Metallo-β-Lactamase Mediated Antimicrobial Resistance and Progress in Inhibitor Discovery

Yongqiang Yang[1,2,§], Yu-Hang Yan[3,§], Christopher J. Schofield[4], Alan McNally[5], Zhiyong Zong[1,2,6], Guo-Bo Li[3*]*

[1]Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China

[2]Center for Pathogen Research, West China Hospital, Sichuan University, Chengdu, China

[3]Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry and Sichuan Province, Department of Medicinal Chemistry, West China School of Pharmacy, Sichuan University, Chengdu, China

[4]Department of Chemistry, Chemistry Research Laboratory and the Ineos Oxford Institute for Antimicrobial Research, University of Oxford, Oxford, United Kingdom

[5]Institute of Microbiology and Infection, College of Medical and Dental Science, University of Birmingham, Birmingham, United Kingdom

[6]Division of Infectious Diseases, State Key Laboratory of Biotherapy, Chengdu, China

[§] Y. Yang and Y.-H. Yan are co-first authors.

**E-mail: zongzhiy@scu.edu.cn; liguobo@scu.edu.cn*

Abstract

Resistance to β-lactam antibiotics is rapidly growing, substantially due to the spread of serine-β-lactamases (SBLs) and metallo-β-lactamases (MBLs), which efficiently catalyse β-lactam hydrolysis. Combinations of a β-lactam antibiotic with an SBL inhibitor have been clinically successful, however, no MBL inhibitors have been developed for clinical use. MBLs are a worrying resistance vector, because they catalyse hydrolysis of all β-lactam antibiotic classes, except the monobactams, and they are being disseminated across many bacterial species worldwide. Here we review the classification, structures, substrate profiles, and inhibition mechanisms of MBLs, highlighting current clinical problems due to MBL-mediated resistance and progress in understanding and combating MBL-mediated resistance.

MBLs represent a major challenge in health care settings

MBL history and classification

β-Lactams are the most important antibiotics, but their use is increasingly compromised by resistance due to β-lactamases, which catalyse their hydrolysis. Whilst clinically useful inhibitors of the serine β-lactamases (SBLs), such as clavulanic acid, have been developed, inhibitors of metallo-β-lactamases (MBLs), which have more recently emerged as a major clinical problem, are not yet available in clinic. The first discovered MBL was isolated from *Bacillus cereus* in 1966 [1], but it was not until the late 1980s that several MBLs were identified in clinically relevant species; these included CcrA in *Bacteroides fragilis* [2] and CphA in *Aeromonas hydrophila* [3]. In 1991, 1999, and 2008, three important types of MBLs, *i.e.* the IMPs (imipenemases), VIMs (Verona integron-encoded metallo-β-lactamases), and NDMs (New Delhi metallo-β-lactamases), were identified in many human pathogens including Enterobacterales and *Pseudomonas aeruginosa* [4]. These three MBL types are an increasing challenge for clinical care, and in some regions MBL mediated resistance is endemic. New MBLs are continuously being reported, as exemplified by the reports of the CrxA, GMB-1, and PJM-1 MBLs in 2022 [5-7]. As of writing, there are 94 identified types of MBLs with >480 variants (https://www.ncbi.nlm.nih.gov/pathogens/refgene/) (Table S1). *In silico* analysis of publicly available genomes and metagenomes predicts the presence of more than 2,000 MBLs [8], implying that a large number of MBLs, particularly with respect to clinical significance, are waiting to be characterised.

In the Ambler β-lactamase classification, MBLs belong to class B [9], which are Zn(II)-dependent hydrolases. Phylogenetic analyses based on amino acid sequences indicate

that the identified MBLs can be further divided into three major groups, corresponding to Ambler subclasses B1, B2, and B3, plus two singletons, which in part reflects differences in metal ion use and active sites (Figure 1). The B1 MBLs (*e.g.*, IMPs, VIMs, and NDMs) are presently the most clinically relevant MBLs [10]; they have a broad substrate selectivity and catalyse hydrolysis of bicyclic β-lactams, such as penicillins, cephalosporins, cephamycins, oxacephems, and carbapenems [9]. They can also hydrolyse β-lactam containing SBL inhibitors, including clavulanic acid, sulbactam and tazobactam [11], though do not hydrolyse monobactams and only poorly hydrolyse the non β-lactam containing SBL inhibitor avibactam [12]. Thus, they represent one of the most worrying bacterial resistance vectors and pose a great threat to public health.

MBLs are a common clinical problem associated with poor outcomes

MBLs are present in a large variety of Gram-negative bacteria of clinical significance, including *A. baumannii*, *Achromobacter* spp., *P. aeruginosa*, *S. maltophilia*, *Vibrio cholerae*, and many Enterobacterales species. Enterobacterales and *P. aeruginosa* are the two major producers of acquired MBLs and are also major human pathogens causing both community-acquired and healthcare-associated infections worldwide. Three global surveillance studies of Enterobacterales collected 38,266 isolates from 40 countries during 2012-2014 [13], 103,960 from 55 countries during 2008-2014 [14], and 81,781 from 39 countries during 2012-2017 [15], respectively. These studies reported ~0.4% [13, 14] or ~0.7% [15] prevalence of MBLs in Enterobacterales, accounting for ~24% [15] or ~30% of all carbapenemases [14]. Although the current prevalence may not appear to be high, considering the enormous number of clinically relevant Enterobacterales isolated

worldwide, infections due to MBL-producing Enterobacterales are frequently encountered in healthcare settings. NDMs, IMPs, and VIMs are the three MBLs commonly detected in Enterobacterales [13-15], amongst which NDMs were the most common (44-69%), followed by VIMs (22-39%), then the IMPs (9-17%) [13-15]. For *P. aeruginosa*, a global surveillance study has found MBL-encoding genes in 3.8% (n=308) of 8,010 isolates collected from 40 countries during 2012-2014, amongst which VIMs accounted for ~88% MBLs [13]. The prevalence of MBLs varies substantially among different species across countries and regions, which is well documented (see *e.g.*, [16, 17]). More continuous surveillance programs at all levels (institutional to international) with rigorous methodology on MBLs and MBL-producing bacteria are needed to monitor the trend and to guide empiric antimicrobial use. In particular, surveillance data on certain vulnerable patient subgroups such as neonates and ICU patients or stratified by infection sites would provide more precise information to guide antimicrobial therapy.

Recent clinical data have revealed that infections due to MBL-producing bacteria are commonly associated with unfavourable outcomes [18-21]. The in-hospital mortality of patients with infections caused by NDM-producing *K. pneumoniae*, IMP-producing Enterobacterales, VIM-producing *P. aeruginosa*, and SPM-producing *P. aeruginosa* could be as high as 42% [18], 39% [19], 48% [20], and 51% [21], respectively, implying the great health threats and clinically therapeutic challenges for MBL-producing pathogens. The excess mortality due to infections caused by MBL-producing bacteria warrants further investigations, which would ideally comprise subgroup analysis according to the MBL type, patient population, infection site, and bacterial species and comparison with those caused by bacteria producing SBL-type carbapenemases.

Current therapeutic issues against MBL-producing bacteria

The production of MBLs in Gram-negative pathogens substantially narrows options for antimicrobial therapy. In healthcare settings, the combination of ceftazidime-avibactam (CZA) and aztreonam (ATM) and cefiderocol are the two recommended options for combating MBL-producing Enterobacterales [22, 23], *P. aeruginosa* [23] and *S. maltophilia* [24].

The CZA/ATM combination is effective against MBL-producing pathogens because ATM is stable to MBL hydrolysis and avibactam (AVI) is able to protect ATM from hydrolysis by SBLs that are commonly co-produced by MBL-producing bacteria. A small-scale three-site study of patients with bloodstream infection due to MBL-producing Enterobacterales has demonstrated that CZA/ATM (n=52) manifested a substantially lower 30-day mortality rate (19.2% vs. 44%, p=0.007) compared to alternatives such as polymyxin and tigecycline (n=50) [25]. In two other small-scale studies, comprising 24 and 40 patients with various types of infection due to VIM-producing Enterobacterales/*P. aeruginosa* and NDM-producing Enterobacterales, CZA/ATM treatment was associated with a 17% [26] and 23% [27] mortality, respectively. However, resistance to ATM/AVI in Enterobacterales and *P. aeruginosa* has emerged [28, 29], which involves multifactorial mechanisms. In *E. coli*, a four-amino-acid insertion (*e.g.*, YRIK and YRIN) in PBP3, the main target of ATM, has been identified as a common mechanism rendering reduced susceptibility to ATM/AVI [30, 31]. In *K. pneumonia* and *Enterobacter* spp., alteration and/or decreased production of outer membrane porins OmpK35 (OmpF) and/or OmpK36 (OmpC) are a major mechanism to ATM/AVI resistance [32, 33]. Some β-

lactamases (*e.g.*, CMY-42, CTX-M-15, and KPC SBLs) that enable efficient hydrolysis of ATM, in particular when overproduced, could overwhelm the inhibition by AVI and confer resistance to ATM/AVI [31, 33]. Alternatively, overexpression of the AcrAB-TolC efflux pump has been detected in ATM/AVI-resistant strains [32], though its exact role remains unclear. In *P. aeruginosa*, the PER-type and OXA-427 SBLs could confer resistance to ATM/AVI [34]; mutations in a two-component regulatory system sensor-encoding gene named *PA4292* were identified recently as another possible mechanism for ATM/AVI resistance by overproducing pyocyanin and reducing drug influx [35].

It is worthwhile to note that, vaborbactam and relebactam, two non-β-lactam containing SBL inhibitors, have been approved for clinical use in combination with meropenem and imipenem, respectively, in many countries. Although vaborbactam and relebactam do not inhibit MBLs, both are able to protect ATM from SBL-medicated hydrolysis except OXA-type SBLs. The combinations of meropenem/vaborbactam or imipenem/relebactam plus ATM are alternative options, which manifest suboptimal coverage compared to CZA/ATM.

Cefiderocol, a siderophore containing cephalosporin, was approved in 2019 by FDA, but is not yet available in many countries. Cefiderocol is stable to most clinically relevant MBLs and SBLs, likely in part owing to its C-3 pyrrolidinium and C-7 carboxypropanoxyimino groups presenting steric hinderance to impede MBL/SBL hydrolysis [36, 37]. Cefiderocol has another important feature, *i.e.* good outer membrane permeability, stemming from its chlorocatechol siderophore which enables active transportation of cefiderocol into the periplasm space by chelating/binding to outer membrane iron transporters [38].

In a multicentre randomised trial named CREDIBLE-CR, the subgroup analysis of infections due to MBL-producing Gram-negative bacteria (without specifying the species) has revealed that cefiderocol (n=16) achieved a higher cure rate (75% vs. 14%) and a much lower all-cause mortality rate (6.3% vs. 57.1%) compared with the best-available alternative therapies, mostly containing polymyxin (n=7) [39]. In another multicentre study of nosocomial pneumonia due to MBL-producing Enterobacterales, *P. aeruginosa*, or *A. baumannii* named APEKS-NP [40], cefiderocol (n=8) led to similar rates of clinical cure (5/8) at end of treatment and 28-day survival (6/8) to high-dose (6 g per day) meropenem (n=3, 2/3 for both rates) [40]. Notably, in both CREDIBLE-CR and APEKS-NP studies, cefiderocol has a less favourable clinical cure (56.3% vs. 100%) and 28-day survival (18.8% vs. 0) for infections by NDM producers than those due to non-NDM MBL producers, consistent with the *in vitro* susceptibility results [41, 42]. Alarmingly, cefiderocol resistance has emerged in clinical settings [43, 44]. One resistance mechanism is the modifications of iron acquisition genes such as *cirA* in Enterobacterales [45, 46], *pirA* and *piuA* in *A. baumannii* [47, 48], and *piuD* and *pirR* in *P. aeruginosa* [49]. Some MBL (*e.g.*, NDMs and SPM-1) and SBL variants (*e.g.*, SHV-2a, and OXA-427) confer reduced susceptibility to cefiderocol [50-54]. In cefiderocol-resistant *A. baumannii*, the OXA-23 SBL together with penicillin-binding protein mutations were observed [50]; similarly, co-existence of iron transporter alterations, chromosomally AmpC overproduction, and efflux pump gene overexpression were observed in cefiderocol-resistant *P. aeruginosa* [55, 56]. Collectively, cefiderocol is confronting multiple resistance mechanisms, implying an uncertain clinical prospective.

Most MBL-producing clinical isolates of Enterobacterales are susceptible to polymyxins, fosfomycin [57], and newer tetracyclines (tigecycline, amadacycline, and eravacycline) [58, 59]. Polymyxins also have activities against MBL-producing *P. aeruginosa* and *S. maltophilia*, while fosfomycin and newer tetracyclines may cover MBL-producing *P. aeruginosa* and *S. maltophilia*, respectively. However, due to concerns about emerging resistance (including heteroresistance) [60, 61], efficacy [23, 62, 63] and safety [64], these agents are not recommended for the first-line therapy. Since some aminoglycosides (*e.g.*, plazomicin) are effective against MBL-producing Enterobacterales and *P. aeruginosa* [59, 65, 66], they might be used alone or in combination with other antibiotics for treating UTI or other infections caused by MBL-producing bacteria if susceptible [22, 23]. Taken together, the currently available data indicate that MBL-producing pathogens are associated with relatively high failure rates, for which the available antimicrobial therapies are limited and/or supported by scarce clinical data, and are increasingly challenged by emerging antimicrobial resistance, revealing an unmet clinical need of drugs targeting MBL-mediated antimicrobial resistance. To meet such clinical need, direct targeting MBLs by inhibitors appears to be an unavoidable approach.

Progress in understanding and combating MBL-mediated resistance

MBL structure feature and substrate profile

MBLs have a conserved characteristic αβ/βα sandwich fold (MBL fold), which is the core scaffold both of the 'true' MBLs and many other structurally related proteins [67]. B1 MBLs (*e.g.* IMP-1, VIM-2, and NDM-1) have two Zn(II) binding sites; a water molecule (W_1 in Figure 2) or hydroxide is observed (by crystallography) to bridge the two zinc ions in the

resting enzyme and is activated by Zn_1 and Zn_2 for nucleophilic reaction with the substrate β-lactam, a process which is mechanistically equivalent to initial reaction of the nucleophilic serine with the substrate β-lactams, during SBL catalysis. The active sites are located in a shallow groove that is flanked by two flexible loops, the L3 and L10 loops, which likely undergo conformational changes upon substrate binding, and thus help enable the enzymes to accommodate various substrates, including all bicyclic β-lactams [68]. Notably, B1 MBLs have a structurally equivalent, positively charged residue (*e.g.* IMP-1/NDM-1 K_{224} and VIM-2 R_{228}) on L10 loop, which has a vital role in substrate binding by forming electrostatic interactions with a bicyclic β-lactam C-2 carboxylate, so helping to form a catalytically productive conformation [69]; such a residue is conserved across almost all B1 MBLs (except VIM-1 type variants) and is referred as an 'anchoring residue' in this review to highlight its importance in substrate recognition and inhibitor discovery [70] (Figure 2). The flexible L3/L10 loops make variable interactions with substrates leading to different ability in substrate hydrolysis.

By comparison with B1 MBLs, B2 MBLs possess a relatively narrow substrate-binding pocket, and generally have a strong preference for carbapenem substrates, with poorer activity in hydrolysing penicillins and cephalosporins [11]. Crystallography reveals that the active sites of B2 MBLs, contain only one zinc ion which is located at the Zn_2 site, with a water molecule W_1 apparently occupying the Zn_1 site of the B1 MBLs. Binding of a second zinc ion to the Zn_1 site of B2 MBLs is inhibitory. Similar to the B1 MBLs, B2 MBLs (*e.g.*, CphA and Sfh-1) have flexible L3/L10 loops and anchoring residue K_{224} for substrate selectivity (Figure 2). However, B2 MBLs apparently have an extended and

kinked α-helix (α3) at the entrance of their active site, narrowing the catalytic pocket and restricting their substrate scope.

The B3 MBLs hydrolyse all bicyclic β-lactams and are di-zinc enzymes with similar zinc ion binding as in the B1 MBLs (Figure 2) [11], but have different features in the active site flexible loops. For example, L1 has two elongated loops, and has characteristic S_{221} and S_{223} residues on its L10 loop that play a similar role as the IMP anchoring residue K_{224} (or equivalent residue in B1 and B2 MBLs) for substrate binding by interacting with the bicyclic β-lactam C-2 carboxylate (or equivalent).

For all MBLs, binding of one or two active site Zn(II) ions is essential both to maintain structural stability and for catalytic activity [71]. During bacterial infection, the host reduces the availability of Zn(II) ions in the plasma through nutritional immunity, thus leading to a Zn(II)-limited environment in bacteria [72]. Under condition of Zn(II) limitation, MBLs undergo a process of demetallation to generate *apo*-MBLs that are unstable and prone to be degraded and/ or aggregated in the periplasm [73]. However, Zn(II) starvation is likely to drive the evolution of MBLs, allowing them to acquire increased apo-form stability (*e.g.* by the membrane-anchoring manner [74]) and/or higher Zn(II) binding affinity [75, 76]. Understanding MBL structural features and Zn(II) utilization is an important prerequisite for efficient inhibitor discovery targeting clinically relevant MBLs.

Inhibitor classes targeting MBLs

Over the last two decades, structurally diverse MBL inhibitors have been developed, and three drug candidates (*i.e.* VNRX-5133 (Taniborbactam), QPX7728 (Xeruborbactam) and QPX7831, all dual MBL/SBL inhibitors) are being evaluated in clinical trials [77-79]. MBL

inhibition has been reviewed in detail (see *e.g.* ref [76]). Here we outline the principles of MBL inhibition and present an MBL inhibitor classification scheme (type I-V MBL inhibitors) according to the proposed mechanisms of MBL-catalysed hydrolysis (Figure 3A), though it should be noted that some inhibitors could be placed in more than one class.

Type I inhibitors

Many MBL inhibitors have been developed that replace the Zn(II)-activated water nucleophilic hydroxyl W_1, which we categorise as type I inhibitors. Type I inhibitors contain **metal binding pharmacophores** (MBPs) to coordinate, and/or bridge between, Zn_1 and/or Zn_2. For example, the 3-aminophtalic acids show potent inhibition of IMPs (Table S2) *via* a binding mode involving coordination with Zn_1 and Zn_2 and electrostatic interactions with the anchoring residue K_{224} (Figure 3B) [80]. By comparison, thioenolates and sulfamoylpyrrole-3-carboxylates have broad-spectrum inhibition of multiple B1 MBLs, though manifest similar binding modes as the 3-aminophtalic acids (Figure 3B) [81, 82]. Heteroaryl substituted phosphonates manifest dual-inhibition of the MBLs NDM-1, VIM-2 and the SBL KPC-2 [83]. In the binding mode of such an inhibitor with NDM-1, the phosphonate group bridges the two zinc ions and is positioned to make hydrogen-bonds with N_{233} on the L10 loop; the heteroaryl ring is positioned to interact with the L3 loop. Note the sulfamoylpyrrole-3-carboxylates and phosphonates could also be regarded as type III inhibitors (see below). Other examples of type I inhibitors are given in Table S2.

Type II inhibitors

Type II MBL inhibitors are characterised by mimicking the proposed initial binding mode of intact β-lactams, including with respect to maintenance of the Zn(II)-bound hydroxyl W_1 and electrostatic interactions with the anchoring residues (*e.g.*, NDM-1 K_{224} and VIM-2

R_{228}) that are important in substrate recognition. The indole-2-carboxylates (InCs) are representative type II MBL inhibitors; they were identified by a high-throughput screen on NDM-1 [84]. The optimized C-7 Substituted InCs (*e.g.*, InC-**58**, Figure 3C) show nanomolar potency for inhibition of B1 MBL classes of major clinical relevance, revealing their potential to restore carbapenem against multiple drug-resistant Gram-negative bacteria. They manifest good *in vivo* efficacy in mouse infection models and InC-**58** is currently in the pre-clinical evaluation phase. The InCs interact with Zn_2 *via* their carboxylate (which also binds to the substrate carboxylate anchoring residue) and trap the di-zinc bridging hydroxyl, so demonstrating the practicality of intact β-lactam mimicking to achieve broad-spectrum MBL inhibition and potent cell-based activity.

The 3-oxoisoindoline-4-carboxylates (OiC) also have features that mimic initial binding of β-lactams, *e.g.*, chemically stable OiC five-membered lactam approaching to the hydroxyl W_1 and a carboxylate that forms electrostatic interactions with the VIM-2 anchor R_{228} [85]. By contrast with the InCs, the OiC chemotype does not involve chelation with active site Zn(II), but is likely to retain the water molecule W_2 that is an important species in the β-lactam hydrolytic pathway (Figure 3A and 3C). However, OiCs only show narrow spectrum inhibition to VIM-type MBLs, probably due to their requirement for engagement with active site bordering flexible loop residues (in particular, F_{61} and Y_{67} on L3 loop).

Type III inhibitors

Type III MBL inhibitors are exemplified by boronate compounds, which in their sp^3/tetrahedral form mimic the unstable tetrahedral intermediates in β-lactam hydrolysis. The bicyclic boronates (*e.g.*, taniborbactam [77] and xeruborbactam [78]) are perhaps the

most representative type III MBL inhibitors (Figure 3D). The taniborbactam/cefepime combination is being evaluated in patients with complicated urinary tract infections in Phase III (ClinicalTrials.gov no.: NCT03840148), and Xeruborbactam is already in the early clinical evaluation stage (ClinicalTrials.gov nos.: NCT04380207 and NCT05072444). They have similarly positioned bicyclic-ring and carboxylate groups as the bicyclic β-lactams, enabling them to mimic the initial mode of β-lactam binding to the MBL active sites [77, 78]. More specifically, the sp^2 hybridised boronate with a vacant *p*-orbital is positioned to accept an electron pair from zinc-bound hydroxyl W_1 mimicking the natural reaction; thus, in their sp^2 form, bicyclic boronates may be regarded as intact β-lactam substrate mimics (type II inhibitors). Reaction of the sp^2 boronate with a hydroxide ion/water transforms the inhibitor into the sp^3 hybridization state that is structurally similar to the tetrahedral intermediate(s) in both B1 MBL and SBL catalysis. The sp^3 hybridised boronates manifest slow off rates and form stable interactions with MBL active sites, *e.g.*, by displacing the nucleophilic hydroxyl, coordinating with both zinc ions and hydrogen bonding with N_{223} of VIM-2/NDM-1 (Figure 3D). Bicyclic boronates show promise as dual action MBL/SBL inhibitors and, maybe, as antibiotics *via* transpeptidase inhibition. Note, although reaction of boronates with the MBL zinc-bound hydroxyl likely requires binding of an sp^2 hybridised state, in principle the sp^3 state could bind with displacement of the zinc bound hydroxide [79]. Some monocyclic boronates have also been developed as β-lactamase inhibitors, notably the SBL inhibitor vaborbactam which has been approved for clinical use in combination with meropenem. However, the current monocyclic boronates are not potent broad spectrum MBLs [86], possibly in part due to conformational mobility compared to the bicyclic boronates.

Type IV inhibitors

Type IV MBL inhibitors are defined as those more closely mimic binding of the hydrolysed products. Type IV inhibitors bind to make bidentate interactions with Zn_2, the key point of difference with type II inhibitors. The thiazole-4-carboxylic acids (ThCs) and imidazole-2-carboxylic acids (ImCs) are representative type IV inhibitors, both of which coordinate with Zn_2 and make electrostatic interactions with an anchoring residue R_{228}/K_{224} (Figure 3E) [87, 88]. In addition, the reported potent type IV inhibitors are generally positioned to interact with the L3/L10 loops (Table S2).

Type V inhibitors

By contrast with types I-IV, type V inhibitors degrade/modify the metal ion binding active sites, *e.g.*, by zinc ion removal, zinc ion replacement, or covalent reaction with the metal ligating cysteine. Several metal-depriving compounds have been shown to actively eject the active site zinc ions or to sequester free zinc ions that have exited from the MBL active site. For example, aspergillomarasmine A (AMA), a fungal natural product, shows potent inhibition of NDM-1 and VIM-2 by active site metal deprivation (Figure 3F) [89]. AMA is able to fully restore meropenem activity against multiple clinical isolates possessing either VIM or NDM-type alleles and shows efficacy in mice infected with NDM-1-expressing *K. pneumoniae*, suggesting that this mechanism may be effective for combatting MBL-mediated resistance. Other metal-ion chelator chemotypes, such as dipicolinic acid, have been shown to potentiate β-lactam antibiotics against MBL-expressing bacteria (Figure 3F and Table S2) [90-93]. Interestingly, the antimicrobial drug colloidal bismuth subcitrate (CBS) shows potent inhibition of NDM-1, VIM-2 and IMP-4 by a mode of action reported to involve binding of one Bi(III) ion to displace the two B1 MBL active site Zn(II) ions.[94]

CBS can restore meropenem efficacy against NDM-1-producing bacteria *in vitro* and *in vivo*. Ebselen is reported to be an NDM-1 inhibitor by reacting to form an S–Se bond with the NDM-1 active site C_{221} [95]. *p*-Chloromecuribenzoate and mercaptoacetic acid thiol esters have also been reported to react with the MBL active site cysteine [96, 97]. Despite the effective MBL inhibition by type V inhibitors, there is a concern of off-target toxicity, for example due to reaction with other metalloenzymes or cysteine proteases.

Optimised MBL inhibitors should target multiple MBLs of major clinical relevance, but should not inhibit human metalloenzymes [98]. Although further work is required, some of the reported inhibitor chemotypes, including bicyclic boronates, indole-2-carboxylates and thiazoles, are promising in this regard and manifest potent, broad-spectrum inhibition of B1 MBLs. The binding of such inhibitors to MBLs is proposed to mimic key features of complexes involved in β-lactam binding and reaction, albeit in different phases of catalysis; they, appear to be useful chemotypes for development of clinically useful MBL inhibitions.

Other strategies against MBL-mediated resistance

Development of new non-β-lactam antibiotics targeting penicillin binding proteins (PBPs) is another option to combat MBL-mediated resistance. For example, the diazabicyclooctane derivative zidebactam shows activity against PBP2, in addition to having SBL inhibition. Zidebactam manifests strong synergistic antimicrobial activity in combination with a β-lactam against MBL- and/or SBL-producing Enterobacterales and *P. aeruginosa* [99]. Currently, the combination of zidebactam and cefepime is being evaluated in clinical trials (ClinicalTrials.gov no.: NCT02707107 and NCT02674347).

Concluding Remarks and Future Perspectives

MBL-mediated β-lactam antibiotic resistance has attracted global attention in recent years. New MBLs are continuously being reported and many more will inevitably be identified, reflecting an expanding bacterial arsenal to mediate resistance to one of the most classes of anti-infective drugs. The global spread of MBL-encoding genes represents a serious challenge to public health, since MBLs enable resistance to all classes of β-lactam drugs, with the exception of monobactams. Currently, a preferred first-line therapeutic option for MBL-mediated resistance is cefiderocol or the CZA/ATM combination; however, bacterial resistance to cefiderocol and/or CZA/ATM has emerged, and the available data suggests that the efficacy of these drugs against MBL-producing bacteria is unsatisfactory, with treatment being associated with high, albeit reduced mortality. There is thus a clear unmet clinical demand for MBL inhibitors particularly covering major clinically relevant MBL enzymes. We hope that the classification of MBL inhibitors presented here will be useful for developing clinically useful MBL inhibitors. In such development, important factors for optimisation in addition to potency, include Gram-negative bacterial membrane permeability, the balance between broad-spectrum activity, selectivity over human MBL fold and other metallo-enzymes, and the frequency of resistance.

Many bacterial species that are native to aquatic and other environments harbour MBL-encoding genes, which could be transferred to clinically relevant bacterial species and evolve into new genes/variants under antimicrobial selective pressure in agricultural or healthcare situations. This possibility reinforces the need for rigorous multi-site surveillance of MBLs and MBL-producing bacteria in both environmental and clinical settings. In this regard, broad and narrow spectrum MBL inhibitors might be developed as cost-effective probes for the active surveillance of the MBLs in the environment and in

patients with bacterial infections, in order to guide use of antimicrobials, both at the level of individual patients and in populations.

Highlights

Production of MBLs is a common mechanism of resistance to carbapenems and most other β-lactam antibiotics in various Gram-negative bacteria of clinical significance, posing a serious challenge to antimicrobial therapy and leading to poor outcomes for affected patients.

In silico analysis have predicted >2,000 types of MBLs, and 94 types have been identified so far and were divided into three major groups (subclasses B1, B2, and B3) with NDMs, IMPs, and VIMs (all of B1) being the most common seen in human infections.

Cefiderocol and aztreonam in combination of ceftazidime-avibactam are the two recommended options against MBL-producing bacteria but are associated with relatively high failure rates and emerging resistance, suggesting an unmet clinical need for targeting MBL-mediated antimicrobial resistance.

The challenges in developing broad-spectrum inhibitors targeting multiple structurally different MBLs can be moderated by understanding the common MBL catalytical and structural features, *e.g.,* the anchoring residue that is conserved across almost all B1 MBLs, and the flexible L3/L10 loops that affect binding of different substrates and inhibitors.

The MBL inhibition principles and inhibitor classification scheme (type I-V MBL inhibitors) according to the proposed mechanisms of MBL-catalysed hydrolysis offer relatively clear guidelines for developing clinically useful MBL inhibitors/probes.

Glossary

β-Lactamases: enzymes produced by bacteria that catalyse the hydrolysis of β-lactam antibiotics, such as penicillins, cephalosporins, and carbapenems.

Serine-β-lactamases: β-lactamases that employ a nucleophilic serine residue during hydrolysis of β-lactam antibiotics.

Metallo-β-lactamases: β-lactamases that use zinc ion(s) to activate water molecules for β-lactam hydrolysis.

Ambler class: a classification system for β-lactamases that has four classes (A, B, C, and D) based on their amino acid sequences, as proposed by Richard P Ambler.

Anchoring residue: a residue that has a vital role in catalytically productive substrate recognition and binding, *e.g.*, NDM-1 K_{224} specifically interacts with a penicillin C-2 carboxylate group (or equivalent).

Metalloenzyme: enzymes that contain structurally and functionally important metal ions for bioactivity, structural, signalling, or other reasons.

Metal binding pharmacophore: a chemotype that can coordinate with active site metal ion(s).

Outstanding questions

How can antimicrobial regimes for treatment of infections by MBL producers be improved based on currently available β-lactams and β-lactamase inhibitors considering their well-established safety profiles? Are there regimes that could be developed against MBL-producing strains with resistance to aztreonam-avibactam and/or cefiderocol?

What is the excess mortality due to infections caused by MBL-producing bacteria as a whole and/or stratified by the major MBL types (NDM, IMP, and VIM), bacterial groups or species (*e.g.*, Enterobacterales and *P. aeruginosa*), or risk patient populations (*e.g.*, neonates, ICU patients, and those with haematological neoplasms)?

How to develop effective guidelines or strategies for the parallel optimization of MBL inhibition potency, bacterial outer membrane permeability, the balance between broad-spectrum activity and selectivity over human MBL fold enzymes, and frequency of resistance?

Could MBL inhibitors be employed to develop diagnostic methods for MBL-mediated resistance in infected patients to achieve precision treatment (*e.g.*, use of MBL inhibitors in future)?

Acknowledgments

We thank the following for support National Natural Science Foundation of China [81861138055 to Z. Zong; 82122065, 82073698, 81874291 to G.B.L.], Sichuan Science and Technology Program [2022YFH0027 to G.B.L.], and West China Hospital (1.3.5

project for disciplines of excellence, grant number ZYGD22001). CJS thanks the Ineos Oxford Institute for Antimicrobial Research for support.

Declaration of interests

No interests are declared.

References

1. Sabath, L.D. and Abraham, E.P. (1966) Zinc as a cofactor for cephalosporinase from *Bacillus cereus* 569. *Biochem. J.* 98, 11C-13C.

2. Rasmussen, B.A. *et al.* (1990) Cloning and sequencing of the class B β-lactamase gene (*ccrA*) from *Bacteroides fragilis* TAL3636. *Antimicrob. Agents Chemother.* 34, 1590-1592.

3. Massidda, O. *et al.* (1991) The *Aeromonas hydrophila cphA* gene - molecular heterogeneity among class-B metallo-β-lactamases. *J. Bacteriol.* 173, 4611-4617.

4. Bush, K. (2013) The ABCD's of β-lactamase nomenclature. *J. Infect. Chemother.* 19, 549-559.

5. Sóki, J. *et al.* (2022) A novel Bacteroides metallo-β-lactamase (MBL) and its gene (*crxA*) in *Bacteroides xylanisolvens* revealed by genomic sequencing and functional analysis. *J. Antimicrob. Chemother.* 77, 1553-1556.

6. Schauer, J. *et al.* (2022) Characterization of GMB-1, a novel metallo-β-lactamase (MBL) found in three different Enterobacterales species. *J. Antimicrob. Chemother.* 77, 1247-1253.

7. Yamada, *et al.* (2022) Biochemical characterization of the subclass B3 metallo-β-lactamase PJM-1 from *Pseudoxanthomonas japonensis*. *Antimicrob. Agents Chemother.* 66, e00691-00622.

8. Berglund, F. *et al.* (2021) An updated phylogeny of the metallo-β-lactamases. *J. Antimicrob. Chemother.* 76, 117-123.

9. Palzkill, T. (2013) Metallo-β-lactamase structure and function. *Ann. N. Y. Acad. Sci.* 1277, 91-104.

10. Mojica, M.F. *et al.* (2016) B1-metallo-β-lactamases: where do we stand? *Curr. Drug Targets* 17, 1029-1050.

11. Boyd Sara, E. *et al.* (2020) Metallo-β-Lactamases: structure, function, epidemiology, treatment options, and the development pipeline. *Antimicrob. Agents Chemother.* 64, e00397-00320.

12. Abboud, M.I. *et al.* (2016) Interaction of avibactam with class B metallo-β-lactamases. *Antimicrob. Agents Chemother.* 60, 5655-5662.

13. Kazmierczak, K.M. *et al.* (2016) Multiyear, multinational survey of the incidence and global distribution of metallo-β-lactamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 60, 1067-1078.

14. Karlowsky, J.A. *et al.* (2017) *In vitro* activity of imipenem against carbapenemase-positive *Enterobacteriaceae* isolates collected by the SMART Global Surveillance Program from 2008 to 2014. *J. Clin. Microbiol.* 55, 1638-1649.

15. Kazmierczak, K.M. *et al.* (2021) Epidemiology of carbapenem resistance determinants identified in meropenem-nonsusceptible Enterobacterales collected as part of a global surveillance program, 2012 to 2017. *Antimicrob. Agents Chemother.* 65, e0200020.

16. Tenover, F.C. *et al.* (2022) Carbapenemase-producing *Pseudomonas aeruginosa* -an emerging challenge. *Emerg. Microbes Infect.* 11, 811-814.

17. Bush, K. and Bradford, P.A. (2020) Epidemiology of β-lactamase-producing pathogens. *Clin. Microbiol. Rev.* 33, 00047-00019.

18. Snyder, B.M. *et al.* (2019) Risk factors and epidemiologic predictors of blood stream infections with New Delhi Metallo-β-lactamase (NDM-1) producing *Enterobacteriaceae*. *Epidemiol. Infect.* 147, e137.

19. Soneda, K. *et al.* (2022) Clinical characteristics and treatment of IMP-type carbapenemase-producing *Enterobacteriaceae* bacteremia: case series and literature review. *J. Infect. Chemother*. 29, 28-32.

20. Persoon, M.C. *et al.* (2019) Mortality related to Verona integron-encoded metallo-β-lactamase-positive *Pseudomonas aeruginosa*: assessment by a novel clinical tool. *Antimicrob. Resist. Infect. Control.* 8, 107.

21. Zavascki, A.P. *et al.* (2006) The influence of metallo-β-lactamase production on mortality in nosocomial *Pseudomonas aeruginosa* infections. *J. Antimicrob. Chemother.* 58, 387-392.

22. Paul, M. *et al.* (2022) European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative *bacilli* (endorsed by European society of intensive care medicine). *Clin. Microbiol. Infect.* 28, 521-547.

23. Tamma, P.D. *et al.* (2021) Infectious Diseases Society of America Guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR-*P. aeruginosa*). *Clin. Infect. Dis.* 72, e169-e183.

24. Tamma, P.D. *et al.* (2022) Infectious diseases society of america guidance on the treatment of AmpC β-lactamase-producing Enterobacterales, carbapenem-resistant *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia* Infections. *Clin. Infect. Dis.* 74, 2089-2114.

25. Falcone, M. *et al.* (2021) Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by metallo-β-lactamase-producing Enterobacterales. *Clin. Infect. Dis.* 72, 1871-1878.

26. Sempere, A. *et al.* (2022) Ceftazidime-avibactam plus aztreonam for the treatment of infections by VIM-type-producing Gram-negative bacteria. *Antimicrob. Agents Chemother*. 66, e0075122.

27. Nagvekar, V. *et al.* (2021) Clinical outcome of patients on ceftazidime-avibactam and combination therapy in carbapenem-resistant *Enterobacteriaceae*. *Indian J. Crit. Care Med.* 25, 780-784.
28. Karlowsky, J.A. *et al.* (2017) *In vitro* activity of aztreonam-avibactam against *Enterobacteriaceae* and *Pseudomonas aeruginosa* Isolated by clinical laboratories in 40 countries from 2012 to 2015. *Antimicrob. Agents Chemother.* 61, e00472-17.
29. Biedenbach, D.J. *et al.* (2015) *In vitro* activity of aztreonam-avibactam against a global collection of Gram-negative pathogens from 2012 and 2013. *Antimicrob. Agents Chemother.* 59, 4239-4248.
30. Alm, R.A. *et al.* (2015) Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. *J. Antimicrob. Chemother.* 70, 1420-1428.
31. Ma, K. *et al.* (2020) Struggle to survive: the choir of target alteration, hydrolyzing enzyme, and plasmid expression as a novel aztreonam-avibactam resistance mechanism. *mSystems* 5, e00821-20.
32. Satapoomin, N. *et al.* (2022) *Klebsiella pneumoniae* mutants resistant to ceftazidime-avibactam plus aztreonam, imipenem-relebactam, meropenem-vaborbactam, and cefepime-taniborbactam. *Antimicrob. Agents Chemother.* 66, e0217921.
33. Mendes, R.E. *et al.* (2021) Investigation of mechanisms responsible for decreased susceptibility of aztreonam/avibactam activity in clinical isolates of Enterobacterales collected in Europe, Asia and Latin America in 2019. *J. Antimicrob. Chemother.* 76, 2833-2838.
34. Poirel, L. *et al.* (2022) Impact of acquired broad-spectrum β-lactamases on susceptibility to cefiderocol and newly developed β-lactam/β-lactamase inhibitor combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 66, e0003922.
35. Zhao, X. *et al.* (2022) Mutation of PA4292 in *Pseudomonas aeruginosa* increases β-lactam resistance through upregulating pyocyanin production. *Antimicrob. Agents Chemother.* 66, e0042122.
36. Ito-Horiyama, T. *et al.* (2016) Stability of novel siderophore cephalosporin S-649266 against clinically relevant carbapenemases. *Antimicrob. Agents Chemother.* 60, 4384-4386.
37. Aoki, T. *et al.* (2018) Cefiderocol (S-649266), A new siderophore cephalosporin exhibiting potent activities against *Pseudomonas aeruginosa* and other gram-negative pathogens including multi-drug resistant bacteria: structure activity relationship. *Eur. J. Med. Chem.* 155, 847-868.
38. Ito, A. *et al.* (2016) Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 60, 7396-7401.
39. Timsit, J.F. *et al.* (2022) Cefiderocol for the treatment of infections due to metallo-β-lactamase-producing pathogens in the CREDIBLE-CR and APEKS-NP phase 3 randomized studies. *Clin. Infect. Dis.* 75, 1081-1084.

40. Wunderink, R.G. et al. (2021) Cefiderocol versus high-dose, extended-infusion meropenem for the treatment of Gram-negative nosocomial pneumonia (APEKS-NP): a randomised, double-blind, phase 3, non-inferiority trial. *Lancet Infect. Dis*. 21, 213-225.

41. Mushtaq, S. *et al.* (2020) *In vitro* activity of cefiderocol, a siderophore cephalosporin, against multidrug-resistant Gram-negative bacteria. *Antimicrob. Agents Chemother.* 64, e01582-20.

42. Longshaw, C. *et al.* (2020) *In vitro* activity of the siderophore cephalosporin, cefiderocol, against molecularly characterized, carbapenem-non-susceptible Gram-negative bacteria from Europe. *JAC Antimicrob. Resist.* 2, dlaa060.

43. Wang, Q. *et al.* (2022) Occurrence of high levels of cefiderocol resistance in carbapenem-resistant *Escherichia coli* before Its approval in China: a report from China CRE-Network. *Microbiol. Spectr.* 10, e0267021.

44. Nurjadi, D. *et al.* (2022) New Delhi metallo-β-lactamase facilitates the emergence of cefiderocol resistance in *Enterobacter cloacae*. *Antimicrob. Agents Chemother.* 66, e0201121.

45. Lan, P. *et al.* (2022) Catecholate siderophore receptor CirA impacts cefiderocol susceptibility in *Klebsiella pneumoniae*. *Int. J. Antimicrob. Agents* 60, 106646.

46. Klein, S. *et al.* (2022) Rapid development of cefiderocol resistance in carbapenem-resistant *Enterobacter cloacae* during therapy is associated with heterogeneous mutations in the catecholate siderophore receptor *cirA*. *Clin. Infect. Dis.* 74, 905-908.

47. Malik, S. *et al.* (2020) Cefiderocol resistance in *Acinetobacter baumannii*: roles of β-lactamases, siderophore receptors, and penicillin binding protein 3. *Antimicrob. Agents Chemother.* 64, e01221-20.

48. Smoke, S.M. *et al.* (2022) Evolution and transmission of cefiderocol-resistant *Acinetobacter baumannii* during an outbreak in the burn intensive care unit. *Clin. Infect. Dis*. ciac647.

49. Streling, A.P. *et al.* (2021) Evolution of cefiderocol non-susceptibility in *Pseudomonas aeruginosa* in a patient without previous exposure to the antibiotic. *Clin. Infect. Dis.* 73, e4472-e4474.

50. Nordmann, P. *et al.* (2022) Mechanisms of reduced susceptibility to cefiderocol among isolates from the CREDIBLE-CR and APEKS-NP clinical trials. *Microb. Drug Resist.* 28, 398-407.

51. Poirel, L. *et al.* (2021) Contribution of PER-type and NDM-type β-lactamases to cefiderocol resistance in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 65, e0087721.

52. Lan, P. *et al.* (2022) Emergence of high-level cefiderocol resistance in carbapenem-resistant *Klebsiella pneumoniae* from bloodstream infections in patients with hematologic malignancies in China. *Microbiol. Spectr.* 10, e0008422.

53. Tiseo, G. *et al.* (2021) Meropenem-vaborbactam as salvage therapy for ceftazidime-avibactam-, cefiderocol-resistant ST-512 *Klebsiella pneumoniae*-producing KPC-31, a D179Y variant of KPC-3. *Open Forum Infect. Dis.* 8, ofab141.

54. Jacob, A.S. *et al.* (2021) No *in vitro* activity of cefiderocol against OXA-427-producing Enterobacterales. *J. Antimicrob. Chemother.* 76, 3317-3318.
55. Sadek, M. *et al.* (2022) Progressive *in vivo* development of resistance to cefiderocol in *Pseudomonas aeruginosa*. *Eur. J. Clin. Microbiol. Infect. Dis*. 42, 61-66.
56. Lopez-Causape, C. *et al.* (2022) Cefiderocol resistance genomics in sequential chronic *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Clin. Microbiol. Infect*. S1198-743X(22)00586-9
57. Vardakas, K.Z. *et al.* (2016) Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. *Int. J. Antimicrob. Agents* 47, 269-285.
58. Johnston, B.D. *et al.* (2020) Activity of cefiderocol, ceftazidime-avibactam, and eravacycline against carbapenem-resistant *Escherichia coli* isolates from the United States and international sites in relation to clonal background, resistance genes, coresistance, and region. *Antimicrob. Agents Chemother.* 64, e00797-20.
59. Lutgring, J.D. *et al.* (2020) Antibiotic susceptibility of NDM-producing Enterobacterales collected in the United States in 2017 and 2018. *Antimicrob Agents Chemother* 64, e00499-20.
60. Band, V.I. *et al.* (2021) Colistin heteroresistance is largely undetected among carbapenem-resistant Enterobacterales in the United States. *mBio* 12, e02881-20.
61. Lim, T.P. *et al.* (2020) *In vitro* pharmacodynamics of fosfomycin against carbapenem-resistant *Enterobacter cloacae* and *Klebsiella aerogenes*. *Antimicrob. Agents Chemother.* 64, e00536-20.
62. van Duin, D. *et al.* (2018) Colistin versus ceftazidime-avibactam in the treatment of infections due to carbapenem-resistant *Enterobacteriaceae*. *Clin. Infect. Dis.* 66, 163-171.
63. Tumbarello, M. *et al.* (2019) Efficacy of ceftazidime-avibactam salvage therapy in patients with infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. *Clin. Infect. Dis*. 68, 355-364.
64. Wagenlehner, F. *et al.* (2021) Systematic review on estimated rates of nephrotoxicity and neurotoxicity in patients treated with polymyxins. *Clin. Microbiol. Infect*. S1198-743X(20)30764-3.
65. Johnston, B.D. *et al.* (2021) Activity of plazomicin against carbapenem-intermediate or -resistant *Escherichia coli* isolates from the United States and international sites in relation to clonal background, resistance genes, co-resistance, and region. *J. Antimicrob. Chemother.* 76, 2061-2070.
66. Stone, G.G. *et al.* (2020) *In vitro* activity of ceftazidime-avibactam against Gram-negative isolates collected in 18 European countries, 2015-2017. *Int. J. Antimicrob. Agents* 56, 106045.
67. Bebrone, C. (2007) Metallo-β-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. *Biochem. Pharmacol.* 74, 1686-1701.
68. Materon, I.C. *et al.* (2004) Analysis of the context dependent sequence requirements of active site residues in the metallo-β-lactamase IMP-1. *J. Mol. Biol.* 344, 653-663.

69. Softley Charlotte, A. *et al.* (2020) Structure and molecular recognition mechanism of IMP-13 metallo-β-lactamase. *Antimicrob. Agents Chemother.* 64, e00123-00120.
70. Yan, Y.-H. *et al.* (2020) Principles and current strategies targeting metallo-β-lactamase mediated antibacterial resistance. *Med. Res. Rev.* 40, 1558-1592.
71. Morán-Barrio, J. *et al.* (2009) Secretion of GOB metallo-β-lactamase in *Escherichia coli* depends strictly on the cooperation between the cytoplasmic DnaK chaperone system and the Sec machinery: completion of folding and Zn(II) ion acquisition occur in the bacterial periplasm. *Antimicrob. Agents Chemother.* 53, 2908-2917.
72. Antelo, G.T. *et al.* (2021) Molecular evolution of transition metal bioavailability at the host–pathogen interface. *Trends Microbiol.* 29, 441-457.
73. Lopez, C. *et al.* (2022) Deciphering the evolution of metallo-β-lactamases: A journey from the test tube to the bacterial periplasm. *J. Biol. Chem.* 298, 101665.
74. Gonzalez, L.J. *et al.* (2016) Membrane anchoring stabilizes and favors secretion of New Delhi metallo-β-lactamase. *Nat. Chem. Biol*. 12, 516-522.
75. Bahr, G. *et al.* (2017) Clinical evolution of New Delhi Metallo-β-Lactamase (NDM) optimizes resistance under Zn(II) deprivation. *Antimicrob. Agents Chemother.* 62, e01849-01817.
76. Bahr, G. *et al.* (2021) Metallo-β-lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design. *Chem. Rev.* 121, 7957-8094.
77. Liu, B. *et al.* (2020) Discovery of taniborbactam (VNRX-5133): a broad-spectrum serine- and metallo-β-lactamase inhibitor for carbapenem-resistant bacterial infections. *J. Med. Chem.* 63, 2789-2801.
78. Hecker, S.J. *et al.* (2020) Discovery of cyclic boronic acid QPX7728, an ultrabroad-spectrum inhibitor of serine and metallo-β-lactamases. *J. Med. Chem.* 63, 7491-7507.
79. Reddy, K.R. *et al.* (2021) Selection of QPX7831, an orally bioavailable prodrug of boronic acid β-lactamase inhibitor QPX7728. *J. Med. Chem.* 64, 17523-17529.
80. Hiraiwa, Y. *et al.* (2014) X-ray crystallographic analysis of IMP-1 metallo-β-lactamase complexed with a 3-aminophthalic acid derivative, structure-based drug design, and synthesis of 3, 6-disubstituted phthalic acid derivative inhibitors. *Bioorganic Med. Chem. Lett.* 24, 4891-4894.
81. Brem, J. *et al.* (2014) Rhodanine hydrolysis leads to potent thioenolate mediated metallo-β-lactamase inhibition. *Nat. Chem.* 6, 1084-1090.
82. Wachino, J.-I. *et al.* (2020) Sulfamoyl heteroarylcarboxylic acids as promising metallo-β-lactamase inhibitors for controlling bacterial carbapenem resistance. *mBio* 11, e03144-19.
83. Pemberton, O.A. *et al*. (2019) Heteroaryl phosphonates as noncovalent inhibitors of both serine- and metallocarbapenemases. *J. Med. Chem.* 62, 8480-8496.
84. Brem, J. *et al.* (2022) Imitation of β-lactam binding enables broad-spectrum metallo-β-lactamase inhibitors. *Nat. Chem.* 14, 15-24.

85. Li, G.-B. *et al.* (2017) NMR-filtered virtual screening leads to non-metal chelating metallo-β-lactamase inhibitors. *Chem. Sci.* 8, 928-937.
86. Langley, G.W. *et al.* (2019) Profiling interactions of vaborbactam with metallo-β-lactamases. *Bioorganic Med. Chem. Lett.* 29, 1981-1984.
87. Leiris, S. *et al.* (2019) SAR studies leading to the identification of a novel series of metallo-β-lactamase inhibitors for the treatment of carbapenem-resistant *Enterobacteriaceae* infections that display efficacy in an animal infection model. *ACS Infect. Dis.* 5, 131-140.
88. Dai, Q. *et al.* (2021) AncPhore: a versatile tool for anchor pharmacophore steered drug discovery with applications in discovery of new inhibitors targeting metallo-β-lactamases and indoleamine/tryptophan 2,3-dioxygenases. *Acta Pharm. Sin. B* 11, 1931-1946.
89. King, A.M. *et al.* (2014) Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature* 510, 503-506.
90. Chen, A.Y. *et al.* (2017) Dipicolinic acid derivatives as inhibitors of New Delhi metallo-β-lactamase-1. *J. Med. Chem.* 60, 7267-7283.
91. Zhang, E. *et al.* (2018) NOTA analogue: a first dithiocarbamate inhibitor of metallo-β-lactamases. *Bioorganic Med. Chem. Lett.* 28, 214-221.
92. Falconer, S.B. *et al.* (2015) Zinc chelation by a small-molecule adjuvant potentiates meropenem activity *in vivo* against NDM-1-producing *Klebsiella pneumoniae. ACS Infect. Dis.* 1, 533-543.
93. Shi, X.-F. *et al.* (2019) H_2depda: an acyclic adjuvant potentiates meropenem activity *in vitro* against metallo-β-lactamase-producing Enterobacterales. *Eur. J. Med. Chem.* 167, 367-376.
94. Wang, R. *et al.* (2018) Bismuth antimicrobial drugs serve as broad-spectrum metallo-β-lactamase inhibitors. *Nat. Commun.* 9, 439.
95. Chiou, J. *et al*. (2015) Ebselen as a potent covalent inhibitor of New Delhi metallo-β-lactamase (NDM-1). *ChemComm* 51, 9543-9546.
96. Payne, D.J. *et al.* (1997) Inhibition of metallo-β-lactamases by a series of mercaptoacetic acid thiol ester derivatives. *Antimicrob. Agents Chemother.* 41, 135-140.
97. Thomas, P.W. *et al.* (2013) An altered zinc-binding site confers resistance to a covalent inactivator of New Delhi metallo-β-lactamase-1 (NDM-1) discovered by high-throughput screening. *Bioorg. Med. Chem.* 21, 3138-3146.
98. Yu, J.-L. *et al.* (2022) MeDBA: the metalloenzyme data bank and analysis platform. *Nucleic Acids Res.* 51, D593-D602.
99. Mushtaq, S. *et al.* (2021) Activity of cefepime/zidebactam (WCK 5222) against 'problem' antibiotic-resistant Gram-negative bacteria sent to a national reference laboratory. *J. Antimicrob. Chemother.* 76, 1511-1522.
100. Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792-1797.

101. Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688-2690.
102. Letunic, I. and Bork, P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res*. 44, W242-W245.
103. King, D. and Strynadka, N. (2011) Crystal structure of New Delhi metallo-β-lactamase reveals molecular basis for antibiotic resistance. *Protein Sci.* 20, 1484-1491.
104. Hinchliffe, P. *et al.* (2016) Cross-class metallo-β-lactamase inhibition by bisthiazolidines reveals multiple binding modes. *Proc. Natl. Acad. Sci. U.S.A.* 113, E3745-E3754.
105. Chen, J.Z. *et al.* (2020) Comprehensive exploration of the translocation, stability and substrate recognition requirements in VIM-2 lactamase. *Elife* 9, e56707.
106. Garau, G. *et al.* (2005) A metallo-β-lactamase enzyme in action: crystal structures of the monozinc carbapenemase CphA and its complex with biapenem. *J. Mol. Biol.* 345, 785-795.
107. Fonseca, F. *et al.* (2011) Crystal structure of *Serratia fonticola* Sfh-I: activation of the nucleophile in mono-zinc metallo-β-lactamases. *J. Mol. Biol.* 411, 951-959.
108. Nauton, L. *et al.* (2008) Structural insights into the design of inhibitors for the L1 metallo-β-lactamase from *Stenotrophomonas maltophilia*. *J. Mol. Biol.* 375, 257-269.

Figure Legends

Figure 1. A maximum likelihood-based phylogeny of 94 metallo-β-lactamases based on amino acid sequences. The first named variant of each enzyme is represented by each branch. Different colors are used to highlight each subclass. Multiple sequence alignment was performed using MUSCLE v3.8.15.51 [100]. RAxML v8.2.10 [101] was used to investigate phylogeny from the combined alignment file with 100 bootstraps. The tree was visualized and edited using iTOL v6 [102].

Figure 2. MBLs have a conserved αβ/βα fold but have different active site features. The representative B1 MBLs IMP-1 (PDB ID 5EV6), VIM-2 (PDB ID 5YD7) and NDM-1 (PDB ID 3SPU) possess two conserved Zn(II) binding sites [103-105], structurally equivalent anchoring residues, and flexible loops for binding with various substrates including all bicyclic β-lactam antibiotics. The B2 MBLs CphA (PDB ID 1X8G) and Sfh-I (PDB ID 3SD9) are mono-zinc enzymes with a preference for carbapenem hydrolysis [106, 107]. Similarly to B1 MBLs, the B3 MBL L1 (PDB ID 2FM6) has a di-zinc binding active site and flexible loops that contribute to a wide substrate profile [108].

Figure 3. MBL inhibitor classification scheme. (A) Proposed outline mechanisms for MBL-mediated β-lactam hydrolysis. (B) Class I inhibitors: these displace the nucleophilic W1 and W2, as evidenced by crystal structures of IMP-1:ApA (PDB ID 3WXC), VIM-2:ML302F (PDB ID 4PVT), NDM-1:HaP-**2** (PDB ID 6D1B), and NDM-1:SPC (PDB ID 6KZL) [80-83]. (C) Class II inhibitors: these mimic intact β-lactam binding, as evidenced by crystal structures of NDM-7:InC-48 (PDB ID 7AEZ) and VIM-2:OiC-16 (PDB ID 5LE1) [84, 85]. (D) Class III inhibitors: these mimic tetrahedral intermediates, as evidenced by crystal structures of VIM-2:VNRX-5133 (PDB ID 6SP7) and NDM-1:QPX7728 (PDB ID 6V1M) [77, 78]. (E) Class IV inhibitors: these mimic as evidenced by crystal structures of anionic intermediates or hydrolytic products, exemplified by VIM-2:ANT431 (PDB ID 6HF5) and VIM-2:ImC-1 (PDB ID 7CHV) [87, 88]. (F) Class V inhibitors: these degrade the metal ion binding active sites [89, 90, 94, 95].

732