REVIEW ARTICLE

Metallo- β -lactamases—a new therapeutic challenge

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Introduction

Over the last 30 years, many different β -lactamases with serine at the active site have been identified. Approximately 60 are plasmid mediated and there is probably a similar number of chromosomally mediated enzymes. In addition there is a relatively small group of β -lactamases in which the active site has a metal ion. The first of these, the *Bacillus cereus* II metallo- β -lactamase, was identified in 1966 when the cephalosporinase activity of a β -lactamase produced by a *B. cereus* isolate was shown to be inhibited by EDTA.¹ Since then, many other metallo- β -lactamases have been identified from clinical isolates all over the world (table I). Metallo- β -lactamases are mechanistically different from β -lactamases which have serine at the active site. The metal ion active site appears to decrease their susceptibility to β -lactamase inhibitors and enables them to hydrolyse a broad spectrum of β -lactam agents including carbapenems. Tables I–IV list those enzymes known to be metallo- β -lactamases as well as those suspected to belong to this group by virtue of susceptibility to EDTA or hydrolysis of carbapenems. Comparative data on these enzymes is scarce and some of the enzymes listed may be duplicates.

The first reported metallo- β -lactamases were produced by organisms of minor clinical relevance, e.g., *Flavobacterium odoratum*,² *B. cereus*³ or *Legionella gormanii*,⁴ and were regarded as rare curiosities. However, the recently discovered metallo- β -lactamases have been associated with more clinically relevant genera such as *Serratia*, *Bacteroides* and *Pseudomonas* and have forced a re-evaluation of the clinical importance of these enzymes.

Molecular structure of metallo- β -lactamases

Bacillus spp.

The *B. cereus* II enzyme isolated from *B. cereus* 569/H/9, is the only metallo- β -lactamase which has undergone X-ray crystallographic studies⁵ and most of

the structural and mechanistic information on metallo- β -lactamases is derived from this enzyme.

As with many metallo- β -lactamases, the zinc ion of the *B. cereus* II enzyme can be replaced by different metal ions and still retain some β -lactamase activity⁶ (table IV). Equilibrium dialysis⁶ and ¹H nuclear magnetic resonance (NMR)⁷ indicate that the *B. cereus* II enzyme is capable of binding two zinc ions. However, computer assisted molecular modelling indicates one major metal ion binding site with two minor sites, one of which is close to the major binding site and may affect the activity of the enzyme against certain substrates.⁵ Both the nucleotide⁸ and the amino-acid sequences⁹ of the *B. cereus* II enzyme have been determined and NMR studies have revealed that the zinc ion in the major binding site is surrounded by three histidine residues,⁷ but only two of these may be true ligands.⁵ A cysteine residue, adjacent to the zinc ion binding site, has also been implicated in the coordination of the zinc ion.⁵

The mechanism of action of *B. cereus* II metallo- β -lactamase is thought to involve a water molecule, bound to the zinc ion of the active site, attacking the carbonyl group of the β -lactam ring. It was originally proposed that a glutamate residue, glutamate 37, acted as a general base, deprotonating the water molecule, with the proton being donated to the nitrogen atom of the β -lactam ring to cause cleavage.¹⁰ However, computer models showed that glutamate 37 was too far from the zinc ion to perform this function and that it was more likely that glutamate 212 was involved.⁵ More recently, site directed mutagenesis has revealed that neither glutamate residue is essential for the catalytic function of the enzyme.¹¹ Lim *et al.*¹² have now demonstrated that aspartate 90 is essential for enzyme activity and have proposed that this residue acts as the general base to assist in the hydrolysis of the amide bond of β -lactam substrates. The detailed function of the amino acids in the active site of the *B. cereus* II metallo- β -lactamase requires further elucidation.

A second *B. cereus* metallo- β -lactamase has also been sequenced. This enzyme, produced by *B. cereus* 5/B/6,¹³ a mutant strain of *B. cereus* 569/H/9, differs from the classic *B. cereus* II enzyme by only 18 amino-

Table I. General properties of metallo- β -lactamases

Bacterial species	Producer strain	Enzyme name	Metal ion required	Iso-electric point, pI	Molecular mass	Amino-acid homology with <i>B. cereus</i>	Plasmid encoded	Inducibility	Approx. year of isolation	Country of isolation	Ref. no.
<i>Bact. fragilis</i>	TAL2480*	CfiA	Zn ²⁺	4.7	25,249† 44,000§	32‡		No	≤ 1986	USA	23–25,19
<i>Bact. fragilis</i>	GAI30144		Zn ²⁺	4.7	33,000				1986/7	Japan	17
<i>Bact. fragilis</i>	G 237		¶	4.8	26,000				≤ 1983	Japan	20
<i>Bact. fragilis</i>	BFR 81R	CfiA-type	Zn ²⁺	4.7					1985	France	19
<i>Bact. fragilis</i>	119		¶	4.6					≤ 1986	UK	21
<i>Bact. fragilis</i>	QMCN3	CfiA-type	Zn ²⁺		c.25,249	c.32			≤ 1991	UK	26
<i>Bact. fragilis</i>	QMCN4	CfiA-type	Zn ²⁺		c.25,249	c.32			≤ 1991	UK	26
<i>Bact. fragilis</i>	GAI30079		Zn ²⁺						1986/7	Japan	17,18
<i>Bact. fragilis</i>	10-73		**				Yes		≤ 1991	Japan	51
<i>Bact. distasonis</i>			**	4.6	160,000§				≤ 1991	Sweden	22
<i>B. cereus</i>	569/H/9	II	Zn ²⁺	8.45	24,932† 22,000§	100		Yes	1947	UK	3,8
<i>B. cereus</i>	5/B/6	II-type	Zn ²⁺	8.4/9.1	22,500	90			1975	UK	13,14
<i>A. hydrophila</i>	19	A-2	Zn ²⁺	8.0	31,500			Yes	≤ 1990	USA	29
<i>A. hydrophila</i>	AE036	CphA	Zn ²⁺	8.0	28,000	24.7††		Yes	≤ 1990	Italy	30
<i>F. odoratum</i>	GN14053		**	5.8	26,000			Yes	≤ 1985	Japan	2
<i>L. gormanii</i>	ATCC 33297		**	10.5	25,000				≤ 1986	Japan	4
<i>P. aeruginosa</i>	GN 17203		Zn ²⁺	9.0	28,000		Yes	No	1988	Japan	50
<i>S. marcescens</i>	S6		**	9.7	25,000		No	No	1982	UK	31
<i>X. maltophilia</i>	GN12873	L-1	Zn ²⁺	6.9	118000 26000	0		Yes	≤ 1982	Japan	15,16

* TAL2480 produces a β -lactamase identical to that of *Bact. fragilis* TAL3636 (CcrA).

† Calculated from sequence.

‡ 58–77% homology around active site.²⁴

§ Determined by gel filtration.

|| Determined by SDS-PAGE.

¶ Evidence suggests a metallo- β -lactamase but not tested for.

** Shown to be a metallo- β -lactamase but insufficient evidence to assume it is a Zn²⁺ metallo- β -lactamase.

†† 24.7% and 25% overall amino-acid homology with *B. cereus* II and *Bact. fragilis* CfiA, respectively; 61.6% and 66.2% similarity (after allowing for conservative substitutions) to *B. cereus* II and *Bact. fragilis* CfiA, respectively.³⁰

acid residues,¹⁴ and is classed as a *B. cereus* II-type metallo- β -lactamase.

Xanthomonas maltophilia

The most extensively characterised *X. maltophilia* metallo- β -lactamase is the L-1 enzyme. Only the N-terminal (32 residues) of the L-1 enzyme has been sequenced.¹⁵ It has a molecular mass *c.* four times greater when determined by gel filtration than by SDS-PAGE suggesting a tetramer structure.¹⁶

Bacteroides spp.

Ten *Bacteroides* spp. metallo- β -lactamases have been reported (tables I–IV) but, as discussed above, some of these enzymes may be identical. For example, the metallo- β -lactamases produced by *Bact. fragilis* GAI30144 and *Bact. fragilis* GAI30079 are reported to have similar substrate profiles,¹⁷ the latter enzyme has been characterised in more detail¹⁸ but further work is required to compare these two enzymes. The metallo- β -lactamases produced by *Bact. fragilis* strains TAL2480,¹⁹ GAI30144,¹⁷ G237,²⁰ 119²¹ and BFR81R¹⁹ all have similar iso-electric points (table I) as well as a number of other similar characteristics (tables I–IV) but there are insufficient data to confirm that these enzymes are the same. The only metallo- β -

lactamase derived from a *Bacteroides* spp. other than *Bact. fragilis* is an enzyme isolated from a *Bact. distasonis* strain.²² This enzyme is distinct from other *Bacteroides* spp. metallo- β -lactamases in having a molecular mass of 160 000 (table I) and being inhibited by clavulanic acid and sulbactam. It would be of interest to determine the molecular masses of this and other enzymes in table I by both gel filtration and SDS-PAGE as this may reveal other metallo- β -lactamases which exist as multimers.

Cuchural *et al.*²³ identified metallo- β -lactamases in *Bact. fragilis* TAL3636 (CcrA) and *Bact. fragilis* TAL 2480 (CfiA). The nucleotide sequence of the *ccrA*²⁴ and *cfiA*²⁵ metallo- β -lactamase genes showed that both strains produced identical enzymes. In addition, five strains of *Bact. fragilis* from the UK were probed with a *ccrA* gene probe to establish the prevalence of this metallo- β -lactamase gene.²⁶ DNA from two of the strains hybridised with the probe and the nucleotide sequences of the metallo- β -lactamase genes from these two isolates (*Bact. fragilis* QMCN3 and QMCN4) were *c.* 97% homologous with the *ccrA* β -lactamase gene.²⁶ For the purpose of this review these four highly homologous enzymes will be referred to as CfiA-type metallo- β -lactamases (table I).

Direct sequencing or probing has now confirmed the presence of the CfiA-type metallo- β -lactamase gene in 11 clinical isolates of *Bact. fragilis* from three

Table II. MICs of β -lactams for strains producing metallo- β -lactamases

Strain	MIC (mg/L)													Reference no.
	cephal- oridine	ampi- cillin	peni- cillin G	carbeni- cillin	cephal- othin	cefo- xitin	cefo- taxime	cloxa- cillin	lata- moxef	imi- penem	mero- penem	aztre- onam	pipera- cillin	
<i>Bact. fragilis</i> TAL2480		> 128	> 128	> 128		128	> 128		> 128	128		> 128	> 128*	23
<i>Bact. fragilis</i> GAI 30144						†			High	100				17
<i>Bact. fragilis</i> G 237	200	50	200	100		100		800	100	13			13	20
<i>Bact. fragilis</i> BFR 81R	> 128	> 128†			128	128	128		128	128	256			19
<i>Bact. fragilis</i> 119			> 64‡			64				32				21
<i>Bact. fragilis</i> QMCN3						64				> 16			> 128	26
<i>Bact. fragilis</i> QMCN4						16				1			16	26
<i>Bact. fragilis</i> GAI30079	> 100	> 100	> 100			> 100	> 100			> 100				18
<i>Bact. fragilis</i> 10-73		100†				100			200	200			50	51
<i>Bact. distasonis</i>			> 4096		> 512	> 64				2			> 4096	22
<i>B. cereus</i> 569/H/9	8	64	100		55	> 64	> 64			2	0.5	> 64		8
<i>B. cereus</i> 5/B/6	100	100												11
<i>A. hydrophila</i> 19					> 128		128			128			> 128	29
<i>A. hydrophila</i> AE036										2§				30
<i>F. odoratum</i> GN14053				1600	> 1600		> 1600		400	400				2
<i>L. gormanii</i> ATCC 33297		1.6		0.2	0.4	0.2								4
<i>P. aeruginosa</i> GN17203			> 400						> 400	50	100	25	25	50
<i>S. marcescens</i> S6	512	32		32		16	0.3			16	0.12			31
<i>X. maltophilia</i> GN12873	> 800	400	400	400	> 800	> 800	200	> 800	6	> 100				16

* Resistant to piperacillin + tazobactam and ticarcillin + clavulanic acid combinations.

† Highly resistant to cephamycins.

‡ Also resistant when MIC performed in combination with clavulanic acid.

§ This is the MIC at 10⁶, MIC at 10⁹ = 128.**Table III.** Efficiency of hydrolysis values of β -lactams by metallo- β -lactamases

Strain	V _{max} /Km relative to cephaloridine for													Reference no.
	cephal- oridine	ampi- cillin	peni- cillin G	carbeni- cillin	cephal- othin	cefo- xitin	cefo- taxime	cloxa- cillin	lata- moxef	imi- penem	mero- penem	aztre- onam	pipera- cillin	
<i>Bact. fragilis</i> TAL2480	100	260*	330*	300*	118	6.9	88		14	33				23
<i>Bact. fragilis</i> GAI 30144	100	31							15	21				17
<i>Bact. fragilis</i> G 237	100	38	96	58	105	1.0	72	35	12	12			13	20
<i>Bact. fragilis</i> BFR 81R	100	293*	300		39	1	40		11	7	36			19
<i>Bact. fragilis</i> 119										Hydrolysis				21
<i>Bact. fragilis</i> QMCN3														26
<i>Bact. fragilis</i> QMCN4														26
<i>Bact. fragilis</i> GAI30079	100	16	81			0.4	33			16				18
<i>Bact. fragilis</i> 10-73	100†	178†	252†			8†	90†			127†	184†	ND		51
<i>Bact. distasonis</i>		Hydro- lysis	Hydro- lysis		Hydro- lysis	Low hydrolysis				Low hydrolysis			Hydrolysis	22
<i>B. cereus</i> 569/H/9	100†	146†	333†	270†	160†									13
<i>B. cereus</i> 5/B/6	100†	176†	588†	400†	124†									13
<i>A. hydrophila</i> 19			2131‡		100‡		51‡	89‡		3547‡		94‡		29
<i>A. hydrophila</i> AE036										Hydrolysis				30
<i>F. odoratum</i> GN14053	100	478	1439	612	821	875	739		63	371		< 1		2
<i>L. gormanii</i> ATCC 33297	100	19	17	17	31	18	25	32	8.4	6.5		< 1		4
<i>P. aeruginosa</i> GN17203	100	3.7	6.3	5.8	106	48	55		38	39	40	< 1	1.8	50
<i>S. marcescens</i> S6	100§	198§	70§	102§		56§	84§			916§	151§			31
<i>X. maltophilia</i> GN12873	100	2106	2975	650	344		2806	1125			620			16

ND, not detectable.

* Relative V_{max} value (V_{max} for cephaloridine = 100 %).

† Relative rate of hydrolysis.

‡ V_{max}/Km relative to cephalothin (V_{max}/Km for cephalothin = 100 %).

§ Kcat/Km relative to cephaloridine (Kcat/Km for cephaloridine = 100 %).

different countries.^{19, 24–26} Furthermore, the data in tables I–IV infer that some of the other *Bact. fragilis* enzymes may be CfiA-type metallo- β -lactamases, suggesting that the CfiA-type β -lactamase may be the most prevalent metallo- β -lactamase in clinical isolates of *Bact. fragilis*.

Aeromonas spp.

Aeromonas spp. produce inducible β -lactamases which have hydrolytic activity against imipenem.^{27, 28} In the uninduced form the β -lactamase confers only a low level of resistance to carbapenems. Mutant strains producing β -lactamase constitutively are highly resistant to a broad range of cephalosporins and

imipenem.^{28, 29} Detailed characterisation of one of these enzymes (A-2) showed that it was inhibited by EDTA and that zinc was required for activity²⁹ (table IV).

An interesting phenomenon has been noted with the A-2 metallo- β -lactamase. When ID₅₀ values were determined for clavulanic acid with penicillin G or nitrocefin as reporter substrate, the A-2 β -lactamase was sensitive to clavulanic acid. However, with imipenem as the reporter substrate, the A-2 β -lactamase appeared far less susceptible to clavulanic acid²⁹ (table IV). The authors postulated that this could be caused by clavulanic acid binding close to the active site, blocking the access of penicillin G and nitrocefin, but not imipenem. Alternatively, the A-2

Table IV. Inhibitor profiles of metallo- β -lactamases

Strain	Activity* of EDTA-treated metallo- β -lactamase after addition of																			Reference no.	
	Effect of																Effect of				
	Clavulanic acid	Sulbactam	EDTA	Zn ²⁺	Co ²⁺	Mn ²⁺	Mg ²⁺	Ca ²⁺	Fe ²⁺	Ni ²⁺	Cu ²⁺	Hg ²⁺	MgSO ₄	CuSO ₄	ZnSO ₄	pCMB†	I ₂	Aztrenonam	HgCl ₂		
<i>Bact. fragilis</i> TAL2480	NI	NI	I	+	+	—	—	—	—	—							I	I	NI		23
<i>Bact. fragilis</i> GAI30144	NI	NI	I	112	42	23	0	0									I				17
<i>Bact. fragilis</i> G 237	NI	NI															I	I			20
<i>Bact. fragilis</i> BFR 81R	NI	NI	I														I				19
<i>Bact. fragilis</i> 119	NI	NI															P				21
<i>Bact. fragilis</i> QMCN3			I																		26
<i>Bact. fragilis</i> QMCN4			I																		26
<i>Bact. fragilis</i> GAI30079			I	100		20	0	0													18
<i>Bact. fragilis</i> 10-73	NI		I																		51
<i>Bact. distasonis</i>	I		I																		22
<i>B. cereus</i> 569/H/9	NI	NI	I	+	+/-	+/-	—	—		—		+/-									6
<i>B. cereus</i> 5/B/6	NI	NI	I																		13
<i>A. hydrophila</i> 19	NI†		I	+										NI	NI		P	I		I	29
<i>A. hydrophila</i> AE036			I	+																	30
<i>F. odoratum</i> GN14053	NI	NI	I	52	12	8	0	4	44		0	0	NI (MgCl ₂)	I (ZnCl ₂)	NI		I	I	P	I	2
<i>L. gormanii</i> ATCC 33297	NI	NI	P											I			I	I		I	4
<i>P. aeruginosa</i> GN17203	NI	NI	I	54	7	5	0	7	5		0	0		I	NI (ZnCl ₂)		I	I		I	50
<i>S. marcescens</i> S6	I		I	—		—							NI		I						31
<i>X. maltophilia</i> GN12873	NI		I	94	15	32	14	43	0		0		NI	I	NI		NI	I		I	16

NI, not inhibited; I, inhibited; P, partially inhibited.

* +, β -lactamase active after treatment with metal ion; —, β -lactamase not active after metal ion; +/—, partial activity returned to β -lactamase by metal ion. Figures refer to % activity compared to untreated control.

† p-chloromercuribenzoate.

‡ Clavulanic acid inhibits when nitrocefin or penicillin G were the reporter substrates, but enzyme resistant to clavulanic acid when imipenem was the reporter substrate.²⁹

β -lactamase may have far greater affinity for imipenem than for clavulanic acid. Further kinetic work may reveal information that would otherwise be obtainable only by detailed structural studies. These data indicate that ID₅₀ values for metallo- β -lactamases may vary with different substrates.

CphA is the other *A. hydrophila*-derived metallo- β -lactamase which has been studied in detail.³⁰ The CphA enzyme is inducible and has a similar pI and mol. wt to the A-2 enzyme (table I). The A-2 and CphA metallo- β -lactamase may be identical enzymes.

Serratia marcescens

The metallo- β -lactamase produced by *S. marcescens* S6 is inhibited by zinc sulphate and, furthermore, zinc sulphate does not restore the catalytic activity of the EDTA-treated enzyme³¹ (table IV). This enzyme was thought to be a metallo- β -lactamase but there is insufficient proof that zinc is the metal co-factor.³¹ Similar observations have been made with other metallo- β -lactamases. For example, the *F. odoratum* metallo- β -lactamase has almost the same activity with either zinc or iron as a metal co-factor² (table IV) and the zinc ion of the *X. maltophilia*¹⁶ and *Bact. fragilis* GAI30144¹⁷ metallo- β -lactamases can be replaced with calcium and cobalt ions respectively whilst retaining almost half the activity of the zinc centred enzyme (table IV). Therefore, it is possible that metallo- β -lactamases with active site metal ions other than zinc may exist.

The *S. marcescens* S6 strain is more than 100 times more resistant to imipenem than to meropenem (table II) and the turnover of imipenem by the S6 enzyme is

almost 28 times higher than that of meropenem.³¹ Metallo- β -lactamases may confer resistance to, and hydrolyse, imipenem but not necessarily be cross-resistant to other carbapenems.

A second type of carbapenemase has been identified in *S. marcescens*. This imipenem resistant strain (US12) was isolated in 1970 and produces an enzyme with a pI less than that of the S6 enzyme (preliminary data determined by SmithKline Beecham Pharmaceuticals) but further characterisation is required to deduce whether it is a metallo-enzyme.

Sequence homology between metallo- β -lactamases

Sequence data are available for eight of the 19 enzymes in table I and it is, therefore, possible to deduce the extent of homology between these enzymes. The *A. hydrophila* (CphA) and *Bact. fragilis* (CfiA type) metallo- β -lactamases show 25%³⁰ and 32%²⁵ amino-acid homology, respectively, with the *B. cereus* II β -lactamase. There is 25% homology between the *A. hydrophila* CphA and the *Bact. fragilis* CfiA type metallo- β -lactamases.³⁰ Overall, five regions of homology can be identified in these three metallo- β -lactamases.³⁰ The *B. cereus* II enzyme was the original enzyme of Class B in the Ambler molecular classification of β -lactamases.³² As there is significant homology between the *B. cereus* II, CfiA and CphA metallo- β -lactamases, these enzymes are all considered to be Class B β -lactamases. However, the relatively low homology between the CphA and *B. cereus* enzymes suggests that the CphA protein may represent a subgroup of Class B.³⁰

The limited sequence data available for the *X.*

maltophilia metallo- β -lactamase (L-1) shows little homology with these Class B enzymes.¹⁵ Furthermore, the L-1 enzyme has a tetramer structure. These data suggest that the L-1 enzyme is distinct from other Class B β -lactamases and, until more sequence data is available, this enzyme should be allocated to a new class in the molecular classification of β -lactamases.

The clinical relevance of metallo- β -lactamases

The metallo- β -lactamases hydrolyse all cephamycins, penicillins and all generations of cephalosporins (table III), and generally confer appreciable resistance to these antibiotics (table II). Unlike other β -lactamases, almost all of the metallo- β -lactamases hydrolyse and mediate resistance to imipenem. Of the limited number of metallo- β -lactamases tested, all hydrolyse meropenem, a new carbapenem (table III). Metallo- β -lactamases are produced by a wide variety of organisms and have a broad range of characteristics.

Bacillus spp.

Although the molecular structure and mode of action of *B. cereus* II metallo- β -lactamases have been studied extensively,³ their frequency in clinical strains of *B. cereus* is unknown. With the exception of *B. anthracis* and *B. cereus* in the context of food poisoning, the *Bacillus* spp. are usually dismissed as contaminants or as non-pathogenic when isolated from clinical specimens. However, in the last decade, other *Bacillus* spp., particularly *B. cereus*, have been isolated from both local and systemic infections.^{33,34} Production of a metallo- β -lactamase was not documented in any of these infections but as the importance of *Bacillus* spp. in the clinical setting increases, metallo- β -lactamases may be implicated in treatment failure.

X. maltophilia

As the L-1 enzyme hydrolyses imipenem (table III), it has generally been assumed that carbapenem resistance exhibited by *X. maltophilia* is mediated predominantly by the L-1 metallo-enzyme. However, this may not always hold true. Recently, Cullmann and Dick³⁵ examined the enzymes produced by 20 multi-resistant clinical isolates of *X. maltophilia*. Iso-electric focusing of these enzymes revealed an unexpected heterogeneity with six different types of β -lactamases identified. All 20 strains hydrolysed imipenem and meropenem but, overall, meropenem was a better substrate than imipenem. Surprisingly, inhibition of carbapenem hydrolysis by 1 mM EDTA could not be demonstrated for any of the 20 enzymes; 0.1 mM EDTA causes a 93% inhibition of the L-1 metallo- β -lactamase.¹⁶ None of the strains produced significant amounts of enzyme without induction. Further work is required to ascertain the exact nature of these *X.*

maltophilia β -lactamases. Another aspect to antibiotic resistance in *X. maltophilia* is the low permeability of its outer membrane, which may explain why strains are often also resistant to non- β -lactam agents such as the quinolones.³⁶ In four recent surveys, 180 multi-resistant strains of *X. maltophilia* have been examined; all were resistant to imipenem.³⁷⁻⁴⁰ Garcia-Rodriguez *et al.*³⁷ found that 95% of 42 clinical isolates of *X. maltophilia* were resistant to both imipenem and meropenem; 36 different antimicrobial agents and 33 different combinations of β -lactam and β -lactamase inhibitor were tested *in vitro* and only the combinations of aztreonam + clavulanic acid and amoxycillin + clavulanic acid + aztreonam were effective.

Some years ago, *X. maltophilia* was not recognised as a pathogen but a recent study by Cullmann³⁸ found that it represented 2% of all isolates cultured from nosocomial respiratory tract infections. *X. maltophilia* is now being isolated with increasing frequency and is emerging as an important nosocomial pathogen.^{41,42} How much of the antimicrobial resistance exhibited by *X. maltophilia* is caused by the production of a metallo- β -lactamase is unknown and until this is determined it is not possible to deduce the true clinical relevance of metallo- β -lactamases in *X. maltophilia*.

Bacteroides spp.

An additional worry from the clinical viewpoint is the possibility of silent metallo- β -lactamase genes. Podglajen *et al.*¹⁹ showed that DNA from three carbapenem-sensitive and four carbapenem-resistant clinical strains of *Bact. fragilis* hybridised with a *CfiA* gene probe. The four sensitive strains could each mutate spontaneously to high-level carbapenem resistance (MIC of imipenem $\geq$ 128 mg/L) with a concomitant increase in metallo- β -lactamase production following exposure to imipenem 32 mg/L. Therefore, some strains of *Bact. fragilis* carry silent metallo- β -lactamase genes which are activated following exposure to carbapenems. This work also implies that the production of the metallo-enzyme may be inducible, and that a mutation in the regulatory genes had caused the strains to produce the enzyme constitutively. Such mutations may cause high level carbapenem resistance in the clinical setting in a similar way to the cephalosporin resistance conferred by chromosomally mediated Group 1⁴³ β -lactamases which have become stably de-repressed.⁴⁴

In three recent surveys, a total of 327 strains of *Bacteroides* spp. was examined and 12 clinical isolates were found to produce enzymes which inactivated imipenem.⁴⁵⁻⁴⁷ The limited data available on these enzymes suggest they could be metallo- β -lactamases (these enzymes are excluded from tables I-IV). However, many other large surveys of *Bacteroides* spp. isolates have identified neither imipenem resistance nor imipenem hydrolysing enzymes, suggesting that frequency of metallo- β -lactamases in clinical isolates of *Bacteroides* spp. is low but significant.

Aeromonas spp.

Aeromonas spp. are increasingly implicated in human infections, particularly wound⁴⁸ and enteric infections.⁴⁹ A significant number of clinical isolates of *Aeromonas* spp. produce inducible carbapenemases and there is evidence to suggest these are metallo-enzymes.^{28,29} Where conditions for induction of these enzymes are provided, they could pose difficult therapeutic problems.

The production of metallo- β -lactamases has also been confirmed in single clinical isolates of *Pseudomonas aeruginosa*⁵⁰ and *S. marcescens*.³¹ If metallo- β -lactamases become widespread in these species the current difficulties in selecting antimicrobial agents to treat infections caused by these nosocomial pathogens will be compounded.

Plasmid mediated metallo- β -lactamases

Only two of the metallo- β -lactamases in table I have been shown to be plasmid mediated. *P. aeruginosa* GN17203 produces a metallo- β -lactamase which was transferable by conjugation to other strains of *P. aeruginosa* but not to *E. coli*.⁵⁰ This metallo- β -lactamase confers resistance to third generation cephalosporins and carbapenems. It mediates a higher level of resistance to meropenem than to imipenem (table II) although it hydrolyses both of these compounds with similar efficiency (table III).

The other transferable metallo- β -lactamase is produced by *Bact. fragilis* 10-73 and is transferable to other *Bact. fragilis* strains by conjugation. This enzyme hydrolyses meropenem at a greater rate than imipenem (table III).⁵¹

Conclusions

Metallo- β -lactamases confer a far broader range of resistance than any of the Group I⁵² or Group 2b' β -lactamases⁵³ and it is important to assess the relevance of metallo- β -lactamases in the clinical environment. It must be emphasised that metallo- β -lactamases are not the only cause of resistance to carbapenems. Altered permeability and serine-based chromosomally mediated β -lactamases have also been implicated in imipenem resistance.⁵⁴⁻⁵⁸ However, the recent isolation of plasmid mediated metallo- β -lactamases suggests that there is the potential for rapid dissemination and the incidence of metallo- β -lactamases may increase over the next few years with these enzymes becoming the most common cause of carbapenem resistance.

Metallo- β -lactamases are now being isolated from more established pathogens, such as *S. marcescens*, *P. aeruginosa* and *Bact. fragilis*. Furthermore, other less pathogenic organisms which can produce metallo- β -lactamases are increasingly implicated in infections, particularly in immunocompromised patients. The inducibility of metallo- β -lactamases in *Aeromonas* spp. and *X. maltophilia* and the discovery of covert metallo- β -lactamase genes in *Bact. fragilis* are additional concerns. The increasing use of imipenem together with the imminent launch of meropenem will add to the selective pressure for metallo- β -lactamases.

Combating metallo- β -lactamases may prove to be a difficult problem. Current data suggests that they are a heterogeneous group of enzymes which implies that there could be problems in the design of agents that would be efficient against all metallo- β -lactamases.

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