8h for 24 hours	72 h thigh	No difference	↓CFU
Ma et al., 2019 (36)	Murine peritonitis model	*E. coli* XJ141026 (NDM-1)	8×10^7	MEM: 64	MEM 10 mg/kg single dose i.p.	Thanatin 0.1 mg/kg single dose i.p.	1 h postinoculation	24 h spleen 24 h liver	↓CFU ↓CFU	↓CFU ↓CFU
Yarlagadda et al., 2018 (37)	Murine peritonitis model	*K. pneumoniae* R3934 (NDM-1)	$\sim 10^6$	MEM: >100	MEM 10 mg/kg i.p.	Dipi-van 10 mg/kg i. p.	2 and 24 h postinoculation	48 h liver 48 h kidney 48 h lungs 48 h spleen	↓CFU ↓CFU No difference No difference	↓CFU ↓CFU ↓CFU ↓CFU
Monogue et al., 2018 (39)	Murine neutropenic cUTI model	3 Isolates (NDM-1)	$10^{5.5}$	MEM: 64 to 256	MEM 1 g q8h (HSR, s.c.)	NAC 1.5 g q8h (HSR, s.c.)	3 h postinoculation	48 h kidney	↓CFU (3/3 isolates)	↓CFU (3/3 isolates)
					CAZ 25 mg/kg s.c.	AVI 6.25 mg/kg s.c.	3, 7, 23, and 31 h postinoculation	48 h kidney		No difference (3/3 isolates)
		7 Isolates (KPC, OXA-48)	$10^{5.5}$	MEM: 16 to 512	MEM 1 g q8h (HSR, s.c.)	NAC 1.5 g q8h (HSR, s.c.)	2 h postinoculation	48 h kidney	No difference (5/7 isolates)	↓CFU (7/7 isolates)
					CAZ 25 mg/kg s.c.	AVI 6.25 mg/kg s.c.	3, 7, 23, and 31 h postinoculation	48 h kidney		↓CFU (7/7 isolates)
Sully et al., 2017 (40)	Murine peritonitis model	*E. coli* CVB-1 (NDM-1)	3×10^6 (5% mucin)	MEM: 16	MEM 50 mg/kg s.c.	PPMO 5 mg/kg i.p. with inoculum	0 h postinoculation 0 and 6 h postinoculation 0 and 6 h postinoculation	6 h blood 10 h blood 10 h spleen	No difference ↓CFU ↓CFU	↓CFU ↓CFU ↓CFU
Falconer et al., 2015 (41)	Murine peritonitis model	*K. pneumoniae* (NDM-1)	2×10^6	MEM: >2 μM	MEM 10 mg/kg single dose s.c.	SIT-Z5 10 mg/kg single dose s.c.	0.5 h postinoculation	48 h liver 48 h spleen	No difference No difference	↓CFU ↓CFU
King et al., 2014 (42)	Murine peritonitis model	*K. pneumoniae* N11-2218 (NDM-1)	2×10^6	MEM: 32	MEM 10 mg/kg single dose s.c.	AMA 10 mg/kg single dose s.c.	0.5 h postinoculation	48 h spleen 48 h liver	No difference No difference	↓CFU ↓CFU
Wiskirchen et al., 2014 (43)	Murine thigh model	10 Isolates (wild-type, isogenic-derived and clinical NDM)	10^8	DOR MIC range: 0.03 to >64	DOR 2 g q8h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	↓CFU (10/10 isolates)	
				ETP MIC range: 0.012 to 128	ETP 1 g q24h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	↓CFU (8/10 isolates)	
				CAZ MIC range: 0.25 to >128	CAZ 2 g q8h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	↓CFU (3/10 isolates)	
				ATM MIC range: ≤0.25 to >256	ATM 2 g q6h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	↓CFU (4/10 isolates)	
Wiskirchen et al., 2013 (30)	Murine thigh model	6 Isolates (wild-type, isogenic-derived and clinical NDM)	10^8	ETP MIC range: 0.012 to >32	ETP 1 g q24h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	↓CFU (5/6 isolates)	
				DOR MIC range: 0.03 to >32	DOR 2 g q8h (HSR, s.c.)[b]				↓CFU (6/6 isolates)	
		Isogenic-derived KPC-2	10^8	ETP: 0.012 to 4	ETP 1 g q24h (HSR, s.c.)[b]		2 h postinoculation	24 h thigh	No difference	
				DOR: 0.03 to 1	DOR 2 g q8h (HSR, s.c.)[b]				No difference	
	Murine thigh model	*E. coli* 389 (NDM-1, TEM-1, CTX-M-15)	10^8	ETP: >32	ETP 1 g q24h (HSR, s.c.)[b]		2 h postinoculation	72 h thigh	↓CFU	
				DOR: 8	DOR 2 g q8h (HSR, s.c.)[b]				↓CFU	

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TABLE 2 (Continued)

Reference	Animal infection model	Isolate (genotype)	Inoculum (CFU/ml)	BL MIC (mg/liter)	BL dose and frequency	MBL-I or BL-I dose and frequency	Dose initiation	Measured endpoint	Activity, BL alone	Activity, BL/MBL-I or BL/BL-I
Yoshizumi et al., 2012 (44)	Murine peritonitis model	*E. coli* TUM10702 (NDM-1)	2.7×10^7	IMI: 512	IMI/cilastatin 25 mg/kg single dose s.c.	Ca-EDTA 200 mg/kg single dose s.c.	2 h postinoculation	4 h liver 4 h blood	No difference	↓CFU ↓CFU
	Murine neutropenic peritonitis model	*E. coli* TUM10702 (NDM-1)	6×10^7	IMI: 512	IMI/cilastatin 10 mg/kg single dose s.c.	Ca-EDTA 100 mg/kg single dose s.c.	2 h postinoculation	4 h liver 4 h blood	No difference No difference	↓CFU ↓CFU
Souli et al., 2011 (45)	Rabbit intra-abdominal abscess model	*E. coli* (VIM-1)	10^8	IMI: 1	IMI 70 mg/kg q12h, 30 min i.v. infusion		4 h postinoculation	96 h bacterial abscess	↓CFU	
				MEM: ≤0.25	MEM 125 mg/kg q12h, 10 min i.v. infusion		4 h postinoculation	96 h bacterial abscess	↓CFU	
				ERT: 1.5	ERT 60 mg/kg q12h, i.v. bolus		4 h postinoculation	96 h bacterial abscess	↓CFU	
				ATM: ≤0.25	ATM 70 mg/kg q12h, i.v. bolus		4 h postinoculation	96 h bacterial abscess	↓CFU	

[a]BL, β-lactam; MBL-I, metallo-β-lactamase inhibitor; MEM, meropenem; IMI, imipenem; DOR, doripenem; ETP or ERT, ertapenem; FEP, cefepime; CAZ, ceftazidime; AZT, aztreonam; AVI, avibactam; ZID, zidebactam; NAC, nacubactam; HSR, human simulated regimen; cUTI, complicated urinary tract infection; s.c., subcutaneous; i.p., intraperitoneal; i.v., intravenous; NA, not available; q12h, every 12 hours.

[b]Pretreatment with uranyl nitrate.

assessment (preclinical surrogate for clinical efficacy) (25–27), while Table 2 describes characteristics and outcomes of all other studies.

With the exception of the rabbit intra-abdominal abscess study by Souli et al. (45), all studies were conducted in mice. The most common infection model was the thigh model in 18 studies, followed by peritonitis model (9 studies). In the peritonitis model, CFU was enumerated from a variety of sites, including liver (7 studies), spleen (6 studies), blood (6 studies), kidney (5 studies), and peritoneal fluid (3 studies). Change in bacterial density was assessed at a variety of time points, ranging from 4 to 96 hours postinoculation, with the majority at 24 and 48 hours. A slightly larger proportion of studies utilized the neutropenic model than the immunocompetent model (19 versus 13 studies). Historically, *in vivo* efficacy or PK/PD assessments have utilized the neutropenic model for several reasons. First, immunosuppression allows for the evaluation of antimicrobial activity against the infecting pathogen without the contribution and confounding effect of the immune system (27). Consequently, a larger dose of drug is typically required to achieve a similar change in bacterial burden (i.e., bacteriostasis or 1-log kill) in neutropenic than in immunocompetent infection models (53). Second, inhibition of the immune system is sometimes necessary to enable bacteria to establish an infection *in vivo* (27).

ISOLATE CHARACTERISTICS

Isolate selection in the studies was consistent with real-world prevalence and distribution (54, 55). Indeed, the vast majority of MBL-harboring *Enterobacterales* evaluated in these animal models were clinical *Klebsiella pneumoniae* or *Escherichia coli* isolates harboring NDM-1. Only 5 studies included either a VIM- or IMP-harboring isolate (19, 20, 31, 45, 56). Current surveillance data suggest that among MBLs, NDMs are the most frequently identified worldwide, with NDM-1 being the most detected NDM variant (6, 57, 58). For example, in a multiyear, global distribution study, Kazmierczak et al. reported that among MBL-positive *Enterobacteriaceae*, 44% of isolates carried bla_{NDM}, 39% carried bla_{VIM}, and 17% carried bla_{IMP} (58). However, sporadic outbreaks due to *Enterobacterales* isolates harboring VIM and IMP in parts of Europe as well as an increasing global prevalence suggest preclinical animal studies evaluating novel therapies should not be limited only to NDM producers (59, 60). Furthermore, evidence of different zinc sensitivities between genotypes and the evolution of MBL variants also warrants inclusion of a genotypically diverse isolate collection when evaluating β-lactam and BL/MBL-I efficacy (42, 61–64).

In select studies, investigators utilized wild-type (WT) strains and their isogenic derivatives with inserted plasmids encoding an NDM, VIM, IMP, or KPC as experimental positive and negative controls (Table 1 and 2).

The rabbit model utilized a VIM-producing *E. coli* isolate with a carbapenem-susceptible phenotypic profile determined by broth microdilution (meropenem and imipenem) and Etest (ertapenem). Notably, this isolate was shown to be positive for MBL production with the imipenem-EDTA double-disc synergy test and negative for extended-spectrum β-lactamases (ESBLs) by the isoelectric focusing test. Despite the presence of a MBL (VIM-1 by genotypic confirmation), the isolate was susceptible to imipenem (MIC, 1 mg/liter), meropenem (MIC, $\leq$0.25 mg/liter), ertapenem (MIC, 1.5 mg/liter), and aztreonam (MIC, $\leq$0.25 mg/liter) (45). In contrast, all murine studies in this literature review utilized carbapenem nonsusceptible isolates, with susceptibility routinely determined by conventional broth microdilution. Additional *in vitro* susceptibility testing with zinc-depleted media was reported in 2 studies (19, 20).

Besides reporting the MBL genotype, only a few studies provided details on ESBLs, which are typically coproduced by these clinical strains. Similarly, few studies utilized publicly available or clinical reference strains such as ATCC or NCTC isolates. In the future, reporting of both target (i.e., NDM-1) and coharbored enzymes as well as inclusion of reference strains will enable valuable drug-bug comparisons by other laboratories as drug development advances.

A range of bacterial inoculums (10^5 to 10^8 CFU/ml) were used to establish infections in the studies evaluated, reflecting typical animal inoculation practices observed in the literature.

CHOICE OF β-LACTAM AND DOSING REGIMEN

Meropenem was the preferred β-lactam backbone to pair with MBL-Is in all but one study (imipenem) (44). In contrast, a variety of β-lactam agents were administered in the studies designed to evaluate β-lactam agents alone (i.e., no MBL-I combination therapy), namely, meropenem, doripenem, imipenem, ertapenem, cefepime, ceftazidime, and aztreonam. In a small number of these studies, a serine-based β-lactamase inhibitor such as avibactam was also used in combination (Table 1 and 2).

The β-lactam dose and frequency of administration differed greatly between studies with no clear dose justification in the majority of studies for the MBL-I activity assessed. Notably, 10 mg/kg of body weight meropenem was the most common dose administered and was typically given as a single dose. In comparison, studies designed to evaluate β-lactam agents alone tended to utilize murine doses that resulted in drug exposures similar to those achieved in humans receiving clinical doses.

Understandably, the majority of MBL-I studies are in the early preclinical phase and utilized carbapenem/MBL-I doses sufficient enough to demonstrate proof of concept. Nonetheless, the importance of dosage and dosing frequency selection in preclinical *in vivo* research cannot be understated. Indeed, for studies utilizing approved and existing β-lactams such as meropenem as the backbone therapy, consideration of the well-established pharmacokinetics of meropenem in early studies will provide more accurate assessments and a higher degree of confidence in human translatability upon addition of the investigational inhibitor. For instance, recognizing meropenem's rapid clearance after pharmacokinetic analysis of the β-lactam backbone (meropenem) and MBL-I (compound-272), Ooi et al. utilized a frequent dosing interval (every 2 hour) to compensate for clearance. The half-life of meropenem was determined to be 0.3 hours (18 minutes) compared with 1 hour for compound-272, demonstrating that a single dose or infrequent dosing would have been significantly inadequate (38). With regard to the rabbit experimental model, doses were selected to achieve concentrations comparable to those achievable in humans, with $>$50% time of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions ($T_{>MIC}$) for the respective isolates for the dosing interval (45).

ACTIVITY OF β-LACTAMS ON BACTERIAL BURDEN

Observations of reductions in bacterial density after administration of β-lactam monotherapy were markedly different between studies (Table 1 and 2). In studies that utilized nonoptimized β-lactam exposures (e.g., single-dose frequency) and demonstrated treatment benefit, this evidence of bacterial killing was not limited to any one infection model, immune status, isolate genotype/phenotype, or site of infection. Notably, the administration route (subcutaneous [s.c.] and intravenous [i.v.]) of identical meropenem doses appeared to result in different magnitudes of efficacy for 2 studies with similar infection model designs (*E. coli* IR3; NDM-1) (38, 49). The lack of reported CFU counts from 0-h controls in several studies reviewed precludes a more robust comparison of β-lactam efficacy to established bacteriostatic or 1-log bacterial density reduction endpoints indicative of clinical efficacy. As a result, bacterial density of treatment groups could be compared only with control groups enumerated at the end of study (Table 2). So, while a β-lactam treatment effect relative to endpoint controls may have been observed, this approach does not account for the possibility that net bacterial growth relative to bacterial density at the onset of infection (0-h control) may have still occurred with the treatment regimen. Furthermore, the lack of CFU data from 0-h control animals hinders any efforts to evaluate if an *in vitro-in vivo* inoculum effect exists with MBL-producing bacteria (65).

Several antimicrobial agents, such as meropenem, doripenem, imipenem, ertapenem, ceftazidime-avibactam, aztreonam-avibactam, and aztreonam-ceftazidime-avibactam, demonstrated microbiological activity when administered at clinically achievable exposures (Table 1). The majority of these studies demonstrated bacterial activity ranging from bacteriostasis to 2-$\log_{10}$ bacterial reduction in the thigh, lung, and kidney infection models relative to bacterial density at onset of infection (0-h controls). Of these agents, the activity of aztreonam in combination with avibactam is not unexpected given aztreonam's stability against MBLs and avibactam's role in protecting aztreonam from hydrolysis by coharbored ESBLs. In isolates lacking clinically relevant ESBLs, cefepime alone also demonstrated microbiological *in vivo* activity despite high-level *in vitro* resistance (20).

Notably, a humanized meropenem regimen in the murine thigh study by Moya et al. was reported to be inefficacious against 4 NDM-harboring isolates (47). Unfortunately, the human meropenem dose on which the murine PK profile was based on was not provided, but a 42% free $T_{>MIC}$ ($fT_{>MIC}$) at a MIC of 1 mg/liter and 26% $fT_{>MIC}$ at an MIC of 4 mg/liter were reported (47). For comparison, the percent free time above MIC achieved in humans receiving either 2 g meropenem every 8 hours (q8h) as a 3-h intravenous infusion is 100% $fT_{>MIC}$, at an MIC of 1 mg/liter, and 85% $fT_{>MIC}$, at an MIC of 4 mg/liter, or 1 g meropenem q8h (0.5-h intravenous infusion) is 86% $fT_{>MIC}$, at an MIC of 1 mg/liter, and 50% $fT_{>MIC}$, at an MIC of 4 mg/liter, making interpretation of their results challenging (39, 66–68). In this regard, reporting the human β-lactam dose that is humanized in the animal model and comparison of relevant $T_{>MIC}$ indices is imperative for providing robust translatable data.

In the five studies that included wild-type (WT) and isogenically derived isolates with inserted plasmids, clinically relevant carbapenem and ceftazidime-avibactam exposures resulted in bacterial killing in the WT, WT-bla_{NDM}, and WT-bla_{VIM} strains, while growth was observed with the WT-bla_{KPC} strain (20, 28, 30, 43, 52). Similarly, a select number of studies included clinical KPC- and OXA-48-harboring isolates as controls and demonstrated inefficacy with carbapenems (Table 1 and 2).

In the rabbit model, investigators created an intra-abdominal abscess that was inoculated with a carbapenem-susceptible VIM-1-producing *E. coli* isolate. Concordant with *in vitro* susceptibility and an ESBL-negative profile, treatment with meropenem, imipenem, ertapenem, and aztreonam resulted in significant bacterial reduction (45).

DISCUSSION

Over the last 2 decades, there has been a warranted and growing interest in the discovery and development of MBL-Is (5, 69, 70). As detailed in this review, several investigational MBL-Is with mechanisms of action ranging from zinc chelation to zinc-independent enzyme inhibition and novel gene silencing are in the preclinical pipeline and are being studied in combination with existing β-lactam backbones. In tandem, evidence of unexpected β-lactam efficacy against MBL-producing *Enterobacterales* in animal models has been growing (Fig. 1), but the extent and range of available data have not been comprehensively reviewed until now (19, 46, 51, 71–73). In addition, we recently detailed how currently utilized susceptibility testing media (i.e., Mueller-Hinton broth) may be inappropriate for characterizing MBL resistance due to variable and supraphysiologic zinc cation concentrations (63). When these zinc cations are reduced to mimic physiologic bioactive concentrations elicited by nutritional immunity during an infection, β-lactam MICs are reduced severalfold (19, 20, 63, 74).

Within the limitations of this review, abstracted animal data suggest that (i) β-lactams with intrinsic stability against ESBLs/cephalosporinases (i.e., carbapenems) and (ii) ceftazidime, aztreonam, and cefepime in combination with a broad-spectrum BL-I (i.e., avibactam) result in substantial bacterial reduction among clinical and engineered MBL-harboring *Enterobacterales* when administered at clinically relevant exposures. While these results are potentially promising, it remains to be determined if the addition of a MBL-I to optimized carbapenem therapy results in enhanced efficacy (i.e., further reduction in bacterial burden compared with the carbapenem alone) in the animal model. It is also worth noting that

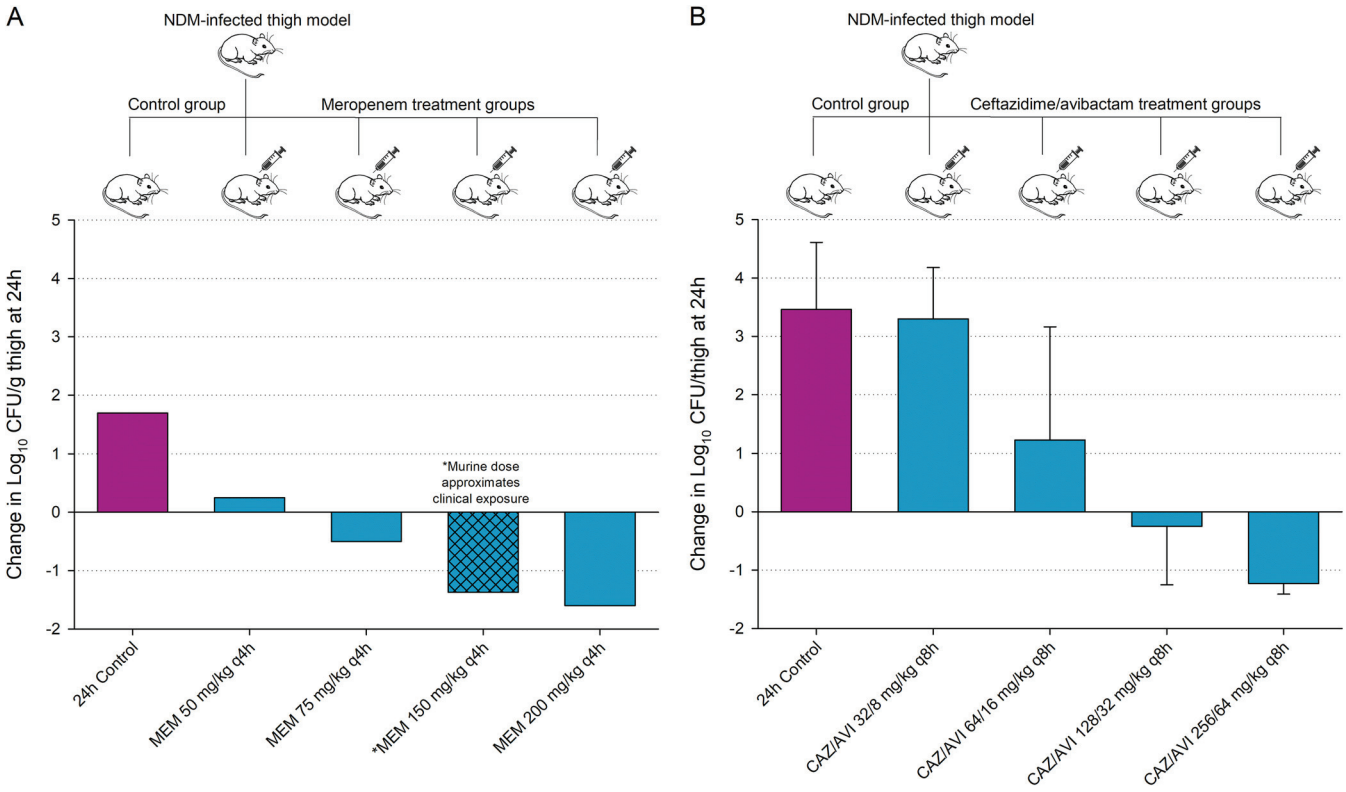

FIG 1 Mean change in bacterial density at 24 hours relative to 0-h controls after administration of meropenem (A) and ceftazidime-avibactam (B) against an NDM-harboring *E. coli* and *K. pneumoniae*, respectively. Data adapted from 2 contemporary β-lactam dose-ranging studies (46, 51).

animal studies that utilize subtherapeutic backbone exposures continue to have a critical role in drug development as a means to dose fractionate or to demonstrate additional or enhanced efficacy upon addition of the BL-I or adjunctive agent. However, key to the design of these studies is the reporting on the efficacy of the full therapeutic exposure of the backbone agent alone (75, 76).

Observed trends in microbiological activity among studies in this review also suggest that despite an apparent *in vitro* resistant profile, inclusion of the β-lactam backbone as a monotherapy arm in future β-lactam/MBL-I animal studies will yield valuable insights and opportunities to further our understanding of MBL *in vitro-in vivo* discordance (77, 78). In addition, a recently published study demonstrating unexpected *in vivo* activity with imipenem and ertapenem against an isogenic derivative *E. coli* with inserted NDM-1 plasmid was not included in this literature review due to a lack of 24-h control groups, which is critical for appropriately evaluating the impact of treatment versus placebo/vehicle over the course of the study period and as an indicator of bacterial fitness (71).

This review highlights the diversity in animal infection models and study endpoints employed within MBL-I development studies. Similar heterogeneity was observed in a recent study aimed at better understanding the range and scientific use of animal lung infection models, resulting in the authors advocating for the need for harmonized consensus models in drug development (79). Nonetheless, animal infection models continue to be an essential translational tool for assessing the toxicity and efficacy of antimicrobial agents. Data generated in these models have been valuable components of regulatory-approval applications (79, 80). However, there is an awareness to remain cautious when drawing conclusions from experimental animal studies especially in regard to MBL-harboring organisms, as our knowledge of zinc variability, MBL-variant evolution, bacterial species differences, and most importantly host factors (mouse versus human) appears to be the tip of the iceberg (7, 19, 20, 62, 81–84). Indeed, our current understanding of the impact of zinc concentration on MBL-mediated resistance is limited to murine models but provides noteworthy insights. For

example, in a murine study by Corbin et al., imaging of metal distribution in a staphylococcal liver abscess revealed a cation-starved environment (i.e., devoid of zinc and manganese) (85). This host versus pathogen interaction raises important clinical and microbiological questions. Similar to the importance of characterizing free antibiotic concentrations at various infection sites, how does bioactive zinc availability compare between infection sites, e.g., soft tissue, blood, epithelial lining fluid, peritoneal fluid, and urine; and importantly, what is the temporal profile of zinc over the course of an infection at each site? Furthermore, how does the ability of the pathogen to acquire zinc from its environment (host or susceptibility media) impact MBL resistance and ultimately β-lactam efficacy? Also, what physiochemicals in the pathogen's environment are required to facilitate this zinc uptake? Clearly, the manifestation of MBL resistance (*in vitro* and *in vivo*) is complex and presents a unique opportunity for multiple disciplines to begin to systematically reassess our current microbiological, PK/PD, and clinical knowledge.

STUDY DESIGN CONSIDERATIONS

The drug pipeline does demonstrate innovative efforts to increase the throughput of lead MBL-I candidates, and their subsequent integration with robust classic and translational PK/PD studies will go a long way to derisk clinical development programs. Previous PK/PD reviews have described general study considerations one may take with respect to the design of animal infection models, drug pharmacokinetics, and study endpoints (27, 66, 80, 86, 87), and we echo those considerations and summarize additional recommendations relevant to this current topic:

Antimicrobial susceptibility testing. Underpinning MBL *in vitro-in vivo* discordance is the MIC. The challenge, therefore, is to elucidate the relationship between MIC and likelihood of outcome (clinical or animal). While there is insufficient data to propose a change in current susceptibility testing methodology, efforts to create a host-mimicking media (zinc-limited) through addition of EDTA or zinc chelation/removal and resupplementation have provided a physiologically plausible means with which to describe *in vivo* data. Studies should consider reporting MICs from conventional (Mueller-Hinton broth) and zinc-limited media to aid in a more complete understanding of exposure-response relationship.

Isolate selection. A sufficient number of clinical and engineered strains (if available) that harbor a variety of MBLs should be evaluated in the animal model to enable robust microbiological activity profiling across genotypes and variants.

One should consider an inclusion of reference strains (i.e., ATCC, NCTC, and CDC AR Bank) to enable drug-bug comparisons across different laboratories.

One should report both target (i.e., NDM-1) and coharbored enzymes (i.e., CTX-M) to discern the activity of the β-lactam backbone and the contribution of MBL-I.

Exposure-response assessment. Due to a lack of clinical data with β-lactams and evidence of MBL *in vitro-in vivo* discordance, (i) a number of efficacy studies in small and large mammals may be required to elucidate β-lactam monotherapy activity against MBLs before being relied upon as a model to bridge to humans, and (ii) each of these animal models should include a β-lactam monotherapy arm in addition to the β-lactam/MBL-I arm as a means with which to interrogate MBL *in vitro-in vivo* resistance correlation.

Faster drug elimination in animal models requires careful design of the dosing strategy. A PK profile at the site of infection is important for the backbone alone, inhibitor alone, and in combination to assess drug exposure and any drug interaction effects.

A variety of dosing strategies can be administered to elucidate a relevant PD index. However, for efficacy studies, one should consider a therapeutic exposure of the backbone if utilizing a clinically approved agent in an effort to derisk the drug development program.

One should report 0-h bacterial density to allow for appropriate preclinical endpoint assessment of the treatment arm, i.e., bacteriostasis, 1-log kill, and 2-log kill.

CONCLUSIONS

Developing effective therapeutic agents against Gram-negative bacteria has always been a challenge in the antimicrobial arena and is more so true for MBL-harboring organisms for which no β-lactamase inhibitor has yet made it into clinical practice.

This review highlights animal studies, albeit heterogeneous in design, in which select β-lactam agents demonstrate activity (with and without a MBL-I) against MBL-harboring *Enterobacterales*, suggesting an urgent need for robust preclinical studies in small and large mammals to both optimize current antimicrobials and advance the translational development of MBL-Is. As more robust data are generated, we can collectively refine our approach to the selection of animal species and model, infection site, dosing strategy, and *in vitro* susceptibility tests to improve data interpretation and our ability to correlate preclinical exposure-response relationships to clinical efficacy.

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