Metallo-β-lactamases

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Chapter 3.2

Metallo-β-lactamases

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Abstract:

Metallo-β-lactamases (MBLs) are a class of enzymes that are produced by a variety of bacterial species and are responsible for conferring resistance to a broad range of β-lactam antibiotics, including carbapenems, which are often considered the "last line of defense" against antibiotic-resistant infections. The spread of MBL-producing organisms and the limited treatment options available for infections caused by these organisms represent serious challenges that they pose to public health. The chapter will cover the current state of research on MBLs, including the structure-function relationship, the genetic diversity, the mechanism of resistance and the development of inhibitors in this field.

Key Words:

metallo-β-lactamases ; carbapenemases ; inhibitors ; antibiotic resistance ; Gram-negative bacteria

[H1 Introduction]

Metallo-β-lactamases (MBLs) are a major public health concern as they are able to hydrolyze most β-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, leading to treatment failure and increased morbidity and mortality. These enzymes have been identified in a wide range of Gram-negative and Gram-positive bacteria, and have been reported in many parts of the world. The emergence and spread of MBL-producing bacteria have led to a rise in treatment-resistant infections and a decrease in the effectiveness of antibiotics.

β-Lactamases can be classified in two different ways, according to their sequence similarity (molecular classification) or to the activity profile (functional classification). The former is known as Ambler classification (1, 2) and organizes the β-lactamases in classes A, C and D, which are serine-β-lactamases (SBLs), and class B, which are MBLs. The MBLs are further organized in three subclasses, B1, B2 and B3 (Figure 3.2.1). The latter is a functional classification (3, 4), based on the specific hydrolysis profile against different β-lactam substrates.

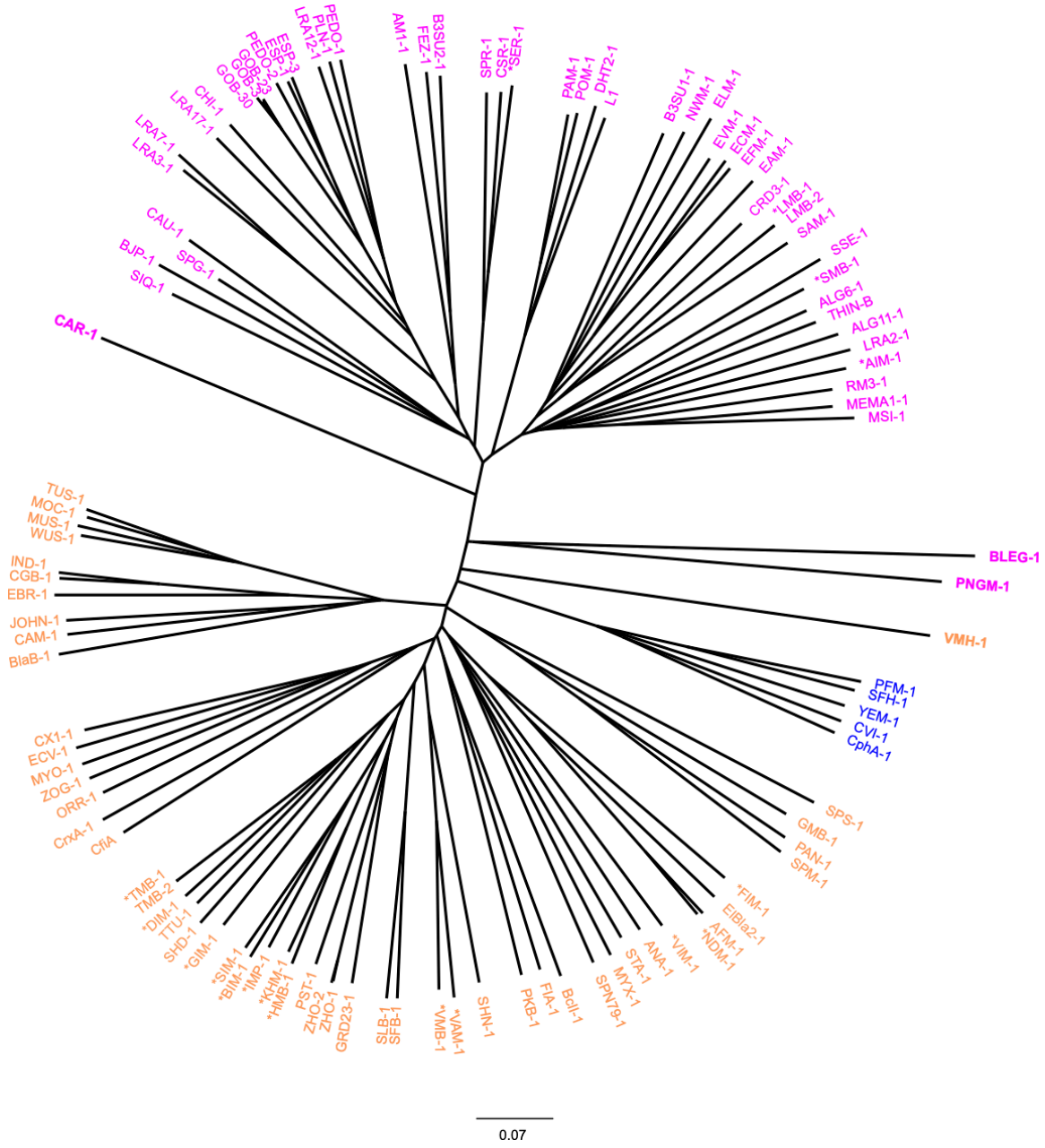
High quality and comprehensive reviews focused on clinically relevant β-lactamases were published over the years, with a special focus on carbapenemases (4-11) or on MBLs (12-27).

[H1 Structure and function of metallo-β-lactamases]

[H2 Phylogenetic comparison and evolution]

The MBLs are organized phylogenically in three distinct clusters, which correspond to subclasses B1, B2 and B3 (Figure 3.2.1). Subclasses B1 and B2 form a distinct phylogenic group (28), separated from the cluster B3 by an intermediary region with enzymes possessing variable active sites and mixed substrate hydrolysis profiles (Figure 3.2.1). A phylogenetic comparison of all members of the MBL superfamily suggested that the β-lactamase activity appeared and evolved separately in the B1+B2 and B3 groups (27, 29). A recent study evidenced the evolutionary traits acquired by several clinically-relevant MBLs in order to adapt to their native environment, depending if they are soluble or membrane-bound proteins. An important factor influencing this evolution is the amount of metal available in the cell under zinc starvation conditions (30).

An *in-silico* analysis based on protein sequence similarity networks (SSNs) applied to the superfamily of MBLs, combined with traditional cladistic and phenetic phylogenetic analysis, evidenced the zinc-coordinating motifs that are specific for each enzymatic reaction. Additionally, the local network interconnectivity was measured using the neighborhood connectivity coloring to detect new protein families within the SSN clusters. This approach evidenced the important number of unexplored protein families in this superfamily (31).



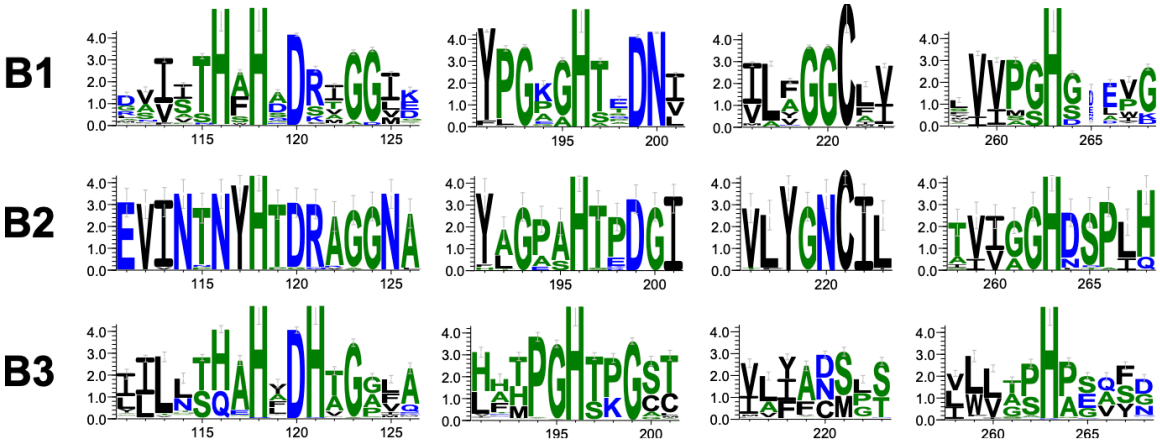
*** Insert Figure 3.2.1 ***

Caption: Phylogenetic tree of MBLs. The enzymes belonging to subclasses B1, B2 and B3 are colored in orange, blue and magenta, respectively. The plasmidic MBLs are labeled with a *, and those situated in the intermediary region are highlighted in bold. The tree was generated using SeaView (32) and the phylogram generated using FigTree (version 1.4.3).

Credit: The authors (Elsa Denakpo, Guillaume Arlet, Alain Philippon, Bogdan I. Iorga)

[H2 Primary structure/sequence analysis]

The sequences of MBLs are not well conserved, with sequence identities as low as 10 % for the most distant members. The most conserved residues are those coordinating the zinc ions (Figure 3.2.2). The B1 subclass is characterized by the motifs ^{116}HxHxDx, ^{196}H, ^{221}C and ^{263}H. The motifs present in the subclass B2 are very similar, differing only at the residue in position 116: ^{116}NxHxDx, ^{196}H, ^{221}C and ^{263}H. The differences are more significant in the subclass B3. First, the Cys residue in position 221 is absent, and replaced by a His in position 121. Second, most sequences have the motif ^{116}HxHxDH, but there are some notable exceptions, with some different motifs: ^{116}QxHxDH (GOB, CPS, ESP, PLN, SIQ, LRA12, LRA17, LRA19 and PEDO-1/2 families), ^{116}ExHxDH (SIE and SSE families) and ^{116}HxRxDQ (SPR, CSR and SER families). For the B3 subclass it was proposed to use the active site amino acid changes to distinguish the variants (i.e., B3-RQK, B3-Q, B3-E) (33).



*** Insert Figure 3.2.2 ***

Caption: Residue conservation in the sequences of MBLs in the regions neighboring the zinc-coordinating residues (positions 116, 118, 120, 196, 221 and 263 for subclasses B1 and B2, and positions 116, 118, 120, 121, 196 and 263 for subclass B3). The image was generated with WebLogo version 3 (34).

Credit: The authors (Elsa Denakpo, Guillaume Arlet, Alain Philippon, Bogdan I. Iorga)

A standard numbering scheme for MBLs (named BBL) was developed in 2001 and updated in 2004 (35, 36). This scheme is based on the structural alignment of representative members of each subclass and allows the comparison of residues from equivalent positions, which otherwise would have different numbers, specific to each family. The representative MBLs from each subclass (B1, B2 and B3) are presented in Table 1.

[H2 Mutations and spectrum of activity]

Historically, the class A molecular β-lactamases, particularly TEM enzymes, constituted an excellent model of molecular evolution when the first third-generation cephalosporins (ceftazidime and cefotaxime) were introduced into clinical practice in the 1980s. The substitution of certain amino acids in ESBLs (Extended-Spectrum Beta-Lactamases) such as Glu104Lys, Arg164Ser/His, Ala237Thr, Gly238Ser, Glu240Lys/Arg led to the extension of the spectrum due to an improved affinity (37, 38). On the other hand, other substitutions (Met69Leu/Val/Ile, Trp165Arg, Met182Thr, Arg244Cys/Ser/Thr, Val261Ile, Arg275Leu/Gln, Asn276Asp) in the same type of enzyme had an opposite effect, namely a decrease in affinity and therefore resistance to inhibitors such as clavulanic acid and sulbactam (defined as IRT, Inhibitor Resistant TEM) (39).

The evolutionary potential of MBLs appears to be less significant if judged by the extended inactivation spectrum of these enzymes with respect to β-lactams (except for monobactams such as aztreonam), including carbapenems and cefepime, and on the other hand, by a smaller number of variants, especially in transferable MBLs such as IMP, NDM and VIM enzymes, which are mainly involved in clinical medicine. Thus, 249 variants have been identified for TEM type enzymes, 48 for NDM, 83 for VIM, and 98 for IMP (40). Protein sequence heterogeneity is the lowest in the NDM family, ranging from 74 % to 100 %, and several substitution locations (one to three amino acids) have been identified without notable differences in β-lactam resistance levels and kinetic constants (P28A, V88L, D95N, D130G, E152K, M154L, G222D, R264H). Several substitution locations have also been evaluated by directed mutagenesis as indicated in Table 2 for several NDM type enzymes, allowing for a better understanding of this enzyme family, including the importance of some amino acids away from the active site (41-46). It is important to emphasize the absence of mutations that generate a higher level of resistance, especially with respect to carbapenems or aztreonam or cefepime.

Regarding the IMP family, protein heterogeneity ranges from 80 % to 100 %, and several substitutions (in positions 59, 67, 115, 119, 120, 121, 218, 233, 262) have been identified without notable differences in resistance to β-lactams, including carbapenems (47-52).

Several sites tested by directed mutagenesis were also evaluated (Table 3). The potential for expanding the spectrum remains limited again in the absence of mutants generating a higher level of resistance, particularly against carbapenems or aztreonam (47, 53-56).

The VIM family consists of 80 variants, with a sequence identity ranging from 75 % to 100 % and which can be grouped in at least three clusters, including two dominant ones. The

evolutionary potential of this family towards increased resistance to some β-lactams is again limited. This is true for the naturally occurring mutants, which are observed in clinic, but also for synthetic mutants obtained through directed mutagenesis (57-66).

The hyperproduction of the enzyme induced by a strong promoter, obtained by mutation, is a well-known mechanism reported in molecular classes A and C β-lactamases. However, this mechanism remains little described in transferable MBLs (e.g. IMP, NDM, or VIM) (67-73).

Table 1. Representative class B β-lactamases

Sub-class	Metallo-β-lactamase	Origin of name[a]	Accession	Gene[b]	Organism	Number of residues	References
B1	AFM-1	*Alcaligenes faecalis* Metallo-β-lactamase	AYV97588	P	*Alcaligenes faecalis*	267	(74)
B1	ANA-1	*Anaeromyxobacter* sp.	WP_041449074	CHR	*Anaeromyxobacter* sp.	255	(75)
B1	BcII-1	*Bacillus cereus* type II	AAA22276	CHR	*Bacillus cereus*	257	(76)
B1	BIM-1	Belem imipenemase	ANY85569	In	*Pseudomonas putida*	245	–
B1	BlaB-1	Beta-lactamase class B	AAF89154	CHR	*Elizabethkingia meningoseptica*	249	(77)
B1	CAM-1	Central Alberta Metallo-β-lactamase	AVX51087	CHR	*Pseudomonas aeruginosa*	241	(78)
B1	CEMC19-1	Name of clone	Name of clone	CHR	Uncultured bacteria	252	(79)
B1	CfiA	Cefoxitin-imipenem-hydrolyzing enzyme	AAA22907	CHR	*Bacteroides fragilis*	249	(80, 81)
B1	CGB-1	*Chryseobacterium gleum* class B	AAL55263	CHR	*Chryseobacterium gleum*	242	(82)
B1	CrxA-1	Carbapenem resistant *B. xylanisolvens*	MBS5055441		*Bacteroides xylanisolvens*	250	(83)
B1	CX1-1	Name of clone	AGU01687	CHR	Uncultured bacteria	246	(84)
B1	DIM-1	Dutch imipenemase	AGC92784	P, In	*Pseudomonas stutzeri*	251	(85)
B1	EBR-1	*Empedobacter brevis* resistant	AAN32638	CHR	*Empedobacter brevis*	235	(86)
B1	ECV-1	*Echinicola vietnamensis*	AGA78874	CHR	*Echinicola vietnamensis*	257	(75)
B1	ElBla2	*Erythrobacter litoralis* Beta-lactamase 2	ABC63608	CHR	*Erythrobacter litoralis*	261	(87)
B1	FIA-1	*Fibrella aestuarina*	WP_041258349	CHR	*Fibrella aestuarina*	249	(75)
B1	FIM-1	Florence imipenemase	AFV91534	ISCR	*Pseudomonas aeruginosa*	262	(88)
B1	GIM-1	German imipenemase	ALO69078	P, In	*Pseudomonas aeruginosa*	250	(89)

B1	GMB-1	German Metallo-Beta-lactamase	QKF95688	CHR	*Citrobacter freundii*	270	(90)
B1	GRD23-1	Name of clone	APR64493	CHR	Uncultured bacteria	241	(91)
B1	HMB-1	Hamburg Metallo-Beta-lactamase	AMY61250	Tn	*Pseudomonas aeruginosa*	241	(92)
B1	IMP-1	Imipenem-hydrolyzing β-lactamase	ABK27309	Tn, IS	*Serratia marcescens*	246	(93)
B1	IND-1	*Chryseobacterium indologenes*	AAD20273	CHR	*Chryseobacterium indologenes*	239	(94)
B1	JOHN-1	*Flavobacterium johnsoniae*	AAK38324	CHR	*Flavobacterium johnsoniae*	248	(95)
B1	KHM-1	Kyorin Health Science MBL1	BAF91108	P	*Citrobacter freundii*	241	(96)
B1	MOC-1	*Myroides odoratus* Carbapenemase	ANJ59787	CHR	*Myroides odoratus*	248	–
B1	MUS-1	*Myroides odoratimimus*	AAN63647	CHR	*Myroides odoratimimus*	246	(97)
B1	MYO-1	*Myroides odoratimimus*	WP_081048762	CHR	*Myroides odoratimimus*	266	(75)
B1	MYX-1	*Myxococcus xanthus*	ABF86854	CHR	*Myxococcus xanthus*	261	(75)
B1	NDM-1	New Delhi Metallo-β-lactamase	AHM26723	P, In	*Klebsiella pneumoniae*	270	(98)
B1	ORR-1	*Ornithobacterium rhinotracheale*	WP_109545042	CHR	*Ornithobacterium rhinotracheale*	255	(75)
B1	PAN-1	*Pseudobacteriovorax antillogorgiicola*	SMF47935	CHR	*Pseudobacteriovorax antillogorgiicola*	270	(99)
B1	PEDO-3	*Pedobacter roseus*	AJP77076	CHR	*Pedobacter kyungheensis*	237	(100)
B1	PST-1	*Pseudomonas stutzeri*	WP_043942497	CHR	*Stutzerimonas stutzeri*	242	(75)
B1	SFB-1	*Shewanella frigidimarina* Beta-lactamase	AAT90847	CHR	*Shewanella frigidimarina*	244	(101)
B1	SHD-1	*Shewanella denitrificans*	ABE54111	CHR	*Shewanella denitrificans*	269	(75)
B1	SHN-1	*Shewanella denitrificans*	ABE56430	CHR	*Shewanella denitrificans*	237	(75)
B1	SIM-1	Seoul imipenemase	AER61546	In	*Acinetobacter baumannii*	246	(102)
B1	SLB-1	*Shewanella livingstonensis* Beta-lactamase	AAT90846	CHR	*Shewanella livingstonensis*	249	(101)
B1	SPM-1	Sao Paulo Metallo-β-lactamase	AAR15341	Tn	*Pseudomonas aeruginosa*	276	(103)
B1	SPN79-1	Name of clone	APR64486	CHR	*Phenylobacterium*/Proteobacteria	278	(91)
B1	SPS-1	*Sediminispirochaeta smaragdinae*	ADK81930	CHR	*Sediminispirochaeta smaragdinae*	276	(75)

B1	STA-1	*Stigmatella aurantiaca*	WP_109545039	CHR	*Stigmatella aurantiaca*	261	(75)
B1	SZM-1	Shenzhen Metallo-β-lactamase	JAPDUI000000000*	CHR	*Arenimonas* metagenome	231	(104)
B1	TTU-1	*Teredinibacter turnerae*	ACR12883	CHR	*Teredinibacter turnerae*	243	(75)
B1	TMB-1	Tripoli Metallo-Beta-lactamase	CBY88906	In	*Achromobacter xylosoxidans*	245	(105)
B1	TMB-2	Tripoli Metallo-Beta-lactamase	BAM73613	CHR	*Acinetobacter courvalinii*	245	(106)
B1	TUS-1	*Myroides odoratus*	EKB08120	CHR	*Myroides odoratus*	248	(97)
B1	VAM-1	*Vibrio alginolyticus* Metallo-β-lactamase	QTJ60982	P,In	*Vibrio alginolyticus*	246	(107, 108)
B1	VIM-1	Verona Imipenemase	CAC35170	In	*Pseudomonas aeruginosa/A.denitificans*	266	(109)
B1	VMB-1	*Vibrio* Metallo-Beta-lactamase	QGQ32905	In	*Vibrio alginolyticus*	247	(110)
B1	VMH-1	*Vibrio* MetalloHydrolase	ASM98011	CHR	*Vibrio vulnificus*	281	(111)
B1	WUS-1	*Wenzhou Medical University*	UPP01678	CHR	*Myroides albus*	246	(112)
B1	ZHO-1	*Zhongshania aliphaticivorans*	WP_082793656	CHR	*Zhongshania aliphaticivorans*	246	(113)
B1	ZOG-1	*Zobellia galactanivorans*	CAZ94871	CHR	*Zobellia galactanivorans*	247	(75)
B2	CphA	Carbapenem-hydrolyzing Metallo-β-lactamase	CAA40386	CHR	*Aeromonas hydrophila*	254	(114)
B2	ImiS (CphA-11)	Imipenemase *Aeromonas veronii* bv. sobria	CAA71441	CHR	*Aeromonas veronii* bv. sobria	255	(115)
B2	ImiH (CphA-13)	Imipenemase *Aeromonas hydrophila*	CAD69003	CHR	*Aeromonas hydrophila*	254	(116)
B2	CVI-1	*Chromobacterium violaceum*	WP_164461288	CHR	*Chromobacterium violaceum*	255	–
B2	PFM-1	*Pseudomonas fluorescens* Metallo-β-lactamase	QDC33502	CHR	*Pseudomonas fluorescens complex*	253	(117)
B2	SFH-1	*Serratia fonticola* carbapenem hydrolase	WP_024531368	CHR	*Serratia fonticola*	255	(118)
B2	YEM-1	*Yersinia mollaretii*	CQH06348	CHR	*Yersinia mollaretii*	246	(119)
B3	AIM-1	Adelaide imipenemase	CAQ53840	IS	*Pseudomonas aeruginosa*	303	(120)
B3	ALG6-1	Name of clone	APR64488	CHR	Uncultured bacterium	318	(91)

B3	ALG11-1	Name of clone	APR64489	CHR	Uncultured bacterium	295	(91)
B3	AM1-1	Name of clone	AGU01669	CHR	Uncultured bacterium	311	(84)
B3	BJP-1	*Bradyrhizobium japonicum*	BAC51495	CHR	*Bradyrhizobium diazoefficiens*	294	(121)
B3	BLEG-1	*Bacillus lehensis* strain G1	AIC95013	CHR	*Alkalihalobacillus lehensis*	428	(122)
B3	CAR-1	*Erwinia carotovora*	AIA71664	CHR	*Pectobacterium carotovorum*	342	(123)
B3	CAU-1	*Caulobacter crescentus*	CAC87665	CHR	*Caulobacter crescentus*	289	(124)
B3	CPS-1	*Chryseobacterium piscium*	AJP77054	CHR	*Chryseobacterium piscium*	290	(100)
B3	CRD3-1	Name of clone	APR64487	CHR	Uncultured bacterium	303	(91)
B3	CSR-1	*Cronobacter sakazakii* resistant	AKE96626	CHR	*Cronobacter sakazakii*	318	(33)
B3	DHT2-1	Name of clone	APR64485	CHR	Uncultured bacterium	302	(91)
B3	EAM-1	*Erythrobacter aquimaris* Metallo-β-lactamase	AFN85388	CHR	*Erythrobacter aquimaris*	318	(125)
B3	ECM-1	*Erythrobacter citreus* Metallo-β-lactamase	WP_122630824	CHR	*Erythrobacter citreus*	303	(125)
B3	EFM-1	*Erythrobacter flavus* Metallo-β-lactamase	AFN85384	CHR	*Erythrobacter flavus*	302	(125)
B3	ELM-1	*Erythrobacter longus* Metallo-β-lactamase	AFN85386	CHR	*Erythrobacter longus*	313	(125)
B3	ESP-1	*Epilithonimonas* sp.	AJP77085	CHR	*Epilithonimonas tenax*	290	(100)
B3	EVM-1	*Erythrobacter vulgaris* Metallo-β-lactamase	AFN85385	CHR	Qipengyuania *vulgaris*	305	(125)
B3	FEZ-1	*Fluoribacter gormanii* endogenous Zn β-lactamase	CAB96921	CHR	*Fluoribacter gormanii*	282	(126)
B3	GOB-1	*Elizabethkingia meningoseptica* class B	AAF04458	CHR	*Elizabethkingia meningoseptica*	290	(127)
B3	L1	Labile β-lactamase from *S. maltophilia*	CAA52968	CHR	*Stenotrophomonas maltophilia*	290	(128)
B3	LMB-1	Linz Metallo-Beta-lactamase	AMK49163	P	*Enterobacter cloacae*	304	(129, 130)
B3	LMB-2	Linz Metallo-Beta-lactamase	SEI10464	CHR	*Rheinheimera pacifica*	304	–
B3	LRA2-1	Beta-lactam resistance from Alaska	ACH58985	CHR	Uncultured bacterium	302	(131)

B3	LRA3-1	Beta-lactam resistance from Alaska	ACH58987	CHR	Uncultured bacterium	302	(131)
B3	LRA7-1	Beta-lactam resistance from Alaska	ACH58998	CHR	Uncultured bacterium	302	(131)
B3	LRA8-1	Beta-lactam resistance from Alaska	ACH58988	CHR	Uncultured bacterium	305	(131, 132)
B3	LRA12-1	Beta-lactam resistance from Alaska	ACH58990	CHR	Uncultured bacterium	293	(131)
B3	LRA17-1	Beta-lactam resistance from Alaska	ACH58994	CHR	Uncultured bacterium	294	(131)
B3	LRA19-1	Beta-lactam resistance from Alaska	ACH59005	CHR	Uncultured bacterium	300	(131)
B3	MEMA1-1	Meropenem resistance A1	KY705336*	CHR	Uncultured bacterium	285	(133)
B3	MIM-1	Maynooth Imipenemase-1	AIT78529	CHR	*Novosphingobium pentaromativorans*	299	(134)
B3	MSI-1	*Massilia* sp.	AJP77057	CHR	*Massilia oculi*	341	(100)
B3	NWM-1	North Rhine-Westphalia Metallo-β-lactamase	QXM27671	CHR	*Pseudomonas aeruginosa*	310	–
B3	PAM-1	*Pseudomonas alcaligenes* Metallo-β-lactamase	BAO01151	CHR	*Pseudomonas alcaligenes*	287	(135)
B3	PAM-2	*Pseudomonas alcaligenes* Metallo-β-lactamase	BCG24254	CHR	*Pseudomonas tohonis*	287	(136)
B3	PEDO-1	*Pedobacter* sp.	AJP77059	CHR	*Pedobacter roseus*	286	(100)
B3	PEDO-2	*Pedobacter* sp.	AJP77071	CHR	*Pedobacter borealis*	285	(100)
B3	PJM-1	*Pseudoxanthomonas japonensis* Metallo-β-lactamase	WP_213603971	CHR	*Pseudoxanthomonas japonensis*	301	(137)
B3	PLN-1	*Pedobacter lusitanus*	KIO75746	CHR	*Pedobacter lusitanus*	289	(138)
B3	POM-1	*Pseudomonas otitidis* Metallo-β-lactamase	ABY56045	CHR	*Pseudomonas otitidis*	286	(139)
B3	PNGM-1	Papua New Guinea Metallo-β-lactamase	AWN09461	CHR	Uncultured bacterium	386	(140)
B3	RM3-1	Name of clone	AGU01679	CHR	Uncultured bacterium	302	(84, 141)
B3	SAM-1	*Simiduia agarivorans* Metallo-β-lactamase	AFV00127	CHR	*Simiduia agarivorans*	295	(134)
B3	SER-1	*Salmonella enterica*	APY61104	?	*Salmonella enterica* ser Hillingdon	294	(33)

B3	SIE-1	*Sphingobium indicum* B3-E	KEY99315	CHR	*Sphingobium indicum*	307	(142)
B3	SIQ-1	*Sphingobium indicum* B3-Q	SMF77924	CHR	*Allosphingosinicella indica*	290	(33)
B3	SMB-1	*Serratia* Metallo-Beta-lactamase	BAL14456	IS	*Serratia marcescens*	280	(143)
B3	SPG-1	*Sphingomonas* sp.	AJP77080	CHR	*Sphingomonas* sp.	285	(100)
B3	SPR-1	*Serratia proteamaculans*	ABV42357	CHR	*Serratia proteamaculans*	298	(144)
B3	SSE-1		SBV32245	CHR	Uncultured *Sphingopyxis sp.*	288	(33)
B3	B3SU1-1	Sub-class B3 and author	QWJ89341	CHR	Uncultured bacterium	303	–
B3	B3SU2-1	Sub-class B3 and author	QWJ89342	CHR	Uncultured bacterium	293	–
B3	THIN-B	*Janthinobacterium lividum* class B	CAC33832	CHR	*Janthinobacterium lividum*	316	(145)

[a] The underlined letters form the name of the β-lactamase

[b] Chr, Chromosome; In, integron; IS, insertion sequence; P, plasmid; Tn, transposon; –, unknown.

* No GenBank accession available.

Table 2. Synthetic mutants of NDM-1 : bacteriological (MIC) and enzymatic (kinetic constants) changes

Mutation	**MIC[a] (µg.ml^{-1})**						**K_m (µM) ; k_{cat} (s^{-1}) ; k_{cat}/K_m ($\mu M^{-1}.s^{-1}$)**						**Reference**
	AMP[b]	**FOT[b]**	**CAZ[b]**	**FOX[b]**	**IMP[b]**	**MER[b]**	**AMP[b]**	**FOT[b]**	**CAZ[b]**	**FOX[b]**	**IMP[b]**	**MER[b]**	
WT	4096	256			16		143 550 3.84	6 85 13.79			65 238 3.71		(46)
F64H	4096 → →	256→			16→		188 793 4.27	6 83 14.80			164 409 2.50		(46)
D84E	256↘	16↘			1↘		82 597	16 113			70 153		(46)

				7.29	6.77	2.20	
W87P	2048↘	32↘	2↘	518	6	295	(46)
				1823	46	351	
				3.53	8.50	1.20	
K121R	512↘	64↘	4↘	61	4	31	(46)
				26	13	36	
				0.43	3.69	1.20	
K121T	512↘	64↘	2↘	231	10	117	(46)
				246	50	50	
				1.06	5.13	0.43	
G195A	4096 →	256→	8↘	57	4	52	(46)
				248	32	94	
				4.43	7.74	1.84	
D199E	1024↘	64↘	2↘	11	6	6	(46)
				23	48	17	
				2.10	7.65	2.81	
K224H	256↘	128↘	0.5↘	906	26	ND	(46)
				330	111	ND	
				0.36	4.24	0.08	
						6	
K224R	2048↘	256→	4↘	179	3	69	(46)
				294	39	62	
				1.65	12.81	0.90	
D225E	4096 →	128↘	16→	41	4	50	(46)
				102	38	117	
				2.54	9.78	2.37	
G232A	2048↘	64↘	1↘	178	24	ND	(46)
				417	90	ND	
				2.39	3.80	0.63	
N233H	4096 →	256→	8↘	56	3	134	(46)
				591	55	286	
				10.58	20.42	2.22	
N233Q	2048↘	256→	2↘	160	1.1	565	(46)
				279	25	288	

							1.74	22.95			0.51		
WT	>256	>256	64	32	8	16	94.5 471 4.98	53 332 6.26	99.5 114 1.15	41.3 23 0.56	73.5 567 7.71	53.5 237 4.43	(41)
W93A	32↘	32↘	16↘	4↘	2↘	2↘	128.9 394 3.06	81.9 286 3.49	121.6 92 0.76	67 17 0.25	98.8 439 4.44	87.9 197 2.24	(41)
WT	>256					16	193 139 0.72					59 35 0.59	(45)
Y229W	128↘					8↘	297 253 0.85					268 435 1.62	(45)
WT		128	>128	8	>64	>64					35 64 1.83	80 75 0.94	(43, 44)
L209F		1↘	16↘	8→	0.25↘	<0.0625↘					33 0.55 0.01 7	50 0.86 0.017	(43, 44)
Y229W		>128→	>128→	8→	>64→	>64→					81 38 0.47	259 820 3.17	(44)
L209F/Y229W		32↘	32↘	4↘	64↘	>64→					120 55 0.46	88 208 2.36	(44)
V88L/M154L (NDM-5)	>500				>50	>50	117.5 1912 16				6.32 44 7	11.8 190.7 16.1	(42)
V88L/E152A/M154L	8↘				0.8↘	0.4↘	207.3 239 1.15				39.3 94 2.3	33.2 81.1 2.4	(42)

[a] The arrows represent the variation of CMI compared to WT.

[b] AMP : ampicillin ; FOT : cefotaxime ; CAZ : ceftazidime ; FOX : cefoxitin ; IMP : imipenem ; MER: meropenem

Table 3. Synthetic mutants of IMP-1 : bacteriological (MIC) and enzymatic (kinetic constants) changes

	MIC[a] (µg.ml^{-1})						K_m (µM) ; k_{cat} (s^{-1}) ; k_{cat}/K_m (µM^{-1}.s^{-1})						
Mutation	**AMP[b]**	**FOT[b]**	**CAZ[b]**	**FOX[b]**	**IMP[b]**	**MER[b]**	**AMP[b]**	**FOT[b]**	**CAZ[b]**	**FOX[b]**	**IMP[b]**	**MER[b]**	**Reference**
WT	128		256		2	2	300		16.7		27	9	(54)
							46		1.1		3.9	3.7	
							0.15		0.066		0.144	0.41	
S262G/E126G (IMP-3)	8↘		64↘		0.25↘	8↗							(54)
S262G (IMP-6)	8↘		64↘		0.25↘	4↗	300		91		170	6.5	(54)
							4.9		5.9		11.6	10	
							0.016		0.065		0.07	1.54	
V67F (IMP-10)	16↘		>512↗		4↘	8↗	ND		36		92	17	(54)
							ND		7.2		15.2	17.9	
							0.006		0.2		0.17	1.1	
S262G/G235S (IMP-25)	8↘		128↘		0.25↘	8↗							(54)
E59K (IMP-30)	64↘		256→		1↘	1↘							(54)
E126G (IMP-34)	128→		256→		2→	2→							(54)
V67F/F87S (IMP-40)	4↘		256→		4↗	8↗							(54)
G63R (IMP-42)	64↘		512↗		1↘	2→							(54)
L82I/V96G (IMP-52)	128→		256→		1↘	4↗							(54)
E207K (IMP-60)	64↘		128↘		0.5↘	0.5↘							(54)
N129I (IMP-61)	128→		256→		2→	4↗							(54)

V66F (IMP-66)	32↘	128↘	1↘	8↗							(54)
L304I (IMP-70)	128→	256→	2→	1↘							(54)
V67A (IMP-76)	8↘	128↘	0.5↘	8↗							(54)
V67F/L250S (IMP-77)	16↘	>512↗	2→	8↗							(54)
V67F/S262G (IMP-78)	4↘	128↘	0.25↘	32↗	600 2.3 0.004		132 20.5 0.16		270 20.3 0.075	20.2 24.8 1.23	(54)
K249R (IMP-79)	128→	256→	2→	4↗							(54)
V67F/S262A (IMP-80)	8↘	512↗	1↘	8↗							(54)
L82I	128→	256→	2→	2→							(54)
WT								1.6 12.7 8	19 41 2.2		(56)
K161R								480 28 0.57	>1000 >1 0.0013		(56)
K161A								800 9 0.011	>1000 >7.1 0.0077		(56)
K161E								1100 1.3 0.003	>1000 >2.8 0.0045		(56)
N167A								33 90 2.7	>1000 >140 0.34		(56)
N167D								77 38 0.5	>1000 >100 0.16		(56)
WT	125	250	3		97 62 0.64	0.98 16 17			59 98 1.7		(47)

N233A	31↘	63↘	0.25↘	110 230 2.1	30 495 16	>1000 >970 0.97	(47)
N233C	<2↘	<2↘	0.25↘	1098 9.1 0.0083	76 51 0.67	>2000 >5.4 0.0027	(47)
N233D	250↗	500↗	3→				(47)
N233E	125→	250→	3→	92 416 4.5	8 34 4.3	178 111 0.62	(47)
N233F	125→	250→	3→				(47)
N233G	125→	500↗	0.38↘				(47)
N233H	125→	125↘	0.5↘				(47)
N233I	16↘	125↘	0.38↘				(47)
N233K	16↘	63↘	0.38↘	1445 132 0.091	98 595 6.1	>2000 >336 0.17	(47)
N233L	16↘	250→	0.5↘				(47)
N233M	16↘	500↗	0.75↘				(47)
N233P	125→	125↘	0.38↘				(47)
N233Q	125→	500↗	0.2↘				(47)
N233R	31↘	250→	0.38↘	501 83 0.17	73 391 5.3	>2000 >134 0.067	(47)
N233S	125→	500↗	1.5↘				(47)
N233T	16↘	500↗	0.38↘				(47)
N233V	31↘	500↗	0.38↘				(47)
N233W	250↗	63↘	1.5↘				(47)
N233Y	125→	125↘	3→				(47)

[a] The arrows represent the variation of CMI compared to WT.

[b] AMP : ampicillin ; FOT : cefotaxime ; CAZ : ceftazidime ; FOX : cefoxitin ; IMP : imipenem ; MER: meropenem

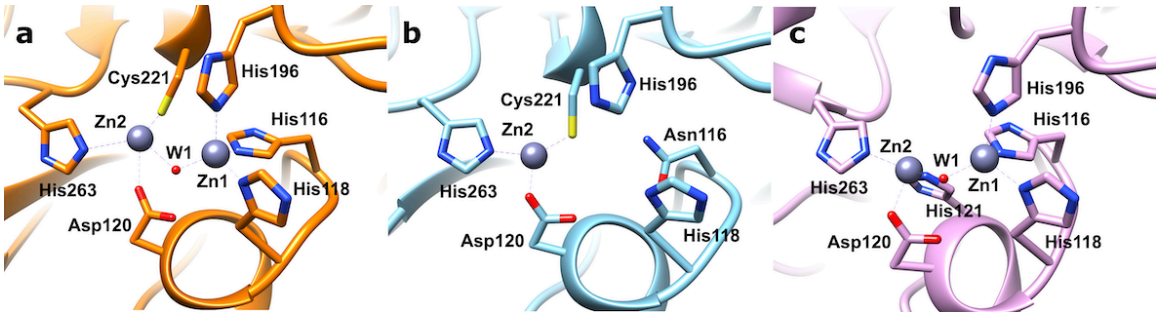
[H2 Secondary and tertiary structure]

The typical three-dimensional structure of class B β-lactamase contains a characteristic αβ/βα motif known as the MBL fold. Structurally, class B β-lactamases belong to the *Lactamase_B* PFAM family (PF00753) and share the same overall fold with many other enzymes that catalyze very different biochemical reactions (27). Indeed, MBLs are part of a larger group of binuclear metallohydrolases that require two closely spaced transition metal ions to carry out a great variety of hydrolytic reactions (146). It was proposed that MBLs present a modular structure in their active sites that can be dissected into two halves: one providing the attacking nucleophile, and the second one stabilizing a negatively charged reaction intermediate (147).

The X-ray structure of the MBL from *Bacillus cereus* BcII (strain 569/H/9) was published in 1995 (148), revealing a new fold. This represents the first structure of a protein from this family (subclass B1). In 1998 was published the first structure of a B3 subclass MBL (L1 from the opportunistic pathogen *Stenotrophomonas maltophilia*) (149) and in 2005 the first structure of a B3 subclass MBL (CphA from *Aeromonas hydrophila*) (150).

The active site is organized around one (subclass B2) or two (subclasses B1 and B3) zinc ions that coordinate six very conserved residues specific for each subclass (Figure 3.2.3). These zinc ions are essential for the enzymatic activity and play key roles in the catalytic mechanism, including facilitating nucleophilic attack on the amide carbonyl by the zinc-bound hydroxide ion, stabilizing the anionic tetrahedral intermediate and coordinating the departing amine nitrogen (151). In some cases, steric constraints imposed by the N-terminus may limit the affinity of the active site for β-lactams (33, 141).

Quantum mechanical and molecular dynamics simulations provided insight into the conformational flexibility of these enzymes, the mechanism for carbapenem hydrolysis and the binding mode of different MBL substrates and inhibitors (152-154).

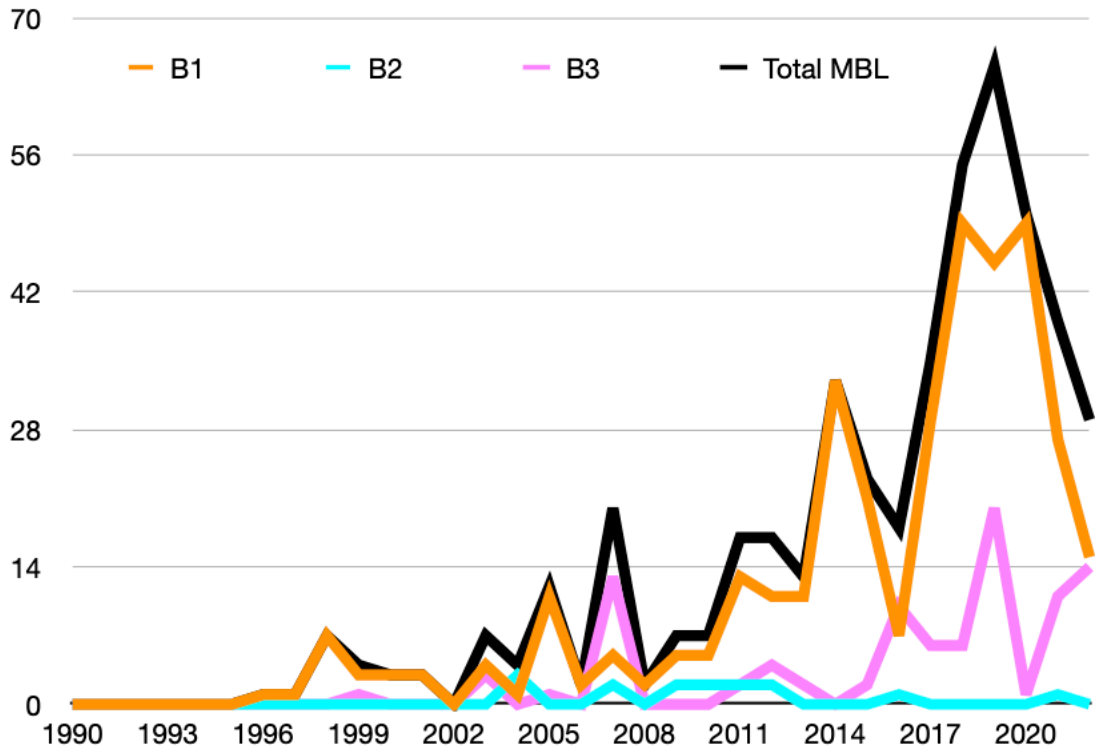


*** Insert Figure 3.2.3 ***

Caption: Active site residues coordinating the zinc ions in representative members of subclass B1 (**a**, BcII, orange), B2 (**b**, CphA, cyan) and B3 (**c**, L1, pink) MBLs. The zinc ions and the bridged water molecules are represented as gray and red spheres, respectively. The residues are numbered according to the BBL standard numbering scheme (35, 36).

Credit: The authors (Elsa Denakpo, Guillaume Arlet, Alain Philippon, Bogdan I. Iorga)

The number of MBL structures deposited in the Protein Data Bank (PDB) has increased significantly during the recent years, especially for the subclass B1 (Figure 3.2.4). This increase is likely due to the importance of structural studies to guide and accompany the synthetic efforts focused on the development of efficient inhibitors targeting clinically-relevant MBLs (e.g. NDM-1, VIM-1, IMP-1, all belonging to subclass B1).



*** Insert Figure 3.2.4 ***

Caption: Evolution of the number of MBL structures deposited in the Protein Data Bank (PDB) over the years. Data was extracted from the Beta-Lactamase DataBase (http://bldb.eu/BLDB/S-BLDB.php).

Credit: The authors (Elsa Denakpo, Guillaume Arlet, Alain Philippon, Bogdan I. Iorga)

[H1 Physiologic and pathologic role of metallo-β-lactamases]

[H2 Metallo-β-lactamases in clinical microbiology]

The emergence of these enzymes in human infections is the consequence of the use of carbapenems in therapy. Indeed, MBLs are able to hydrolyze all β-lactams except monobactams such as aztreonam. The transferable carbapenemases that currently predominate are KPCs (class A), OXA-48-like (class D) and MBLs (class B). Among these

MBLs, the most important ones belong to subclass B1, IMP (Imipenemase), VIM (Verona integron-encoded metallo-β-lactamase) and NDM (New Delhi metallo-β-lactamase) (155). IMP-1 was described in the 90s in Japan in *Pseudomonas aeruginosa* and *Serratia marcescens* and currently has more than 90 variants (http://bldb.eu/BLDB.php?prot=B1#IMP) (26, 40). The first VIM-like enzyme was described in Italy in 1997 in *P. aeruginosa* and more than 80 variants are currently reported (http://bldb.eu/BLDB.php?prot=B1#VIM) (26, 40). NDM-1 was identified in Sweden in 2008 (patient returning from India) in *Klebsiella pneumoniae* and more than 45 variants were characterized to date (http://bldb.eu/BLDB.php?prot=B1#NDM) (26, 40).

The IMP and VIM enzymes are encoded by gene cassettes in integrons which are usually integrated into transposons (156). The bla_{NDM} genes are carried by composite transposons like *Tn*125 bordered by insertion sequences, in particular *IS*Aba125, but multiple recombinations were subsequently observed (25).

While these enzymes have been described all over the world, it seems that IMP- and VIM-type enzymes are predominant in some specific countries: IMP in Japan, Australia and Thailand, and VIM in Greece, Italy and neighboring countries (26, 155).

NDM-like enzymes are endemic in India and Pakistan and neighboring countries. Since 2010, we assist to a worldwide dissemination of NDM-1 (24-26). These genes are carried by a wide variety of plasmids (both narrow and broad spectrum), and in particular from the IncX3 group (24, 25). On these plasmids, NDM enzymes are often associated with resistances to other families of antibiotics (aminoglycosides, fluoroquinolones) (24). The hosts harboring these plasmids are both Enterobacterales and particularly *E. coli* and *K.*

pneumoniae, as well as *P. aeruginosa* and *Acinetobacter baumannii*. In addition, NDM enzymes have been also reported in animals and in the environment (157).

[H2 Metallo-β-lactamases in veterinary medicine and environment]

Considering the animal ecosystem and the enzymatic carbapenem resistance (ECR) mediated by MBLs, numerous publications showed the implication of several transferable types such as IMP-1, NDM-5, VIM-1 and even SIM-1 produced by Enterobacterales (mainly *Escherichia coli*, *K. pneumoniae*, and *Salmonella enterica*), but also by *P. aeruginosa* and *Acinetobacter* spp. (158-165). Various animal species were implicated such as chickens, pigs, calves, boars, flies, birds and particularly animal companion (dogs, cats). Such enzymatic resistance is widespread in countries such as in Algeria, Australia, Brazil, China, Egypt, India, Japan, USA and in Europe. Considering that carbapenems are not authorized in veterinary medicine, such results indicate the importance of a rational use of antibiotics in this field and their prohibition as growth promoters. Nevertheless, the impact of animal reservoirs on human health still remains unclear and debatable; there are several examples of direct links suggesting a potential transmission way of the ECR plasmids between humans and companion animals (158, 166).

A wide range of Gram-negative bacteria issued from nonclinical samples, distributed in various ecosystems such as soil, sediments, sewage, tap water, river, sea water or costal mud, are able to naturally produce various MBLs (see Table 1 for representative examples). The phylum Bacteroidota (formely Bacteroidetes) is composed of several classes of bacteria that can be opportunistic pathogens, including the following genera: *Bacteroides*, *Chitinophaga*, *Chryseobacterium*, *Echinicola*, *Elisabethkingia*, *Empedobacter*,

Epilithonimonas, *Fibrella*, *Flavobacterium*, *Myroides*, *Myxococcus*, *Ornithobacterium*, *Pedobacter*, *Pontibacter*, *Sphingobacterium*, and *Zobellia*. Nevertheless, other MBL-producing Proteobacteria are also mentioned in Table 1, such as *Chromobacterium*, *Erythrobacter*, *Hirschia*, *Pseudobacteriovora*, *Shewanella*, *Sediminispirochaeta*, *Stigmatella*, and *Teredinibacter*. Well-known transferable carbapenem resistance genes (IMP-, NDM- and VIM-types) have been previously reported to spread rapidly to humans via pathogen bacteria such as Enterobacterales, *P. aeruginosa* and *Acinetobacter* spp. (167-171). Such resistance genes encoding MBLs were mainly located on mobile genetic elements (plasmids, transposons, integrons, insertion sequences), which likely contribute to their rapid spread to different bacteria. Such global dissemination of carbapenem-resistant potential opportunistic bacteria is now recognized as a worldwide threat to public health, particularly from a One Health perspective, and a continuous monitoring of some environments is required (172, 173).

[H2 Treatment options for infections mediated by metallo-β-lactamases]

The treatment options for MBLs-producers and in particular for bacteria producing NDM are very limited. For serious infections, combination therapy including a polymyxin is the preferred option. However, resistance to polymyxins is emerging (174). Although the introduction of new antibiotics allows the treatment of certain strains of carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant *P. aeruginosa* (CRPA) with polymyxin-sparing regimens, the use of polymyxins is currently still necessary in carbapenem-resistant *A. baumannii* (CRAB), and in CRE and CRPA harboring MBLs

(175). The activity of β-lactam antibiotics against MBL-producing Enterobacterales in animal infection models was recently reviewed (176).

Alternatively, cefiderocol is a siderophore cephalosporin antibiotic with potent activity against carbapenem-resistant Gram-negative bacteria, including both serine- and metallo-carbapenemase positive strains (177, 178). Cefiderocol is the only of the four new agents with efficacy against MBLs and resistant *Acinetobacter* species, but comparator studies using best available therapy for carbapenem-resistant Gram-negative bacterial infections show higher mortality rates with this new drug, making its role in clinical therapy still to be determined (179).

With the exception of taniborbactam (VNRX-5133) and xeruborbactam (QPX7728), none of the novel β-lactam-β-lactamase inhibitor combinations (avibactam, nacubactam, taniborbactam, zidebactam) present *in vitro* activity against Enterobacterales or *P. aeruginosa* producing MBLs or against carbapenemase-producing *A. baumannii* (180). However, aztreonam can be used in combination with these inhibitors against multidrug resistant (MDR) Enterobacterales and *P. aeruginosa* producing MBLs (181). The aztreonam/avibactam combination is efficient against MBL-producing enteric bacteria owing to the stability of the monobactam to these enzymes, but resistance is still an issue for MBL-producing non-fermentative bacteria (182).

[H1 Classes of metallo-β-lactamase inhibitors and their design]

For many years, the design of a clinically-useful MBL inhibitors was an elusive task (183). Many excellent reviews included a comprehensive description of these studies, on β-lactamase inhibitors in general (184-188) or more specifically on MBL inhibitors (189-

206). A detailed description of these studies is outside the scope of this chapter, and only a few representative examples will be detailed below.

[H2 Metallo-β-lactamase specific inhibitors]

In an early study, Kurosaki *et al.* have exploited the common features of substrate and inhibitor binding to MBLs to generate a new class of irreversible thiol inhibitor (e.g. 3-(3-mercaptopropionylsulfanyl)propionic acid pentafluorophenyl ester) (207, 208).

Bisthiazolidines (BTZs) were designed as substrate-mimicking scaffolds for the inhibition of the NDM-1 carbapenemase. Inspired by known interactions of MBLs with β-lactams, these compounds share some features with the β-lactam substrates, which can be modified with metal-binding groups to target the MBL active site (209-212). Further studies showed that 2-mercaptomethyl thiazolidines (MMTZs) inhibit all MBL subclasses (B1, B2, B3) by maintaining a conserved binding mode (213, 214).

Another MBL inhibitor, ANT431, was shown to potentiate the activity of meropenem against a broad range of MBL-producing CRE and restore its efficacy against an *Escherichia coli* NDM-1-producing strain in a murine thigh infection model (215, 216). An optimized derivative, ANT2681, is a specific, competitive inhibitor of MBLs with potent activity against NDM enzymes. Susceptibility studies evidenced the efficacy of ANT2681 in combination with meropenem against MBL-positive Enterobacterales (including NDM-CRE) (217, 218).

Some of the novel inhibitory approaches involve the use of chelating agents or metal-based drugs that displace the native metal ion (219). For example, a fungal natural product, aspergillomarasmine A (AMA), was identified as a rapid and potent inhibitor of two

clinically relevant MBLs, NDM-1 and VIM-2. AMA also fully restored the activity of meropenem against Enterobacterales, *Acinetobacter* spp. and *Pseudomonas* spp. possessing either VIM or NDM-type alleles (220-222). Mechanistic studies showed that AMA inhibits the MBLs by removal of the active site metal ions required for β-lactam hydrolysis (223, 224). Significant improvements were obtained through the total synthesis (225-229), solid-phase synthesis (230), chemoenzymatic synthesis (231) and fragment-based drug discovery (FBDD) (232) of AMA and its derivatives.

A series of 1,2,4-triazole-3-thione compounds was designed and synthesized as inhibitors of dizinc MBLs (233-239). Computational studies revealed the intermolecular interactions of these compounds with the extended recognition site of VIM-2 (240). Some compounds showed submicromolar activities against VIM-type enzymes and strong NDM-1 inhibition and the mechanism of this inhibition involved at least partially the stripping of the catalytic zinc ions. A strong synergistic activity with meropenem was observed (16- to 1000-fold minimum inhibitory concentration (MIC) reduction) against VIM-type- and NDM-1-producing ultraresistant clinical isolates, including Enterobacterales and *P. aeruginosa*, indicating an efficient penetration of the bacterial cells. Furthermore, selected compounds exhibited no or moderate toxicity toward HeLa cells, favorable absorption, distribution, metabolism, excretion (ADME) properties, and no or modest inhibition of several mammalian metalloenzymes (241).

[H2 Covalent metallo-β-lactamase inhibitors]

A recent review summarizes the current knowledge on the covalent inhibitors of MBLs and their inhibition modes, highlighting the importance of the rational design of covalent

MBL inhibitors and the development of dual-action covalent MBL/SBL inhibitors based on lysine residue of MBLs and serine residue of SBLs (242).

Compounds from the ebsulfur family were reported to inhibit NDM-1 with IC_{50} values ranging from 0.16 to 9 μM. Labelling of NDM-1 using a constructed fluorescent ebsulfur derivative (Ebs-R) suggested that the inhibitor is covalently bound to the target. Moreover, labelling NDM-1 in living *E. coli* cells with Ebs-R by confocal microscopic imaging showed the real-time distribution of the intracellular recombinant protein (243, 244).

The *O*-aryloxycarbonyl hydroxamates are known inactivators of *Enterobacter cloacae* P99 class C SBL with an unusual covalent mechanism targeting both active-site Ser and Lys residues. Unexpectedly, a recent study evidenced that these compounds can also serve as a classical affinity label for NDM-1. Mass spectrometry and ultraviolet photodissociation for extensive fragmentation show a stoichiometric covalent labeling that occurs specifically at Lys211. Moreover, the X-ray crystal structure of the inactivated enzyme reveals that the covalent adduct is bound at the substrate-binding site but not directly coordinated to the active-site zinc cluster (244, 245).

Additional studies also reported 3-bromopyruvate (246), 1,2-isoselenazol-3(2*H*)-one derivatives (247) and disulfiram (248) as potent covalent NDM-1 inhibitors.

[H2 Conjugate metallo-β-lactamase inhibitors]

Cephalosporin prodrugs containing several MBL inhibitors (thiomandelic acid, dipicolinic acid, 8-thioquinoline) were designed and synthesized for the delivery of the active compounds in a spatiotemporally controlled fashion. These conjugates demonstrated potent inhibition of NDM, VIM, and IMP classes of MBLs and displayed potent synergy

with meropenem against MBL-expressing clinical isolates of *K. pneumoniae* and *E. coli* (249, 250). Kinetic experiments showed that thiomandelic acid acts as a slowly turned-over substrate (249).

Similarly, a cephalosporin-tripodalamine conjugate (DPASC) was shown to inhibit different MBLs with high efficacy and low toxicity. The cephalosporin tag blocks the ligand binding site to reduce toxicity and is cleaved by MBLs to release active ligands to inhibit MBLs *in situ* (251).

[H2 Dual serine/metallo-β-lactamase inhibitors]

A recent review describes the current knowledge about the dual SBL/MBL inhibitors, including their modes of inhibition and the crystal structures when available (252).

A series of azetidinimines, imino-analogues of β-lactams, was reported as efficient non-covalent inhibitors of all four classes (A, B, C, D) of β-lactamases. Despite the structural and mechanistic differences between the clinically-relevant carbapenemases KPC-2 and OXA-48 SBLs and NDM-1 MBL, all three enzymes were inhibited with K_i values below 0.3 μM, while the class C cephalosporinase CMY-2 showed 86% inhibition at 10 μM. Selected azetidinimines were also able repotentiate imipenem against a resistant strain of *E. coli* expressing NDM-1 (253).

Similarly, compounds derived from the benzo-[b]-thiophene-2-boronic acid (BZB) and 4-amino-1,2,4-triazole-3-thione scaffolds showed cross-class micromolar inhibition against class A SBL KPC-2 and class B1 MBLs VIM-1 and IMP-1 (254, 255).

The bicyclic boronate VNRX-5133 (taniborbactam) is a new type of β-lactamase inhibitor in clinical development combined with cefepime, for the treatment of infections caused by

β-lactamase-producing CRE and CRPA. VNRX-5133 inhibits SBLs and some clinically important MBLs, including NDM-1, VIM-1 and VIM-2. However, VNRX-5133's activity against IMP-1, and against other B2 and B3 MBLs tested, was lower or not observed. Inhibition is achieved by mimicking the transition state structure and exploiting interactions with highly conserved active-site residues, while employing distinct mechanisms to inhibit both SBLs and MBLs. It is a reversible covalent inhibitor of SBLs with slow dissociation and a prolonged active-site residence time, while in MBLs it behaves as a competitive inhibitor (256-258).

Xeruborbactam (QPX7728) is an ultra-broad-spectrum β-lactamase inhibitor that is currently in clinical development. In addition to potent inhibition of clinically important SBLs it also inhibits many MBLs. This compound displays a remarkably broad spectrum of inhibition, including class B and class D enzymes, and is little affected by porin modifications and efflux. QPX7728 combinations with several β-lactam antibiotics shows broad coverage of Enterobacterales, *A. baumannii* and *P. aeruginosa*. An important aspect is that QPX7728 can also be delivered orally (259-268).

[H1 Metallo-β-lactamase inhibitors in clinical development]

Currently, the most advanced clinical trial targeting MBL-producing bacteria involves the combination cefepime/VNRX-5133 (taniborbactam), which has completed the phase 3 with the sponsorship of Venatorx Pharmaceuticals (code: NCT03840148). The combination aztreonam/avibactam is tested in phase 3 since December 2020 with the sponsorship of Pfizer (code: NCT03580044). Finally, the combination QPX2014/QPX7728 (xeruborbactam) has completed the phase 1 with the sponsorship of

Qpex Biopharma (codes: NCT04380207 and NCT05072444). This information was last updated in January 2023.

[H1 Conclusion]

The emergence of MBL-producing organisms has become a significant public health concern due to their ability to cause multidrug-resistant infections. While there are compounds that have been identified as MBL inhibitors, none are currently clinically-approved. However, several compounds are being evaluated in clinical trials and may be approved in the near future.

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