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MINIREVIEW

Carbapenem-Hydrolyzing β-Lactamases

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INTRODUCTION

Carbapenem-hydrolyzing β-lactamases include some of the more recently described β-lactamases in the repertoire of penicillin-interactive proteins. These enzymes are responsible for conferring resistance to the carbapenems, the β-lactam class with the broadest spectrum of antibacterial activity. The number of these enzymes compared to the number of other β-lactamases remains low, with only 17 carbapenem-hydrolyzing enzymes included in the recent compilation of 190 β-lactamases described as functionally or molecularly distinct enzymes (12). Although carbapenems have remained relatively unscathed by the hydrolytic action of many clinically relevant β-lactamases, an increasing number of carbapenem-hydrolyzing enzymes have been described in the past 4 years, especially from Japan, where the carbapenem class is the market leader among parenteral β-lactams, in contrast to other geographical areas, where carbapenems are used on a more restricted basis (7, 30, 50, 74).

Several recent reviews have summarized the properties of "carbapenemases" (43, 44), or the specific class of carbapenem-hydrolyzing metallo-β-lactamases (57). However, as more of these enzymes have been described, their diversity has become increasingly evident, both genetically and biochemically. Most of these β-lactamases confer resistance not only to carbapenems, but also to other β-lactams. Thus, the term "carbapenemase" used by some authors is misleading, in that cephalosporins or penicillins may be hydrolyzed more efficiently than carbapenems. In this review the term "carbapenemase" will be reserved for that subgroup of metallo-β-lactamases that preferentially hydrolyze carbapenems.

On a molecular level these enzymes can belong either to the class A group of β-lactamases that have serine at the active site or to the class B enzymes that represent the only metallo-β-lactamases that have been identified (2). Although some class C cephalosporinases (33) have been reported to hydrolyze imipenem at a measurable rate (15), these enzymes are usually not considered to be serious carbapenem-hydrolyzing enzymes, because carbapenems do not represent a major substrate in their hydrolytic profile.

In this review production of the carbapenem-hydrolyzing enzymes in their natural isolates will be described, with particular reference to coproduction with other β-lactamases. Biochemical characteristics are summarized, with a new subgrouping of the metallo-β-lactamases proposed on the basis of functional characteristics. The molecular aspects of the chromosomal carbapenem-hydrolyzing enzymes are evaluated, and the regulation of these enzymes is discussed. The sequences of the known enzymes are presented, with three molecular classes proposed for the metalloenzymes on the basis of possible amino acid alignments.

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NATURAL OCCURRENCE

Organisms that produce carbapenem-hydrolyzing enzymes frequently produce more than a single β-lactamase (Table 1). Only in *Flavobacterium odoratum* and *Legionella gormanii* has an imipenem-hydrolyzing chromosomal enzyme been specifically reported as the sole β-lactamase (23, 72). Although *Burkholderia* (*Pseudomonas*) *cepacia* 5JIV with the PCM-1 enzyme was not reported to produce another β-lactamase (9), another chromosomal non-imipenem-hydrolyzing penicillinase has been reported from *B. cepacia* 249 (62), indicating that PCM-1 is not the sole β-lactamase produced by this species. Khushi et al. (37) recently reported the presence of a single CcrA-related β-lactamase in each of five clinical isolates of *Bacteroides fragilis* obtained from five hospitals in the United Kingdom between 1985 and 1987. No attempts were made to identify additional β-lactamases in those isolates, e.g., by looking for induction.

Only three unique serine-based enzymes in functional group 2f have been identified to date from six clinical isolates worldwide (48, 54, 64, 87). Two *Enterobacter cloacae* isolates from the southwestern United States and two *Serratia marcescens* isolates from London were collected in the early 1980s before imipenem had been approved for general clinical use as the first carbapenem. An *S. marcescens* isolate from Minnesota in 1985 produced two enzymes with isoelectric points similar to those seen for the London S6 isolate (48). The sixth isolate originated from Paris in 1990 (52). The organisms that produce group 2f serine β-lactamases also have AmpC-type cephalosporinases. In addition, the IMI-1-producing *E. cloacae* strains and one of the London *S. marcescens* strains produce an apparent plasmid-mediated TEM-like β-lactamase, thereby broadening the resistance profile for these organisms. Livermore (45) has described the group 2f enzymes as "secondary β-lactamases," enzymes which are supplementary to the normal chromosomal β-lactamase of an organism.

Five of the six *Aeromonas* strains that have been well-characterized for β-lactamase production are known to produce three different β-lactamases, not all of which are similar among the strains (26, 27, 73, 82, 85). In four strains enzymes were identified from each of the three major functional groups: groups 1, 2, and 3 (12). This diversity of production crosses species including *Aeromonas hydrophila*, *Aeromonas jandaei*, *Aeromonas salmonicida*, and *Aeromonas sobria*. Although an initial description of *A. hydrophila* 19 included only two β-lactamases in its profile (29), it is quite likely that the A2h enzyme activity was actually due to a combination of a penicillinase and a metalloenzyme, the gene for which is now known and sequenced as *cphA2* (63a). If this were the case, then all the

TABLE 1. Coproduction of chromosomal carbapenem-hydrolyzing β-lactamases with other β-lactamases in naturally occurring isolates[a]

Carbapenem-hydrolyzing β-lactamase in organism	Organism	Strain	Country of origin	Enzymes produced	pI	Functional group[b]	Reference(s)
Serine	*Enterobacter cloacae*	1413B	United States	TEM-1-type	5.4	2b	64
				IMI-1	**7.1**	**2f**	
				(AmpC-type)	8.1	1	
Serine	*Enterobacter cloacae*	NOR-1	France	**NmcA**	**6.9**	**2f**	54
				(AmpC-type)	>9.2	(1)	
Serine	*Serratia marcescens*	S6	England	(AmpC-type)	8.2	1	87
				Sme-1	**9.7**	**2f**	
Serine	*Serratia marcescens*	S8	England	TEM-1 type	5.4	2b	87
				(AmpC-type)	8.2	1	
				Sme-1	**9.7**	**2f**	
Metallo-β-lactamase	*Aeromonas hydrophila*	19	United States	A1h	7.0	1	29
				A2h	**8.0**	**3**	
Metallo-β-lactamase	*Aeromonas hydrophila*	A036	Italy	NN[c]	6.6	NC[d]	73
				NN	7.0	NC	
				CphA[e]	**8.0**	**3**	
Metallo-β-lactamase	*Aeromonas hydrophila*	G19	Scotland	ACE	7.0	1	27
				APE	8.0	2	
				ACP	**ca. 8.2**	**3**	
Metallo-β-lactamase	*Aeromonas jandaei*[f]	14	United States	AsbA1	6.7	1	29, 67
				AsbB1=OXA-12	8.6	2d	
				AsbM1	**9.1**	**3**	85
Metallo-β-lactamase	*Aeromonas salmonicida*	ASA111	Scotland	**ASA-1**	**ND**[g]	**3**	26
				ASA-3	6.0	1	
				ASA-2	7.9	2(b)	
Metallo-β-lactamase	*Aeromonas sobria*	163a	England	CepS	7.0	1	82
				AmpS	7.9	2(c?)[h]	
				ImiS	**9.3**	**3**	80
Metallo-β-lactamase	*Bacillus cereus*	569	Unknown	I	8.6	2a	39
				II	**8.3**	**3**	
Metallo-β-lactamase	*Bacteroides fragilis*	TAL3636	United States	**CcrA**	**5.2**	**3**	69
				(Cephase)[i]	NC	(1)	
Metallo-β-lactamase	*Stenotrophomonas maltophilia*[j]	GN12873	Japan	**L1**	**6.9**	**3**	70, 71
				L2	8.4	2e	
Metallo-β-lactamase	*Stenotrophomonas maltophilia*	5B105	Scotland	**XM-A**	**6.8**	**3**	56
				XM-B	5.2–6.6	2be	

[a] Data for carbapenem-hydrolyzing enzymes (functional group 2f and group 3) are in boldface. Classifications in parentheses are based on presumed assignments.
[b] As defined elsewhere (12).
[c] NN, not named.
[d] NC, not characterized.
[e] Enzyme not named; defined as metallo-β-lactamase produced by the *cphA* gene.
[f] Previously named *Aeromonas sobria* (32a).
[g] ND, not detected.
[h] Carbenicillin-hydrolyzing activity was demonstrated, but neither oxacillin nor cloxacillin was assayed. The possibility of a group 2d enzyme should not be dismissed.
[i] Cephase, cephalosporinase; identified on the basis of cephalosporin-hydrolyzing activity not inhibited by EDTA.
[j] Previously named *Pseudomonas maltophilia* or *Xanthomonas maltophilia.*

Aeromonas spp. reported to date produce a complement of three β-lactamases from groups 1, 2, and 3.

Other organisms known to produce a chromosomal metallo-β-lactamase together with a second β-lactamase include *Bacillus cereus* (39), *B. fragilis* (69), and *Stenotrophomonas maltophilia* (70, 71). Of these *S. maltophilia* presents the greatest risk as a pathogenic organism producing a chromosomal metallo-β-lactamase, with clinical resistance to imipenem virtually universal in all strains. Previously, this species was thought to produce only two enzymes, L1 and L2 (Table 1), but recent reports indicate that *S. maltophilia* may produce a wide variety of β-lactamases, both serine β-lactamases and metalloen-

TABLE 2. Biochemical characteristics of group 2f serine-based carbapenem-hydrolyzing β-lactamases[a]

Enzyme	Original host	Relative rate of hydrolysis[b]								IC_{50} for inhibition (μM)			Molecular mass (kDa)	pI	Reference(s)
		IMP	MER	BIA	PEN	AMP	LOR	FOX	ATM	CA	SUL	TZB			
IMI-1	*Enterobacter cloacae*	100	5.9	11	13	210	2,250	0.34	57	0.28	1.8	0.030	32	7.1	64
NmcA	*Enterobacter cloacae*	100	2.5	ND[c]	50	150	ND	<1	94	0.32[d]	10.0[d]	2.0[d]	30	6.9	54
Sme-1	*Serratia marcescens*	100	8.4	10	32	410	380	6.6	ND	14.0	3.3	3.0	30	9.7	13, 53, 84, 87

[a] Abbreviations: IMP, imipenem; MER, meropenem; BIA, biapenem; PEN, benzylpenicillin; AMP, ampicillin; LOR, cephaloridine; FOX, cefoxitin; ATM, aztreonam; CA, clavulanic acid; SUL, sulbactam; TZB, tazobactam; pI, isoelectric point.
[b] Hydrolysis rates were based on V_{max} data where available.
[c] ND, not determined.
[d] K_i with benzylpenicillin as substrate.

zymes. Like the AmpC cephalosporinases in the family *Enterobacteriaceae*, at least two families of β-lactamases can exist among *S. maltophilia* strains that produce enzymes with similar phenotypes but various isoelectric points (19, 56, 58). Further analysis at both the biochemical and molecular levels will be required to determine how, or whether, these enzymes are related.

Although the carbapenem-hydrolyzing enzymes described before 1990 were all chromosomally produced, plasmid-mediated metallo-β-lactamases have recently been reported in Japan (8, 55, 83). These enzymes are appearing in *B. fragilis*, *Pseudomonas aeruginosa*, and members of the family *Enterobacteriaceae* including *S. marcescens* and *Klebsiella pneumoniae*, organisms that generally produce at least one chromosomal enzyme. To date the spread of these carbapenem-resistant organisms and their associated plasmids has been somewhat limited. Only 6 of 610 Japanese strains of *B. fragilis* in 1991 produced a metallo-β-lactamase (7), with no indication as to whether these were chromosomal or plasmid mediated; note, however, that the sequence for the Japanese plasmid-mediated metallo-β-lactamase from *B. fragilis* is identical to that for the chromosomal CcrA (CfiA) β-lactamases sequenced in the United States (8, 65, 79). Four of 105 *S. marcescens* strains in the Aichi Prefecture in Japan were reported to carry the bla_{IMP} gene in 1993 (55). From 1988 to 1992 nine *P. aeruginosa* isolates from Toyama, Japan, were found to express a plasmid-mediated metallo-β-lactamase conferring resistance to imipenem. High-level resistance, however, was associated with the concomitant loss of the outer membrane protein D2 (50). In another recent report 15 nonclonal strains among 3,700 *P. aeruginosa* isolates collected from 1992 to 1994 from 17 general hospitals in Japan were identified as producing a plasmid-mediated metallo-β-lactamase (74). Although only a small number of metallo-β-lactamase-producing strains have been identified, one can confidently predict that plasmid-mediated transfer will result in a much larger problem due to the increased selective pressure from carbapenem use.

BIOCHEMISTRY

Group 2f serine β-lactamases. Carbapenem-hydrolyzing β-lactamases that utilize a catalytically active serine represent the smallest functional group of β-lactamases (12). Although the Sme-1 β-lactamase was initially thought to be a metallo-β-lactamase due to equivocal studies based on EDTA inhibition, later biochemical and molecular studies established the identity of this enzyme as a serine β-lactamase (53). IMI-1 and NMC-A, closely related at the molecular level (see below), share very similar hydrolytic profiles (Table 2). Functionally, the Sme-1 enzyme is also quite closely related to the other two enzymes. Although all three group 2f enzymes have similar molecular masses, the pI values of the enzymes from *E. cloacae* are close to neutrality, in contrast to the highly basic pI for the Sme-1 enzyme. However, this difference does not result in major functional differences.

The biochemical characteristics of all the group 2f enzymes are summarized in Table 2. On the basis of maximum rate of hydrolysis (V_{max}) values, the group 2f enzymes tend to show higher hydrolysis rates for ampicillin and cephaloridine than for imipenem. IMI-1 has higher k_{cat} values and has a catalytic efficiency for cephaloridine better than those of the AmpC enzymes (Table 3), perhaps explaining the maintenance of these NmcA-type enzymes in strains that have not been exposed to the selective pressure of carbapenems. High V_{max} values are also seen for the monobactam aztreonam, a characteristic very different from that of the metallo-β-lactamases (see below and Table 4). Hydrolysis of imipenem is faster than hydrolysis of the newer carbapenems, meropenem or biapenem, for the IMI-1 and Sme-1 enzymes, resulting in higher MICs of imipenem for the organisms producing them (62, 82).

V_{max} values suggest that these enzymes might well be considered cephalosporinases, but the more complete kinetic data in Table 3 indicate that the Sme-1 group 2f enzyme is a poorly efficient cephalosporinase on the basis of k_{cat}/K_m values, a more accurate measure of hydrolytic capacity (14, 42). The IMI-1 enzyme has a more balanced substrate profile, with almost equivalent hydrolytic efficiencies for imipenem and benzylpenicillin; the values are approximately 30% of that for cephaloridine.

Inhibition of these three enzymes is variable; none of the enzymes is inhibited well by sulbactam, with 50% inhibitory concentrations (IC_{50}s) of >1 μM. The two enzymes from *E. cloacae* are inhibited by clavulanic acid at concentrations of less than 1 μM, whereas the Sme-1 enzyme is poorly inhibited by clavulanate. The IMI-1 enzyme appeared to be better inhibited by tazobactam than the Sme-1 enzymes when they were tested under identical conditions. However, the IMI-1-producing organism did not respond well to the piperacillin-tazobactam combination due to the production of both a TEM-type and an AmpC-type enzyme in the same isolate.

Group 3 metallo-β-lactamases. Group 3 β-lactamases have been assumed to belong to a common functional class of metalloenzymes, primarily on the basis of their ability to hydrolyze imipenem at a readily measurable rate, their ability to be inhibited by metal ion chelators such as EDTA or 1,10-*o*-phenanthroline, and their inability to be inhibited by the commercially available β-lactamase inhibitors (12). However, as

TABLE 3. Comparison of kinetic parameters of carbapenem-hydrolyzing β-lactamases and selected serine β-lactamases, including some that may be coproduced in strains with group 2f or group 3 enzymes

Group	β-Lactamase	Imipenem			Benzylpenicillin			Cephaloridine			Reference(s)
		k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (mM^{-1} s^{-1})	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (mM^{-1} s^{-1})	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (mM^{-1} s^{-1})	
1	P99[a]	0.0020	6.6	0.30	56	8.7	6,400	1,000	680	1,500	14
1	AmpC[b]	0.010	0.80	13	45	4.4	10,200	130	170	760	24, 25
1	S7-AmpC[c]	0.025	18	1.4	5.0	28	180	320	2,100	150	87
2b	TEM-1	0.01	10	1.0	1,600	19	84,000	750	660	1,100	63, 76
2f	Sme-1	11	32	340	3.6	66	54	42	1,100	38	87
2f	IMI-1	89	170	520	36	64	560	2,000	1,070	1,870	64
3a	*B. cereus* II	>100	>1,000	120	680	1,500	450	25	1,400	19	22
3a	CcrA	200	270	740	190	40	4,800	42	5.7	7,400	86
3a	IMP-1	83	30	2,800	370	440	840	51	9.2	5,500	46
3a	L1	65	90	720	1,100	50	22,000	28	300	93	22
3b	CphA	140	86	1,600	3.0	630	4.8	0.3	200	1.5	73
3b	AsbM1	71	230	310	1.2	160	7.5	≤0.06	ND[d]	ND	85

[a] From *E. cloacae* P99, related to the AmpC cephalosporinase in *E. cloacae.*
[b] From *E. coli* K-12.
[c] From *S. marcescens* S7.
[d] ND, not determined.

more of these enzymes have been characterized biochemically, three functional subgroups can be distinguished.

Hydrolysis and inhibition data are presented in Table 4. On the basis of the substrate profiles exhibited by the 15 enzymes in Table 4, two major subgroups have been defined, along with a third subgroup that includes a single enzyme. Subgroup 3a includes the metallo-β-lactamases that have a broad spectrum of hydrolytic activity, hydrolyzing either penicillins or cephalosporins at rates at least 60% of that for imipenem. These enzymes also tend to require added Zn^{2+} for maximal activity or are activated by this divalent cation, suggesting a low-affinity binding for Zn^{2+}. Subgroup 3b comprises those metallo-β-lactamases that hydrolyze carbapenems preferentially. To date, the enzymes in this subgroup are from *Aeromonas* spp., *F. odoratum*, and *B. cepacia*. The last subgroup, subgroup 3c, includes only the enzyme from *L. gormanii*, which exhibits extremely rapid hydrolysis for ampicillin and, especially, cephaloridine. Because this enzyme has not been studied as thoroughly as the other metalloenzymes, it is possible that this enzyme may be reclassified at a later time.

As seen in Table 4, not all the group 3 enzymes have been examined for their ability to hydrolyze carbapenems other than imipenem. Therefore, it is difficult to assess the ability of these enzymes to hydrolyze carbapenems as a class. For those enzymes for which meropenem hydrolysis data have been reported, V_{max} values are generally less for the new carbapenem compared to those observed for imipenem. Two exceptions are the group 3a *B. cereus* II enzyme, with hydrolysis an order of magnitude greater for meropenem than for imipenem, and the group 3b AsbM1 enzyme, with hydrolysis for meropenem approximately three times higher than that for imipenem. None of the group 3 enzymes hydrolyzes aztreonam at significant rates. The enzymes of the group are also poorly inhibited by the commercially available β-lactamase inhibitors: clavulanic acid, sulbactam, and tazobactam.

Subgroup 3a enzymes recognize a wide range of substrates, making them an unequaled single threat to a wide variety of β-lactam-containing agents. These enzymes all hydrolyze penicillins and have V_{max} values comparable to or better than that for imipenem. Cephalosporins are usually hydrolyzed more slowly than imipenem. Many of these enzymes have been shown to require Zn^{2+} for activity. As seen in Table 4 the CcrA and L1 group 3a enzymes are strong penicillinases on the basis of k_{cat}/K_m values, but they are not as efficient as the TEM-1 β-lactamase. CcrA and IMP-1 have the highest catalytic efficiencies for cephaloridine of all the enzymes listed in Table 3, again emphasizing the potency of this group of β-lactamases.

Subgroup 3b contains the true carbapenemases, enzymes that exhibit a strong preference for the hydrolysis of carbapenems. Because the enzymes from *Aeromonas* spp. hydrolyze nitrocefin so poorly, these carbapenemases went unrecognized until recently; their presence was not detected on isoelectric focusing gels or in column fractions. It is now known that a carbapenem must be used as substrate either in a gel overlay or for monitoring activity in column fractions in order to identify this subgroup of metallo-β-lactamase. All the enzymes in group 3b can be inhibited by EDTA, indicating chelation of an active-site metal ion. The addition of Zn^{2+} has been shown to restore activity to many of the enzymes after EDTA addition. However, three of the *Aeromonas* enzymes were shown to be inhibited by Zn^{2+} at concentrations of 15 μM or lower (26, 27, 85). The pronounced specificity of the group 3b enzymes is emphasized in Table 3, where high catalytic efficiencies for imipenem are reported, but very poor hydrolysis of either benzylpenicillin or cephaloridine was observed.

Group 3c is comprised of a single metallo-β-lactamase from *L. gormanii*. This enzyme is distinguished by its very high hydrolysis of cephalosporins, including expanded-spectrum cephalosporins and cephamycins. Upon further evaluations, it is possible that this enzyme could be found to be closely related to the group 3a enzyme.

Multiple β-lactamase production. Biochemical advantages for organisms producing multiple β-lactamases become evident when the data from Tables 1 and 3 are compared, especially when the catalytic efficiencies of various enzymes are evaluated. IMI-1 from *E. cloacae* 1413B is produced with an AmpC-type and TEM-type enzyme. As seen in Table 3 IMI-1 conveys a moderate ability to hydrolyze a broad spectrum of β-lactams, whereas the AmpC and TEM enzymes provide excellent penicillin hydrolysis and moderate hydrolysis of ceph-

TABLE 4. Functional subdivisions of group 3 carbapenem-hydrolyzing metallo-β-lactamases that are inhibited by EDTA[a]

Group	Enzyme	Original host	Relative rate of hydrolysis							IC_{50} for inhibition (μM)			Effect of Zn^{2+}[b]	Molecular mass (kDa)	pI	Reference(s)
			IMP	MER	PEN	AMP	LOR	FOX	ATM	CA	SUL	TZB				
3a	II	*Bacillus cereus* 5/B/6[c]	100[d]	1,200	380	600	16	0.1	<0.1	ND[e]	5,200[f]	ND	E	25	8.3[b]	21, 22, 28
3a	CcrA3	*Bacteroides fragilis* QMCN3	100	ND	130	ND	99	22	ND	ND	ND	1,200	A	26	ND	67, 69
3a	CcrA4	*Bacteroides fragilis* QMCN4	100	ND	69	ND	26	6.4	ND	ND	ND	1,400	A	26	ND	67, 69
3a	CcrA	*Bacteroides fragilis* TAL3636	100	56	95	98	21	5.0	<0.01	>500	>500	400	A, R	26	5.2	18, 69, 84, 86
3a		*Pseudomonas aeruginosa* GN17203	100	23	430	130	60	31	<0.6	>100	>100	ND	E, R	28	9.0	83
3a	IMP-1	*Serratia marcescens* TN9106	100	14	440	1,400	62	35	<2	>100	>100	ND	R	ND	>9.5	46, 55
3a	L1	*Stenotrophomonas maltophilia*[g] GN12873	100	44[h]	420	240	25	ND	ND	>100[i]	25[i]	ND	R	118	6.9	71
3a	L1	*Stenotrophomonas maltophilia* ULA-511	100	60	1,700	270	43	1.7	<0.01	>400[h]	>400[h]	>400[h]	E[j], R	ND	5.9	21, 22
3b	CphA/CphA2	*Aeromonas hydrophila* AE036	100	38	2.1	13[k]	0.07	Inac[l]	<0.01	ND	37	ND	ND	28	8.0	22, 73
3b	A2h	*Aeromonas hydrophila* AER 19	100	ND	250[m]	ND	ND	ND	4.7	>40[n]	ND	ND	R[o]	31.5	8.0	29
3b		*Aeromonas hydrophila* 872	100	ND	1.1	19	14	ND	ND	ND	ND	ND	ND	ND	7.1	75
3b	ACP	*Aeromonas hydrophila* G19	100	ND	ND	<10	<10	ND	ND	2,100	ND	ND	I[p]	31	8.2	27
3b	AsbM1	*Aeromonas jandaei*, 14M[q]	100	310	1.8	0.18	<0.06	<0.06	<0.01	>1,000	>1,000	250	I[q]	34	9.1	85
3b	ASA-1	*Aeromonas salmonicida* subsp. *achromogenes* ASA11	100	ND	ND	<1	<1	<1	ND	>1,000	450	6,300	I[r]	19[s]	>7.9	26
3b	ImiS	*Aeromonas sobria* 163a	100	690	0.3	0.7	ND	ND	ND	ND	ND	ND	E/I[t]	ND	9.3	82
3b	PCM-I	*Burkholderia cepacia* 5JIV	100	18	7.7	3.8	24	ND	<6	<100	ND	<100	R	ND	8.5	9
3b		*Flavobacterium odoratum* GN14053	100	ND	20	43	9.5	7.8	<0.01	>100	>100	ND	R	26	5.8	72
3c		*Legionella gormanii* ATCC 33297	100	ND	140	1,300	1,900	200	<20	>100	>100	ND	ND	25	10.5	23

[a] See footnote *a* of Table 2 for definitions of abbreviations.
[b] A, activates; E, essential for activity; R, required for activity after inhibition by EDTA; I, inhibits.
[c] Similar functional characteristics as enzyme from *B. cereus* 569. Differences in gene sequences for metallo-β-lactamases result in 24 amino acid changes. pI of 8.3 is for enzyme from strain 569.
[d] Data presented are ratios of k_{cat}/K_m; no endpoint for V_{max} was attained for imipenem.
[e] ND, not determined.
[f] K_m.
[g] Previously known as *Xanthomonas maltophilia* or *Pseudomonas maltophilia*.
[h] Data for an L1-type enzyme from *S. maltophilia* 1712.
[i] K_i.
[j] Essential for activity at pH <8 for enzyme from strain I ID 1275 (10); essential for detection on isoelectric focusing gels in multiple strains (58).
[k] Based on ratio of k_{cat}/K_m; no endpoint for V_{max} was attained for ampicillin.
[l] Inac, inactivation.
[m] High benzylpenicillin hydrolysis may be due to a second β-lactamase activity (see text).
[n] Imipenem as substrate. IC_{50} of 0.4 μM reported when either nitrocefin or benzylpenicillin was the substrate.
[o] Effects of Zn^{2+} were determined by using nitrocefin as the substrate.
[p] IC_{50} = 15 μM.
[q] IC_{50} = 8 μM.
[r] IC_{50} = 10 μM.
[s] Determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[t] Optimal concentration, 50 μM; protein precipitate at 10 mM Zn^{2+}.

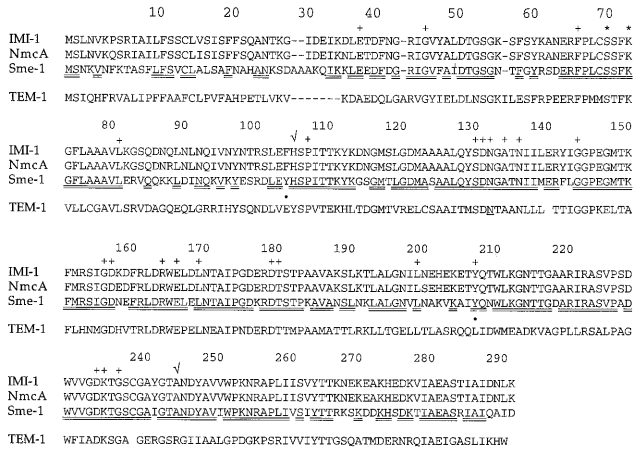

FIG. 1. Amino acid sequence alignment the three class A carbapenem-hydrolyzing β-lactamases and comparison with the class A TEM-1 β-lactamase sequence. IMI-1, *E. cloacae* (GenBank accession number U50278) (64); NmcA, *E. cloacae* (GenBank accession number Z21956) (52); Sme-1, *S. marcescens* (GenBank accession number Z28968) (51), and TEM-1 (GenBank accession number A93821) (5). Dashes indicate gaps that were introduced to optimize the alignment. Numbering is according to Ambler et al. (3). Amino acids conserved among IMI-1, NmcA, and Sme-1 are double underlined. The plus signs and asterisks indicate residues that are highly conserved among all class A serine β-lactamases (35, 53). The asterisks indicate the conserved Ser and Lys of the Ser-X-X-Lys active-site residues of serine β-lactamases. Amino acid positions 105 and 244 are indicated with checkmarks. Amino acid positions 104 and 207 are indicate with a dot above the TEM-1 sequence.

alosporins. The Sme-1 enzyme, when produced by an *S. marcescens* strain with the chromosomal S7-AmpC enzyme, allows the organism to attack imipenem via Sme-1, with more efficient hydrolysis of penicillins and cephaloridine occurring by the AmpC enzyme. Although organisms producing only a subgroup 3a enzyme may be able to withstand assault by most β-lactams, the group 3b enzymes definitely need to be produced in organisms with β-lactamases exhibiting a wider substrate profile than just carbapenems.

SEQUENCES

Three class A serine carbapenem-hydrolyzing enzymes, IMI-1, NMC-A, and Sme-1, have been cloned and their DNA sequences have been determined (52, 54, 64). The two enzymes from *E. cloacae*, IMI-1 and NMC-A, are highly homologous, differing at only seven amino acids, two of which lie within the proposed signal sequence. The third enzyme, Sme-1, from *S. marcescens* S6, is less closely related, but it still shares greater than 70% identity with the two *Enterobacter* enzymes. All three enzymes contain the Ser-X-X-Lys active-site consensus sequence and most amino acid features characteristic of class A β-lactamases (Fig. 1) (35, 52). Asn-132, which is conserved in all the class A β-lactamases, has been proposed to form a hydrogen bond with the hydroxyl of the C-6 hydroxyethyl side chain of carbapenems and may explain the good binding of imipenem to many class A enzymes (78).

When aligned with the functional group 2b TEM-1 enzyme (5), a class A β-lactamase that hydrolyzes imipenem slowly, one major difference in critical amino acids is noted, and this difference may reveal an aspect of why the group 2f class A enzymes hydrolyze carbapenems more efficiently than the TEM enzymes do. Slow hydrolysis of imipenem by the TEM β-lactamase has been described as a biphasic reaction (78). It has been proposed by Easton and Knowles (20) and supported by physical evidence from the laboratory of Taibi and Mobashery (78) that the biphasic property appears to be due to tautomerization of the carbapenem after formation of the acyl enzyme complex. Tautomerization is facilitated by the donation of a proton provided by a water molecule coordinated to the side chain of Arg-244. When Arg-244 was specifically mutated to serine in TEM-1, hydrolysis of imipenem proceeded by monophasic kinetics with a k_{cat} of only 0.04 s^{-1} (78). It should be noted that all three carbapenem-hydrolyzing serine enzymes have alanine in place of arginine at residue 244. Therefore, one can predict that for these enzymes carbapenem hydrolysis would proceed in a linear fashion in the absence of tautomerization. As expected, a monophasic hydrolysis rate for imipenem was observed with the IMI-1 enzyme (11a). A second role for Arg-244 has been proposed: that this residue interacts with the C-3 carboxylate group of the substrate after acylation (34). Replacement of the Arg with Ala would alter this interaction and may result in a faster turnover rate for the group 2f β-lactamases.

Other amino acid substitutions that may contribute to the improved carbapenem hydrolysis rate include amino acid 105 in the conserved box III (35) that is a histidine in all three group 2f enzymes instead of a tyrosine in other class A β-lactamases and a tyrosine at residue 207 in the group 2f enzymes rather than a leucine as in most other class A enzymes. Finally, Glu-104, hydrogen bonded in the TEM-1 enzyme to the conserved Asn-132 and proposed to be critical to the positioning and hydrolysis of β-lactam substrates in class A β-lactamases (38), appears at position 103 in the carbapenem-hydrolyzing class A enzymes. This shift in sequence may affect the positioning of the catalytic water molecule, placing it in a more favorable orientation for the hydrolysis of carbapenems.

Carbapenem-hydrolyzing metallo-β-lactamases are a more diverse group of enzymes than the group 2f β-lactamases. These enzymes all require a metal cofactor. The biological cofactor is probably Zn^{2+}, although other divalent cations, particularly Co^{2+}, can give a functional enzyme (1, 10, 46, 71, 72, 86). The sequences of 12 metallo-β-lactamases from seven different species have been determined (Fig. 2). Four metallo-β-lactamase-encoding genes have been cloned from independent *B. fragilis* isolates: *ccrA* from isolate TAL3636, *cfiA* from isolate TAL2480, and *ccrA3* and *ccrA4* from isolates QMCN3 and QMCN4, respectively (65, 66, 79). The encoded enzymes differ in amino acid sequence by zero to four amino acids; CcrA and CfiA are identical. The metallo-β-lactamase genes *cphA* and *cphA2*, from two independent *A. hydrophila* isolates, have been cloned and sequenced (47, 63a). The two enzymes encoded by these genes share greater then 95% amino acid identity. The sequences of three different *Bacillus* metallo-β-lactamases (β-lactamase II), two from *B. cereus* isolates and one from an alkalophilic *Bacillus* sp. (4, 28, 36, 41), have been determined by amino acid sequence analysis or DNA sequence analysis, or both. The other metallo-β-lactamases whose sequences have been determined are individual representatives each from a different species. Interestingly, two of the enzymes, both identified in Japan, one from *S. marcescens* (IMP-1) (55), and the other from *K. pneumoniae* (GenBank accession number D29636), are identical. The L1 metallo-β-lactamase gene from *S. maltophilia* has also been cloned and sequenced (81).

On a molecular basis, the 12 carbapenem-hydrolyzing metalloenzymes can be divided into three subclasses (Fig. 2). The *Bacillus* spp., *B. fragilis*, *S. marcescens*, and *K. pneumoniae* enzymes form one subclass. These enzymes are all of a similar size (approximately 28 kDa) and share ≥23% sequence identity as a group, and their amino acid sequences can easily be aligned with no or only a few small gaps. Each of these en-

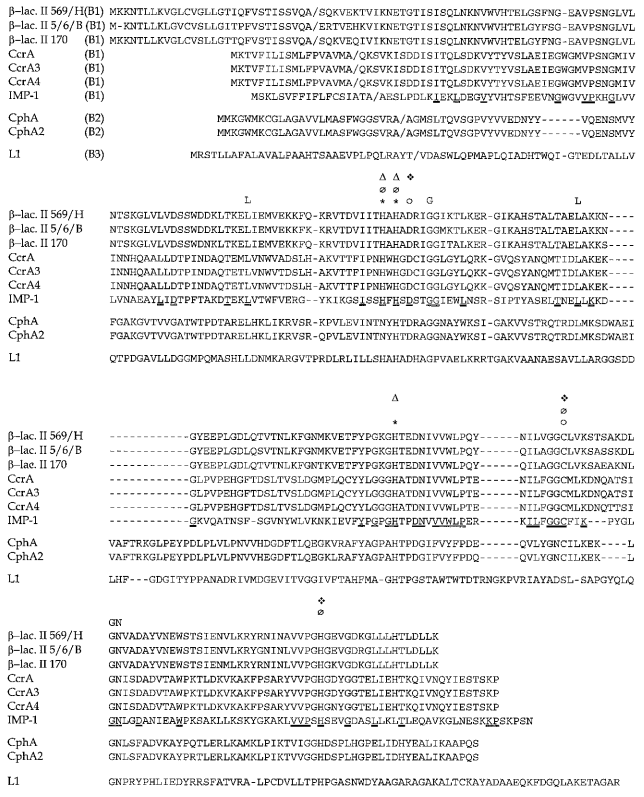

FIG. 2. Amino acid sequence alignment of the 10 unique metallo-β-lactamase sequences. Sequence alignment was performed by first aligning the proteins by using the DNAStar Megalign program. Following this adjustments were made to reduce the number of gaps, place the remaining gaps within predicted loop regions (loops were determined from the secondary structure generated from X-ray crystallographic data of the *B. cereus* β-lactamase II 569H/9 (16), and maintain alignment of the identified active residues. β-lac. II 569/H, *B. cereus* 569/H (GenBank accession number M11189) (28); β-lac. II 5/6/B, *B. cereus* 5/6/B (GenBank accession number M19530) (41); β-lac. II 170, alkalophilic *Bacillus* sp. strain 170 (GenBank accession number M15350) (36); CcrA, *B. fragilis* TAL3636 (identical in amino acid sequence to CfiA from *B. fragilis* TAL2480) (GenBank accession number M63556) (65, 79); CcrA3, *B. fragilis* QMCN3 (66); CcrA4, *B. fragilis* QMCN4 (66); IMP-1, *S. marcescens* TN9106 (GenBank accession number D50438) (55) (identical in amino acid sequence to that of the *K. pneumonia* metallo-β-lactamase from plasmid pDK4-4) (GenBank accession number D29636) (11); CphA, *A. hydrophila* AE036 (GenBank accession number X57102) (47); CphA2, *A. hydrophila* 19 (GenBank accession number U60294); L1, *S. maltophilia* IID 1275 (accession number S45349) (81). Dashes indicate gaps that were introduced to optimize the alignment. Members of the same molecular subclass, B1, B2 or B3, are indicated and grouped. The ø indicates amino acids that were identified by biochemical analysis as interacting with a Zn^{2+} cofactor (6, 28). The asterisks indicate amino acids that were identified by crystal structure as participating in the binding of the Zn^{2+} cofactor in the *B. cereus* 569H/9 enzyme (16). The ○ indicates amino acids that were identified by crystal structure as interacting with a water molecule located within the active site (16). The open triangle indicates amino acids that were identified by crystal structure analysis of the *B. fragilis* CcrA3 enzyme as interacting with Zn1 (17). The ❖ symbol indicates the amino acids that were identified in the CcrA crystal structure as interacting with Zn2 (17). Other fully conserved amino acids are indicated above the alignment. Fully conserved amino acids among subclass B1 are double underlined. Determined and predicted signal sequence cleavage sites are indicated with a slash.

zymes contains the three histidine and one cysteine residues that have been proposed to participate in Zn^{2+} and/or water binding within the active site (Fig. 2) (6, 16, 17, 28).

The *A. hydrophila* enzymes CphA and CphA2 constitute a second subclass. Although they are approximately the same size as the enzymes described above, the CphA enzymes share only ~11% identity (23 of the 50 subclass B1 conserved amino acids) with the first subclass. This second subclass is less well aligned with the other metallo-β-lactamases; several large gaps must be introduced to align the proposed active-site residues. Among these residues CphA and CphA2 contain an asparagine in place of the first histidine residue involved with Zn^{2+} binding.

The *S. maltophilia* L1 enzyme, currently the only member of the third subclass, is the only metallo-β-lactamase that has been identified to function as a multimer composed of four subunits (56, 71). The L1 enzyme shares the least identity with any of the other metallo-β-lactamase enzymes (Fig. 2). Among the proposed active-site residues, the cysteine residue that is believed to participate in Zn^{2+} or water binding has been replaced by a serine.

On the basis of the alignment (Fig. 2), there are only nine fully conserved amino acids among all three subclasses of metallo-β-lactamases. A functional role for most of these amino acids has not been proposed. The proposed molecular subclassification of the metallo-β-lactamases parallels much of the biochemical activity of the various enzymes, the only exception being the separation of the L1 enzyme on a molecular basis from the functional group 3a β-lactamases. It is interesting, therefore, to speculate whether the His-to-Asn change observed in the CphA enzymes is responsible for the limited substrate profile of the group 3b CphA enzymes (73). If so, this substitution is predicted to be present in other *Aeromonas* β-lactamases whose substrate profiles are also restricted to carbapenems.

CRYSTALLOGRAPHY

Although a crystal structure of the metallo-β-lactamase II from *B. cereus* was first reported in 1987 (77), the crystals from which the early data were generated were unstable and difficult to work with. Therefore, the information gathered from the analysis was limited and focused mainly on the metal-binding site. Recently an X-ray crystallographic analysis of a *B. cereus* β-lactamase II was obtained at 2.5-Å resolution (16), followed by the recent publication of the structure of the *B. fragilis* CcrA3 enzyme refined to 1.85-Å resolution (17). Crystal structures of the two enzymes indicate similar folding patterns for the two enzymes, but only one zinc atom was identified in the *B. cereus* enzyme (16), whereas two zinc atoms were at the active site of the *B. fragilis* CcrA3 enzyme (17).

From the *B. cereus* structure three histidine residues, at positions 86, 88 and 149 (Fig. 2), were identified as ligands to a single Zn^{2+} moiety located within the active site. The conserved His-210 and Asp-90 residues, together with the Zn^{2+} moiety, were proposed to interact with an active-site water molecule, resulting in β-lactam hydrolysis (16). These amino acid interactions differ from the earlier biochemical analyses in which the Cys and a different His residue were proposed to interact with one of two Zn^{2+} moieties within the holoenzyme (Fig. 2) (6, 28). An essential role for the proposed water-binding Asp residue is supported by the observation that substitution by either an Asn or Glu residue results in an enzymatically inactive enzyme (40). However, this does not confirm that the essential role of the Asp is to bind water.

In contrast, the model of the *B. fragilis* enzyme (17) is quite different from that suggested for the *B. cereus* enzyme. It is proposed that the two zinc atoms in the *B. fragilis* enzyme share a water (hydroxide) moiety that serves as a ligand for both metal atoms. This hydroxide could be responsible for nucleophilic attack on the carbonyl carbon of a β-lactam. The tetrahedral intermediate formed during the reaction is thought to be stabilized as a result of formation of an oxyanion hole

flanked by the side chain of the conserved Asn-193 and the tetrahedral zinc. Because both of these models are based on native enzyme crystal structures obtained with no substrate or inhibitor at the active site, further studies are necessary to define the role of the catalytic residues more specifically.

PRODUCTION

Production of multiple β-lactamases in the same organism can follow a number of patterns. Expression of all three class A carbapenem-hydrolyzing enzymes is inducible, but is not coordinated with the regulation of other β-lactamases within the cell. For example, imipenem induces expression of both the cephalosporinase and Sme-1 in *S. marcescens*, while cefoxitin is an inducer of the AmpC cephalosporinase only (51). The same induction pattern is seen in *E. cloacae* isolates expressing IMI-1 and NmcA. IMI-1 is inducible with imipenem but not cefoxitin, while cephalosporinase expression is inducible with either β-lactam (64). Induction of these class A enzymes involves a LysR-like regulatory protein, ImiR, NmcR, or SmeR, encoded by an open reading frame immediately upstream of the β-lactamase gene (51, 52, 64). These divergently expressed regulator proteins have been shown to share promoter regions with their respective β-lactamase genes. In the case of *nmcA*, the gene encoding the structural β-lactamase, and *nmcR*, the promoters are overlapping (52). With *smeA* and *smeR* the promoters are face to face but nonoverlapping (51). Studies in which the regulatory protein gene has been deleted have demonstrated that NmcR and SmeR are positive regulators in both the presence and absence of inducer (51, 52). Although induction is observed in response to the addition of β-lactams to the medium, it is not known if the β-lactam or a product of cell wall degradation, resulting from inhibition of penicillin-binding protein activity, is the true inducer (52). No β-lactam-binding motifs, such as a S-X-X-K sequence or a KS/TG sequence, are present within the SmeR, NmcR, or ImiR proteins (51, 52, 64), suggesting that these proteins do not interact with β-lactams.

Expression of the metallo-β-lactamase genes can be either inducible or noninducible. Some of the most interesting expression is seen among the 2.5 to 4% of *B. fragilis* isolates harboring the metallo-β-lactamase gene (7, 60). In the majority of these isolates the gene is expressed at very low levels and does not impart clinical resistance. Induction, leading to increased expression of these genes, has not been observed upon exposure to any β-lactam compound (59, 61). Increased expression leading to clinically relevant resistance has been found to occur either by multiple changes within the promoter (66) or more commonly by insertion sequence (IS) element insertion within the promoter region just upstream from the Shine-Dalgarno box (60, 61, 68). The IS element provides the transcription initiation signals required to produce high-level constitutive expression of the transcript. It is interesting that >80% of *B. fragilis* isolates with a metallo-β-lactamase gene also carry one of three *B. fragilis* IS elements. Two of these IS elements, IS*942* and IS*1186*, are known to promote high-level expression of the metallo-β-lactamase, and the third, IS*4351*, promotes moderate expression of the enzyme when inserted within the β-lactamase promoter (60). These IS elements are rare among *B. fragilis* isolates that do not harbor the metallo-β-lactamase gene (60).

The metallo-β-lactamases produced by *Bacillus* spp., *S. maltophilia*, *A. hydrophila*, and *A. jandaei* are all inducible upon exposure of the cells to various β-lactam compounds. Regulation in *A. jandaei* appears to involve a classical "two-component" signal transduction regulatory system (1a). Constitutive expression of several *A. jandaei* β-lactamases in *E. coli* is dependent on a mutation within the *Escherichia coli creA* to *creD* operon. This operon encodes four proteins that comprise a signal transduction regulatory system of unknown function. An *A. jandaei* chromosomal DNA fragment has been cloned. When this fragment is harbored on a multicopy plasmid in *E. coli*, it leads to the constitutive expression of *A. jandaei* β-lactamases in *E. coli*. When the fragment is introduced into *A. jandaei* on a multicopy plasmid, the expression of all three *A. jandaei* β-lactamases, the AsbM1 metallo-β-lactamase and two serine β-lactamases, AsbA1 and AsbB1, becomes constitutive. DNA sequence analysis indicates that this fragment encodes a protein with characteristics of response regulator proteins (1a). Whether a signal transduction regulatory system is unique to *A. jandaei* or is also used to regulate the expression of other inducible metallo-β-lactamases is unknown.

The majority of the carbapenem-hydrolyzing β-lactamase genes are chromosomally encoded. This has certainly contributed to the slow spread of these enzymes, and thus to the slow increase in β-lactamase-mediated resistance to carbapenems. However, resistance patterns may be changing, and enzymes that have for many years been relatively rare may become a real and increasingly visible threat to the use of β-lactam chemotherapy. The metallo-β-lactamase of *B. fragilis* now lies on a relatively small plasmid of 13.6 kb that appears to be self-transmissible (8). In Japan resistance to carbapenems among *B. fragilis* isolates has increased from 2.0% in 1987 to 5.9% in 1991 (7). It is not known to what extent low-level resistance is β-lactamase mediated. However, high-level resistance (50 μg/ml) is associated with the expression of a metallo-β-lactamase. One of the most sobering reports is the identification of a plasmid-associated gene encoding a metallo-β-lactamase from *K. pneumoniae* (GenBank accession number D29636). The coding sequence of this metallo-β-lactamase gene is identical to that of the metallo-β-lactamase gene cloned from *S. marcescens* (34). However, the upstream DNA sequence diverged approximately 20 base pairs 5′ of the initiation codon. A search of the GenBank DNA sequences (11) against the upstream sequence of the *K. pneumoniae* gene indicated nearly 100% identity between this sequence and the *tnpI* integrase gene of Tn*21*-like transposons (49, 88). The metallo-β-lactamase gene is located within a recombination repeat unit which lies at the 3′ end of *tnpI* (49, 88). This observation indicates that the metallo-β-lactamase gene probably resides on a transposable element, on a plasmid, in a strain known for sharing its plasmids with other bacteria. *K. pneumoniae* has been the originating strain for many of the extended-spectrum serine β-lactamases (31, 32); now it appears that it may be able to contribute to the dissemination of metallo-β-lactamases among other members of the family *Enterobacteriaceae*.

CONCLUSIONS

Perhaps as a result of the restricted use of imipenem for serious nosocomial infections, the distribution of carbapenem-hydrolyzing enzymes has been limited compared to the prevalence of other β-lactamases. However, the recent appearance of plasmid-mediated metallo-β-lactamases in members of the family *Enterobacteriaceae* in Japan demonstrates the ability of carbapenem-hydrolyzing enzymes to be transferred among localized hospitals (30, 50, 74). The functional group 3a enzymes pose a particular threat to most of the β-lactam armamentarium. Transmission appears to be rather promiscuous, because the relatively small outbreaks reported do not seem to be due to clonal strains. In the past carbapenems have been a mainstay of the infectious disease community for serious infections.

However, with more frequent use they will inevitably become responsible for the appearance of more resistant organisms. Therefore, it is critical that these agents be used judiciously in an attempt to minimize the selection of novel carbapenem-hydrolyzing enzymes.

REFERENCES

1. **Abraham, E. P., and S. G. Waley.** 1979. β-Lactamases from *Bacillus cereus*, p. 311–338. *In* J. M. T. Hamilton-Miller and J. T. Smith (ed.), Beta-lactamases. Academic Press, London, United Kingdom.

1a. **Alksne, L. E., and B. A. Rasmussen.** Unpublished data.

2. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. London Biol. **289:**321–331.
3. **Ambler, R. P., A. F. W. Coulson, J.-M. Frère, J.-M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
4. **Ambler, R. P., M. Daniel, J. Fleming, J.-M. Hermoso, C. Pang, and S. G. Waley.** 1985. The amino acid sequence of the zinc-requiring β-lactamase II from the bacterium *Bacillus cereus* 569. FEBS Letters **189:**207–211.
5. **Ambler, R. P., and G. K. Scott.** 1978. Partial amino acid sequence of penicillinase coded by *Escherichia coli* plasmid R6K. Proc. Natl. Acad. Sci. USA **75:**3732–3736.
6. **Baldwin, G. S., S. G. Waley, and E. P. Abraham.** 1979. Identification of histidine residues that act as zinc ligands in β-lactamase II by differential tritium exchange. Biochem. J. **179:**459–463.
7. **Bandoh, K., K. Ueno, K. Watanabe, and N. Kato.** 1993. Susceptibility patterns and resistance to imipenem in the *Bacteroides fragilis* group species in Japan: a 4-year study. Clin. Infect. Dis. **16**(Suppl. 4)**:**S382–S386.
8. **Bandoh, K., K. Watanabe, Y. Muto, Y. Tanaka, N. Kato, and K. Ueno.** 1992. Conjugal transfer of imipenem resistance in *Bacteroides fragilis*. J. Antibiot. (Tokyo) **45:**542–547.
9. **Baxter, I. A., and P. A. Lambert.** 1994. Isolation and partial purification of a carbapenem-hydrolysing metallo-β-lactamase from *Pseudomonas cepacia*. FEMS Microbiol. Lett. **122:**251–256.
10. **Bicknell, R., E. L. Emanuel, J. Gagnon, and S. G. Waley.** 1985. The production and molecular properties of the zinc β-lactamase of *Pseudomonas maltophilia* IID 1275. Biochem. J. **229:**791–797.
11. **Bilofsky, H. S., and C. Burks.** 1988. The GenBank (R) genetic sequence data bank. Nucleic Acids Res. **16:**1861–1864.

11a. **Bush, K.** Unpublished data.

12. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
13. **Bush, K., C. Macalintal, B. A. Rasmussen, V. J. Lee, and Y. Yang.** 1993. Kinetic interactions of tazobactam with β-lactamases from all major structural classes. Antimicrob. Agents Chemother. **37:**851–858.
14. **Bush, K., and R. B. Sykes.** 1986. Methodology for the study of β-lactamases. Antimicrob. Agents Chemother. **30:**6–10.
15. **Bush, K., S. K. Tanaka, D. P. Bonner, and R. B. Sykes.** 1985. Resistance caused by decreased penetration of β-lactam antibiotics into *Enterobacter cloacae*. Antimicrob. Agents Chemother. **27:**555–560.
16. **Carfi, A., S. Pares, E. Dunee, M. Galleni, C. Duez, J. M. Frere, and O. Dideberg.** 1995. The 3-D structure of a zinc metallo-β-lactamase from *Bacillus cereus* reveals a new type of protein fold. EMBO J. **14:**4914–4921.
17. **Concha, N. O., B. A. Rasmussen, K. Bush, and O. Herzberg.** 1996. Crystal structure of the wide-spectrum binuclear zinc β-lactamase from *Bacteroides fragilis*. Structure **4:**823–836.
18. **Cuchural, G. J., Jr., M. H. Malamy, and F. P. Tally.** 1986. β-Lactamase-mediated imipenem resistance in *Bacteroides fragilis*. Antimicrob. Agents Chemother. **30:**645–648.
19. **Cullmann, W., and W. Dick.** 1990. Heterogeneity of β-lactamase production in *Pseudomonas maltophilia*, a nosocomial pathogen. Chemotherapy (Basel) **36:**117–126.
20. **Easton, C. J., and J. R. Knowles.** 1982. Inhibition of the RTEM β-lactamase from *Escherichia coli*. Interaction of the enzyme with derivatives of olivanic acid. Biochemistry **21:**2857–2862.
21. **Felici, A., and G. Amicosante.** 1995. Kinetic analysis of extension of substrate specificity with *Xanthomonas maltophilia*, *Aeromonas hydrophila*, and *Bacillus cereus* metallo-β-lactamases. Antimicrob. Agents Chemother. **39:**192–199.
22. **Felici, A., G. Amicosante, A. Oratore, R. Strom, P. Ledent, B. Joris, L. Fanuel, and J.-M. Frère.** 1993. An overview of the kinetic parameters of class B β-lactamases. Biochem. J. **291:**151–155.
23. **Fuji, T., K. Sato, K. Miyata, M. Inoue, and S. Mitsuhashi.** 1986. Biochemical properties of β-lactamase produced by *Legionella gormanii*. Antimicrob. Agents Chemother. **29:**925–926.
24. **Galleni, M., G. Amicosante, and J.-M. Frère.** 1988. A survey of the kinetic parameters of class C β-lactamases. Cephalosporins and other β-lactam compounds. Biochem. J. **255:**123–129.
25. **Galleni, M., and J.-M. Frère.** 1988. A survey of the kinetic parameters of class C β-lactamases. Penicillins. Biochem. J. **255:**119–122.
26. **Hayes, M. V., C. J. Thomson, and S. G. B. Amyes.** 1994. Three beta-lactamases isolated from *Aeromonas salmonicida*, including a carbapenemase not detectable by conventional methods. Eur. J. Clin. Microbiol. Infect. Dis. **13:**805–811.
27. **Hayes, M. V., C. J. Thomson, and S. G. B. Amyes.** 1996. The 'hidden' carbapenemase of *Aeromonas hydrophila*. J. Antimicrob. Chemother. **37:**33–44.
28. **Hussain, M., A. Carlino, M. J. Madonna, and J. O. Lampen.** 1985. Cloning and sequencing of the metallothioprotein β-lactamase II gene of *Bacillus cereus* 569/H in *Escherichia coli*. J. Bacteriol. **164:**223–229.
29. **Iaconis, J. P., and C. C. Sanders.** 1990. Purification and characterization of inducible β-lactamases in *Aeromonas* spp. Antimicrob. Agents Chemother. **34:**44–51.
30. **Ito, H., Y. Arakawa, S. Ohsuka, R. Wacharotayankun, N. Kato, and M. Ohta.** 1995. Plasmid-mediated dissemination of the metallo-β-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. Antimicrob. Agents Chemother. **39:**824–829.
31. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
32. **Jacoby, G. A., and L. Sutton.** 1991. Properties of plasmids responsible for production of extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**164–169.

32a. **Janda, J. M.** Personal communication.

33. **Jaurin, B., and T. Grundstrom.** 1981. *ampC* cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. Proc. Natl. Acad. Sci. USA **78:**4897–4901.
34. **Jelsch, C., L. Mourey, J.-M. Masson, and J.-P. Samama.** 1993. Crystal structure of *E. coli* TEM1 β-lactamase at 1.8 Å resolution. Proteins Struct. Funct. Genet. **16:**364–383.
35. **Joris, B., J.-M. Ghuysen, G. Dive, A. Renard, O. Dideberg, P. Charlier, J.-M. Frère, J. A. Kelly, J. C. Boyington, P. C. Moews, and J. R. Knox.** 1988. The active-site-serine penicillin-recognizing enzymes as members of the *Streptomyces* R61 DD-peptidase family. Biochem. J. **250:**313–324.
36. **Kato, C., T. Kudo, K. Watanabe, and K. Horikoshi.** 1985. Nucleotide sequence of the β-lactamase gene of alkalophilic *Bacillus* sp. strain 170. J. Gen. Microbiol. **131:**3317–3324.
37. **Khushi, T., D. J. Payne, A. Fosberry, and C. Reading.** 1996. Production of metal dependent β-lactamases by clinical strains of *Bacteroides fragilis* isolated before 1987. J. Antimicrob. Chemother. **37:**345–350.
38. **Knox, J. R.** 1995. Extended-spectrum and inhibitor-resistant TEM-type β-lactamases: mutations, specificity, and three-dimensional structure. Antimicrob. Agents Chemother. **39:**2593–2601.
39. **Kuwabara, S., and E. P. Abraham.** 1967. Some properties of two extracellular β-lactamases from *Bacillus cereus* 569/H. Biochem. J. **103:**27c–30c.
40. **Lim, H. M., R. K. Iyer, and J. J. Pène.** 1991. Site-directed mutagenesis of dicarboxylic acids near the active site of *Bacillus cereus* 5/B/6 β-lactamase II. Biochem. J. **276:**401–404.
41. **Lim, H. M., J. J. Pene, and R. Shaw.** 1988. Cloning, nucleotide sequence, and expression of the *Bacillus cereus* 5/B/6 β-lactamase II structural gene. J. Bacteriol. **170:**2873–2878.
42. **Livermore, D. M.** 1985. Do β-lactamases 'trap' cephalosporins? J. Antimicrob. Chemother. **15:**511–514.
43. **Livermore, D. M.** 1992. Carbapenemases. J. Antimicrob. Chemother. **29:**609–613.
44. **Livermore, D. M.** 1993. Carbapenemases: the next generation of β-lactamases? ASM News **59:**129–135.
45. **Livermore, D. M.** 1995. β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. **8:**557–584.
46. **Marumo, K., A. Takeda, Y. Nakamura, and K. Nakaya.** 1995. Purification and characterization of metallo-β-lactamase from *Serratia marcescens*. Microbiol. Immunol. **39:**27–33.
47. **Massida, O., G. M. Rossolini, and G. Satta.** 1991. The *Aeromonas hydrophila cphA* gene: molecular heterogeneity among class B metallo-β-lactamases. J. Bacteriol. **173:**4611–4617.
48. **Medeiros, A. A., and R. S. Hare.** 1986. Beta-lactamase mediated resistance to penems and carbapenems among *Enterobacteriaceae*, abstr. 116, p. 117. *In* Programs and abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
49. **Mercier, J., J. Lachapelle, F. Couture, M. Lafond, G. Vezina, M. Boissinot, and R. C. Levesque.** 1990. Structural and functional characterization of *tnpI*, a recombinase locus in Tn*21* and related β-lactamase transposons. J. Bacteriol. **172:**3745–3757.
50. **Minami, S., M. Akama, H. Araki, Y. Watanabe, H. Narita, S. Iyobe, and S. Mitsuhashi.** 1996. Imipenem and cephem resistant *Pseudomonas aeruginosa* carrying plasmids coding for class B β-lactamase. J. Antimicrob. Chemother. **37:**433–444.
51. **Naas, T., D. M. Livermore, and P. Nordmann.** 1995. Characterization of an LysR family protein, SmeR from *Serratia marcescens* S6, its effect on expression of the carbapenem-hydrolyzing β-lactamase Sme-1, and comparison of this regulator with other β-lactamase regulators. Antimicrob. Agents Chemother. **39:**629–637.

52. **Naas, T., and P. Nordmann.** 1994. Analysis of a carbapenem-hydrolyzing class A β-lactamase from *Enterobacter cloacae* and of its LysR-type regulatory protein. Proc. Natl. Acad. Sci. USA **91:**7693–7697.
53. **Naas, T., L. Vandel, W. Sougakoff, D. M. Livermore, and P. Nordmann.** 1994. Cloning and sequence analysis of the gene for a carbapenem-hydrolyzing class A β-lactamase, Sme-1, from *Serratia marcescens* S6. Antimicrob. Agents Chemother. **38:**1262–1270.
54. **Nordmann, P., S. Mariotte, T. Naas, R. Labia, and M.-H. Nicolas.** 1993. Biochemical properties of a carbapenem-hydrolyzing β-lactamase from *Enterobacter cloacae* and cloning of the gene into *Escherichia coli*. Antimicrob. Agents Chemother. **37:**939–946.
55. **Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura, and N. Kato.** 1994. Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob. Agents Chemother. **38:**71–78.
56. **Paton, R., R. S. Miles, and S. G. B. Amyes.** 1994. Biochemical properties of inducible β-lactamases produced from *Xanthomonas maltophilia*. Antimicrob. Agents Chemother. **38:**2143–2149.
57. **Payne, D. J.** 1993. Metallo-β-lactamases—a new therapeutic challenge. J. Med. Microbiol. **39:**93–99.
58. **Payne, D. J., R. Cramp, J. H. Pateson, J. Neale, and D. Knowles.** 1994. Rapid identification of metallo- and serine β-lactamases. Antimicrob. Agents Chemother. **38:**991–996.
59. **Podglajen, I., J. Breuil, F. Bordon, L. Gutmann, and E. Collatz.** 1992. A silent carbapenemase gene in strains of *Bacteroides fragilis* can be expressed after a one-step mutation. FEMS Microbiol. Lett. **70:**21–29.
60. **Podglajen, I., J. Breuil, I. Casin, and E. Collatz.** 1995. Genotypic identification of two groups within the species *Bacteroides fragilis* by ribotyping and by analysis of PCR-generated fragment patterns and insertion sequence content. J. Bacteriol. **177:**5270–5275.
61. **Podglajen, I., J. Breuil, and E. Collatz.** 1994. Insertion of a novel DNA sequence, IS*1186*, upstream of the silent carbapenemase gene *cfiA*, promotes expression of carbapenem resistance in clinical isolates of *Bacteroides fragilis*. Mol. Microbiol. **12:**105–114.
62. **Prince, A., M. S. Wood, G. S. Cacalano, and N. X. Chin.** 1988. Isolation and characterization of a penicillinase from *Pseudomonas cepacia* 249. Antimicrob. Agents Chemother. **32:**838–843.
63. **Raquet, X., J. Lamotte-Brasseur, E. Fonzé, S. Goussard, P. Courvalin, and J.-M. Frére.** 1994. TEM β-lactamase mutants hydrolysing third-generation cephalosporins. A kinetic and molecular modelling analysis. J. Mol. Biol. **244:**625–639.

63a. **Rasmussen, B. A.** Unpublished data.

64. **Rasmussen, B. A., K. Bush, D. Keeney, Y. Yang, R. Hare, C. O'Gara, and A. A. Medeiros.** 1996. Characterization of IMI-1 β-lactamase, a novel class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob. Agents Chemother. **40:**2080–2086.
65. **Rasmussen, B. A., Y. Gluzman, and F. P. Tally.** 1990. Cloning and sequencing of the class B β-lactamase gene (*ccrA*) from *Bacteroides fragilis* TAL3636. Antimicrob. Agents Chemother. **34:**1590–1592.
66. **Rasmussen, B. A., Y. Gluzman, and F. P. Tally.** 1991. *Escherichia coli* chromosomal mutations that permit direct cloning of the *Bacteroides fragilis* metallo-β-lactamase gene, *ccrA*. Mol. Microbiol. **5:**1211–1219.
67. **Rasmussen, B. A., D. Keeney, Y. Yang, and K. Bush.** 1994. Cloning and expression of a cloxacillin hydrolyzing enzyme and a cephalosporinase from *Aeromonas sobria* AER 14M in *Escherichia coli*: requirement for an *E. coli* chromosomal mutation for efficient expression of the class D enzyme. Antimicrob. Agents Chemother. **38:**2078–2085.
68. **Rasmussen, B. A., and E. Kovacs.** 1991. Identification and DNA sequence of a new *Bacteroides fragilis* insertion sequence-like element. Plasmid **25:**141–144.
69. **Rasmussen, B. A., Y. Yang, N. Jacobus, and K. Bush.** 1994. Contribution of enzymatic properties, cell permeability, and enzyme expression to microbiological activities of β-lactams in three *Bacteroides fragilis* isolates that harbor a metallo-β-lactamase gene. Antimicrob. Agents Chemother. **38:**2116–2120.
70. **Saino, Y., M. Inoue, and S. Mitsuhashi.** 1984. Purification and properties of an inducible cephalosporinase from *Pseudomonas maltophilia* GN12873. Antimicrob. Agents Chemother. **25:**362–365.
71. **Saino, Y., F. Kobayashi, M. Inoue, and S. Mitsuhashi.** 1982. Purification and properties of inducible penicillin β-lactamase isolated from *Pseudomonas maltophilia*. Antimicrob. Agents Chemother. **22:**564–570.
72. **Sato, K., T. Fujii, R. Okamoto, M. Inoue, and S. Mituhashi.** 1985. Biochemical properties of β-lactamase produced by *Flavobacterium odoratum*. Antimicrob. Agents Chemother. **27:**612–614.
73. **Segatore, B., O. Massidda, G. Satta, D. Setacci, and G. Amicosante.** 1993. High specificity of *cphA*-encoded metallo-β-lactamase from *Aeromonas hydrophila* AE036 for carbapenems and its contribution to β-lactam resistance. Antimicrob. Agents Chemother. **37:**1324–1328.
74. **Senda, K., Y. Arakawa, K. Nakashima, H. Ito, S. Ichiyama, K. Shimokata, N. Kato, and M. Ohta.** 1996. Multifocal outbreaks of metallo-β-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. Antimicrob. Agents Chemother. **40:**349–353.
75. **Shannon, K., A. King, and I. Phillips.** 1986. β-Lactamases with high activity against imipenem and Sch 34343 from *Aeromonas hydrophila*. J. Antimicrob. Chemother. **17:**45–50.
76. **Sowek, J. A., S. B. Singer, S. Ohringer, M. F. Malley, T. J. Dougherty, J. Z. Gougoutas, and K. Bush.** 1991. Substitution of lysine at position 104 or 240 of TEM-1pTZ18R β-lactamase enhances the effect of serine-164 substitution on the hydrolysis or affinity for cephalosporins and the monobactam aztreonam. Biochemistry **30:**3179–3188.
77. **Sutton, B. J., P. J. Artymiuk, A. E. Cordero-Borboa, C. Little, D. C. Phillips, and S. G. Waley.** 1987. An X-ray-crystallographic study of β-lactamase II from *Bacillus cereus* at 0.35 nm resolution. Biochem. J. **248:**181–188.
78. **Taibi, P., and S. Mobashery.** 1995. Mechanism of turnover of imipenem by the TEM β-lactamase revisited. J. Am. Chem. Soc. **117:**7600–7605.
79. **Thompson, J. S., and M. H. Malamy.** 1990. Sequencing the gene for an imipenem-cefoxitin-hydrolyzing enzyme (CfiA) from *Bacteroides fragilis* TAL2480 reveals strong similarity between CfiA and *Bacillus cereus* β-lactamase II. J. Bacteriol. **172:**2584–2593.
80. **Walsh, T. R., S. Gamblin, D. C. Emery, A. P. MacGowan, and P. M. Bennett.** 1996. Enzyme kinetics and biochemical analysis of ImiS, the metallo-β-lactamase from *Aeromonas sobria* 163a. J. Antimicrob. Chemother. **37:**423–441.
81. **Walsh, T. R., L. Hall, S. J. Assinder, W. W. Nichols, S. J. Cartwright, A. P. MacGowan, and P. M. Bennett.** 1994. Sequence analysis of the L1 metallo-β-lactamase from *Xanthomonas maltophilia*. Biochem. Biophys. Acta **1218:**199–201.
82. **Walsh, T. R., D. J. Payne, A. P. MacGowan, and P. M. Bennett.** 1995. A clinical isolate of *Aeromonas sobria* with three chromosomally mediated inducible β-lactamases: a cephalosporinase, a penicillinase and a third enzyme, displaying carbapenemase activity. J. Antimicrob. Chemother. **35:**271–279.
83. **Watanabe, M., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **35:**147–151.
84. **Yang, Y., N. Bhachech, and K. Bush.** 1995. Biochemical comparison of imipenem, meropenem and biapenem: permeability, binding to penicillin-binding proteins, and stability to hydrolysis by β-lactamases. J. Antimicrob. Chemother. **35:**75–84.
85. **Yang, Y., and K. Bush.** 1996. Biochemical characterization of the carbapenem-hydrolyzing β-lactamase AsbM1 from *Aeromonas sobria* AER14M: a member of a novel subgroup of metallo-β-lactamases. FEMS Microbiol. Lett. **137:**193–200.
86. **Yang, Y., B. A. Rasmussen, and K. Bush.** 1992. Biochemical characterization of the metallo-β-lactamase CcrA from *Bacteroides fragilis* TAL3636. Antimicrob. Agents Chemother. **36:**1155–1157.
87. **Yang, Y., P.-C. Wu, and D. M. Livermore.** 1990. Biochemical characterization of a β-lactamase that hydrolyzes penems and carbapenems from two *Serratia marcescens* isolates. Antimicrob. Agents Chemother. **34:**755–758.
88. **Zuhlsdorf, M. T., and B. Wiedemann.** 1993. Functional and physiological characterization of the Tn*21* cassette for resistance genes in Tn*2426*. J. Gen. Microbiol. **139:**995–1002.