Bacterial Resistance

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Aspergillomarasmine A, an Inhibitor of Bacterial Metallo-β-Lactamases Conferring bla_{NDM} and bla_{VIM} Resistance**

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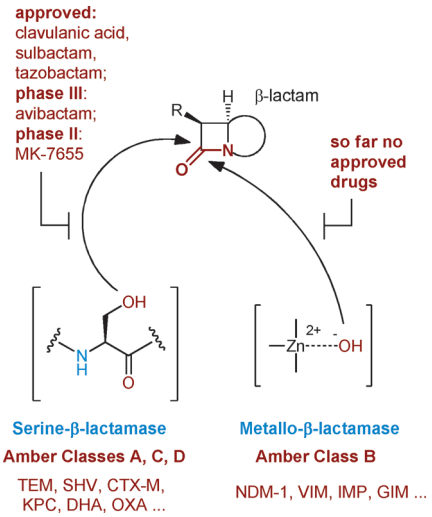
amino acids · antibiotics · lactams · natural products · zinc

Are we standing at the threshold of the post-antibiotic era?[1] A new WHO report states that the threat of antimicrobial resistance is "no more a prediction of the future". Instead it is "happening right now in every region of the world and has the potential to affect anybody".[2] The task for the scientific community is clear. We must come up with ways to tackle bacterial resistance fast; that is, with new biological targets and new antibiotic chemotypes.[3] Otherwise we risk a post-antibiotic era. The consequences would be serious.

Even seven decades after the introduction of penicillin G, the β-lactam class of antibiotics still represents the cornerstone for the treatment of serious infections caused by increasingly multidrug-resistant Gram-negative pathogens, for example, Enterobacteriaceae and nonfermenters like *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Their widespread medical use over decades has contributed to the evolution of several bacterial resistance mechanisms to β-lactams including efflux, reduced permeability, altered transpeptidase β-lactam target enzymes, and—most importantly—chemical inactivation by roughly a thousand β-lactamases (Scheme 1). The worldwide dissemination of extended-spectrum β-lactamases (ESBLs) has driven increased utilization of modern carbapenems which are ESBL-inert and possess a broad spectrum of activity against Gram-positive and Gram-negative bacteria. This in turn triggered the evolution of "superbugs" expressing carbapenem-degrading β-lactamases (carbapenemases).

Serine β-lactamases catalyze amide hydrolysis in the β-lactam ring resulting in ineffective open-chain products, via a covalently Ser-bound acyl-enzyme intermediate (Amber classes A, C, and D). Few drugs inhibiting serine-β-lactamases have reached patients (Scheme 1).[4]

Metallo-β-lactamases (MBLs) require zinc for activity and catalysis does not proceed via a covalent intermediate but rather through nucleophilic attack of an OH anion that is stabilized by zinc in the active site (class B).[5] Organisms



Scheme 1. Classification of β-lactamases.

producing MBLs are resistant to virtually all clinically used β-lactam antibiotics (Scheme 2). Medical need in this area is high as not a single drug fighting MBLs has been approved.

MBLs were discovered five decades ago[6] but initially were not considered to be a serious problem because they were found chromosomally encoded in nonpathogenic organisms. In the 1990s the situation changed with the spread of IMP-[7] and VIM-type[8] MBLs in Gram-negative pathogens. Now, IMP- and VIM-type genes reside with other resistance genes in genetically mobile integron structures that can insert into the bacterial chromosome or into plasmids.[9] Integrons are highly flexible vehicles that accelerate the genetic transfer of resistance determinants among different bacterial strains.

The recently discovered NDM-1 (New Delhi Metallo-β-lactamase-1) provides an alarming example of MBL dissemination. NDM-1 was discovered 2008 in a strain of *Klebsiella pneumoniae* from a Swedish patient who traveled to New Delhi where he acquired a urinary tract infection.[10] The original organism was found to be resistant to all antimicrobial agents tested, except colistin. Detailed analysis further corroborated the remarkable promiscuity of the bla_{NDM-1} plasmids.[11] Since then bla_{NDM-1} has been rapidly spreading

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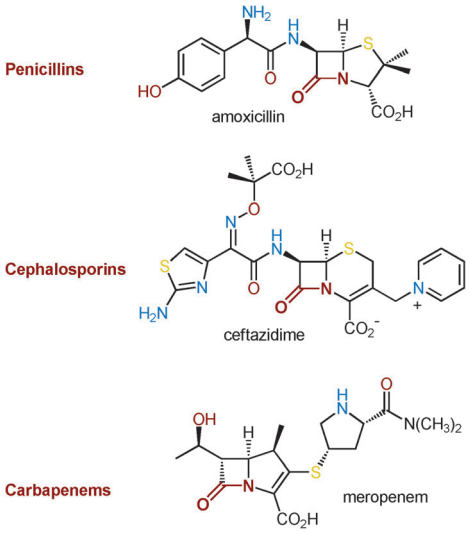


Scheme 2. β-Lactam drug classes affected by MBLs.

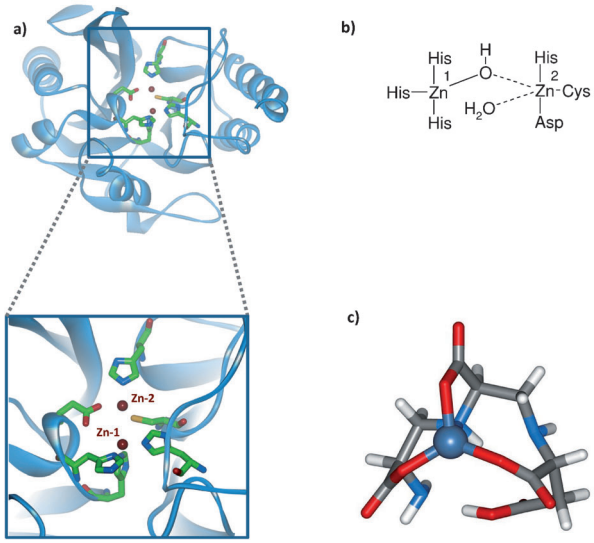


Figure 1. Apo structure[12] of NDM-1 with both Zn^{2+} cations: a) ribbon representation without water molecules and hydroxide ions; b) active site (schematic); c) modeled anionic complex of Zn^{2+} with aspergillomarasminate A (energy-minimized, DFT B3LYP/6-31+G**).

globally. Moreover, NDM-1 has been found in nearly all clinical species of Enterobacteriaceae, *A. baumannii* and *Pseudomonas* spp. indicating the ease with which bla_{NDM-1} plasmids transcend the genus/family barrier.[11] Importantly, the sequencing of bla_{NDM-1} plasmids led to the identification of up to 14 other antibiotic resistance genes, leaving the recipient bacterium susceptible to only tigecycline and colistin.[11] In summary, the severe multidrug-resistant (MDR) phenotype conferred by easily disseminating bla_{NDM-1} plasmids is a serious threat to public health.

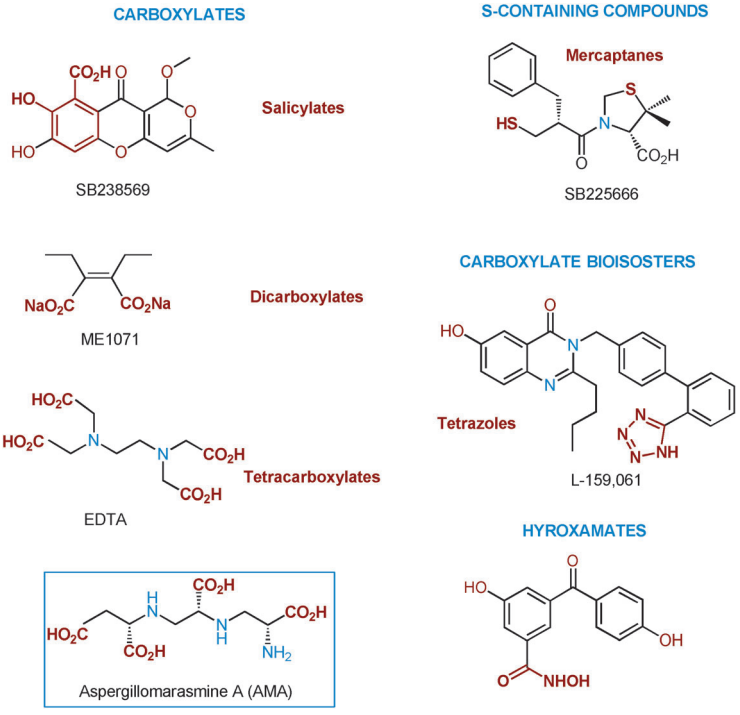
All MBLs are Zn-carrying enzymes. The active site is a dinuclear or a mononuclear zinc site. Ultimately, a Zn^{2+}-bound OH anion is assumed to cleave the β-lactam ring by nucleophilic attack. MBLs are assigned to three subclasses B1, B2, and B3. The sequence identity is high within subclasses but low between them (<20%). Subclass B1 contains most known MBLs including IMP, VIM, and NDM. Subclass B1 enzymes have a dinuclear zinc-containing active site. For optimal turnover both zinc positions are occupied and bridged with the μ-OH. The first zinc site is coordinated by three His side chains, the second by a Asp-Cys-His triad (Figure 1).

Though the search for efficient MBL inhibitors has been in progress for more than a decade, it was not particularly successful. Convincing proof for efficacy in meaningful in vivo models is still rare for MBL inhibitors. Only the dicarboxylate ME-1071 (**5**) has reached the clinic so far, where it has been developed up to phase I as a parenteral drug against MBL-producing bacteria.

All antagonists of MBLs described so far target the active site with varying zinc-binding motifs.[13] Most prominent are carboxylates and sulfur-containing groups (Scheme 3). X-ray analyses show that either the sulfur atom or the carboxylate can kick out water and bridge the two zinc atoms.

In the interest of selectivity and tolerability a good NDM-1 inhibitor should address not only the functional zinc site but also the surrounding amino acids that are structurally different from those of other zinc-containing enzymes. However, selectivity comes at a price. It will lower the chance of a tolerable but "broad-spectrum" MBL inhibitor.

In a recent paper in *Nature* King et al. described the discovery of AMA as an inactivator of NDM-1 and VIM-2 via a cell-based screen carried out with a collection of natural



Scheme 3. Examples of MBL inhibitors.

products.[1] In the development of a test organism, the bla_{NDM-1} β-lactamase gene conferring meropenem resistance was integrated into a sensitized *Escherichia coli* strain by virtue of its increased permeability and reduced efflux. Subsequently, the meropenem susceptibility of this *E. coli* test strain was screened in the presence of test compounds. Only one extract—stemming from *Aspergillus versicolor*—could restore the antibiotic activity of meropenem. Activity-guided purification yielded the known natural product AMA. However, AMA thus far has not been described as an inhibitor of MBLs.

In a biochemical assay AMA inhibited NDM-1 and VIM-2 by 50% at single-digit μM concentrations. A thorough study on the mode of action (MoA) indicated that AMA affects NDM-bound zinc. However, despite displaying a seemingly unspecific inactivation mechanism whereby AMA removes Zn^{2+} similar to known chelators like EDTA,[14] AMA turned out to be potent only against selected representatives of subclass B1, that is, NDM-1 and VIM-2, whereas it showed little carbapenem potentiation against Gram-negative pathogens expressing other members of this subclass like SPM-1 and IMP.

In vitro studies showed that meropenem alone was basically inactive against carbapenem-resistant strains (Table 1). However, in the presence of AMA the activity of meropenem could be restored to a high degree versus bla_{VIM}- and bla_{NDM}-harboring *Pseudomonas* spp., *Actinobacter* spp., and Enterobacteriaceae.

Table 1: AMA reverses meropenem resistance in NDM-1 producing bacteria.

Antibiotic	MIC [μg mL^{-1}][a]	Effect
meropenem	32	inactive
AMA	>128	inactive
meropenem @ 8 μg mL^{-1} AMA	1	"reversed resistance"

[a] Minimal inhibitory concentration versus the NDM-1-positive *K. pneumoniae* N11-2218 strain.[1]

In vitro potency nicely translated into in vivo efficacy. As expected, a lethal NDM-1-positive *K. pneumoniae* infection could not be treated with meropenem or AMA alone. However, when a combination of meropenem (10 mg kg^{-1}) and AMA (30 mg kg^{-1}) was administered subcutaneously, promising survival was observed (>90% for 4 days).

AMA is a new chemotype on the rocky road to clinically meaningful MBL inhibitors. Clearly, the selectivity for NDM-1 and VIM-2 restricts the value of AMA as a pan-MBL lead structure. However, it is also good news as it hints at potentially more specific enzyme binding rather than merely unspecific zinc chelation, such as one would expect for EDTA. Indeed, AMA seems to have lower acute toxicity than EDTA in mice.

Potential side effects have to be taken into consideration early on with a metal-ion-chelating MoA. In principal, direct complexation of Zn^{2+} is feasible with AMA based on modeling (Figure 1). AMA seems to be biochemically equipotent versus NDM-1 (IC_{50} = 4.0 μM) and zinc-binding "off-targets" such as endothelin-converting enzyme (IC_{50} = 3.4 μM) and angiotensin-converting enzyme (IC_{50} 1.2 μM).[15] Further exploration of selectivity will be crucial. Close analogues should be synthesized and tested to outline a structure–activity relationship.

So far, relatively high doses of AMA are needed in vivo to reverse carbapenem resistance. To which extent pharmacokinetic limitations play a role for the highly polar amphoteric structure of AMA is not clear (calc. Log$D_{7.5} \approx -4$).

In summary, King et al.[1] provided an important and most thorough piece of experimental work that should motivate researchers in antibacterials to approach MBLs with new screens and new lead structures.

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