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MINIREVIEW

A Functional Classification Scheme for β-Lactamases and Its Correlation with Molecular Structure

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INTRODUCTION

A classification scheme for β-lactamases based on functional characteristics is presented. Three major groups of enzymes are defined by their substrate and inhibitor profiles: group 1 cephalosporinases that are not well inhibited by clavulanic acid; group 2 penicillinases, cephalosporinases, and broad-spectrum β-lactamases that are generally inhibited by active site-directed β-lactamase inhibitors; and the group 3 metallo-β-lactamases that hydrolyze penicillins, cephalosporins, and carbapenems and that are poorly inhibited by almost all β-lactam-containing molecules. Functional characteristics have been correlated with molecular structure in a dendrogram for those enzymes with known amino acid sequences.

β-Lactamases (EC 3.5.2.6) have been designated by the Nomenclature Committee of the International Union of Biochemistry as "enzymes hydrolysing amides, amidines and other C—N bonds . . . separated on the basis of the substrate: . . . cyclic amides" (323). These enzymes are the major cause of bacterial resistance to β-lactam antibiotics and have been the subject of extensive microbiological, biochemical, and genetic investigations. Investigators have described more than 190 unique bacterial proteins with the ability to interact with the variety of β-lactam-containing molecules that can serve as sub-

TABLE 1. Classification schemes for bacterial β-lactamases

Bush-Jacoby-Medeiros group	1989 Bush group (44)	Richmond-Sykes class (253)	Mitsuhashi-Inoue type (194)[a]	Molecular class (2, 121, 132)	Preferred substrates	Inhibited by:		Representative enzymes
						CA[b]	EDTA	
1	1	Ia, Ib, Id	CSase	C	Cephalosporins	−	−	AmpC enzymes from gram-negative bacteria; MIR-1
2a	2a	Not included	PCase V	A	Penicillins	+	−	Penicillinases from gram-positive bacteria
2b	2b	III	PCase I	A	Penicillins, cephalosporins	+	−	TEM-1, TEM-2, SHV-1
2be	2b′	Not included except K1 in class IV	CXase	A	Penicillins, narrow-spectrum and extended-spectrum cephalosporins, monobactams	+	−	TEM-3 to TEM-26, SHV-2 to SHV-6, *Klebsiella oxytoca* K1
2br	Not included	Not included	Not included	A	Penicillins	±	−	TEM-30 to TEM-36, TRC-1
2c	2c	II, V	PCase IV	A	Penicillins, carbenicillin	+	−	PSE-1, PSE-3, PSE-4
2d	2d	V	PCase II, PCase III	D	Penicillins, cloxacillin	±	−	OXA-1 to OXA-11, PSE-2 (OXA-10)
2e	2e	Ic	CXase	A	Cephalosporins	+	−	Inducible cephalosporinases from *Proteus vulgaris*
2f	Not included	Not included	Not included	A	Penicillins, cephalosporins, carbapenems	+	−	NMC-A from *Enterobacter cloacae*, Sme-1 from *Serratia marcescens*
3	3	Not included	Not included	B	Most β-lactams, including carbapenems	−	+	L1 from *Xanthomonas maltophilia*, CcrA from *Bacteroides fragilis*
4	4	Not included	Not included	ND[c]	Penicillins	−	?	Penicillinase from *Pseudomonas cepacia*

[a] Csase, cephalosporinase; PCase, penicillinase; CXase, cefuroxime-hydrolyzing β-lactamase.
[b] CA, clavulanic acid.
[c] ND, not determined.

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TABLE 2. Group 1: cephalosporin-hydrolyzing β-lactamases poorly inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												
				LOR	LOT	PEN	AMP	CARB	CLOX	OXA	FOX	NCF	TAX	TAZ	ATM	IMP
	ND	*Acinetobacter calcoaceticus*	ML4961	100	470	0.46	<0.1	ND[b]	ND	ND	0.4	ND	<0.1	ND	ND	ND
	Chr	*Acinetobacter calcoaceticus*	NCTC 7844	100[d]	63	3	1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	ND	*Acinetobacter calcoaceticus*	CCM 5593	100	830	24	5	ND	ND	ND	ND	ND	ND	ND	ND	ND
A1	Chr	*Aeromonas hydrophila*	AER19M	ND	100[e]	3	ND	ND	<0.3	ND	ND	370	1.1	0.3	1.5	<0.03
AsbA1	Chr	*Aeromonas sobria*	AER 14M	100	84	32	ND	ND	ND	ND	ND	ND	≤0.3	ND	ND	≤1
	ND	*Bacteroides intermedius*	GAI4874	100	30	ND	<1	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Chr	*Chromobacterium violaceum*		100[g, h]	60	32	1.3	1.9	NDet[i]	NDet	NDet	ND	ND	ND	ND	ND
	Chr	*Citrobacter freundii*	GN346	100	11	1.5[d]	0.07[d]	<0.1[g]	<0.1[g]	ND	0.2	ND	ND	<0.01	ND	ND
AmpC	Chr	*Citrobacter freundii*	OS60[j]	100	29	4.4	0.93	<0.01	<0.01	<0.01	0.05	47	<0.01	ND	<0.01	<0.01
Type A	Chr	*Enterobacter cloacae*	Multiple[l]	100	310	20	0.30	ND	<0.01	<0.01	0.01	130	<0.1	0.01	<0.01	<0.01
P99	Chr	*Enterobacter cloacae*	P99[m]	100	18	1.5	0.02	0.01	0.01	<0.01	0.01	110	<0.1	<0.01	<0.01	<0.01
AmpC	Chr	*Enterobacter cloacae*	MHN1	100	120	3	2	<1	1	ND	<1	ND	<1	<1	ND	ND
AmpC	Chr	*Escherichia coli*	K12[p]	100	230	35	3.2	<0.01	<0.01	ND	0.15	380	0.13	ND	<0.01	<0.01
	ND	*Escherichia coli*	87120702	100[g, q]	130	19	2	<1	<1	ND	<1	ND	<1	3	ND	ND
	ND	*Escherichia coli*	GN5482	100	420	90	<1	<1	<1	<1	<1	ND	<1	ND	ND	ND
BIL-1	P	*Escherichia coli*	BS	100[r]	1.2	NDet	NDet	NDet	ND	ND	ND	170	NDet	NDet	ND	ND
FOX-1	pGLK1	*Klebsiella pneumoniae*[t]	BA32	100	380	1.0	ND	ND	ND	ND	0.7	ND	ND	ND	ND	ND
LAT-1	pHP15	*Klebsiella pneumoniae*	P20	100	130	5	1	<1	<1	ND	<1	ND	<1	1	ND	ND
MIR-1	pMG230	*Klebsiella pneumoniae*	96D	100[g]	120	4	1	<1	1	ND	<1	ND	10	3	ND	ND
MOX-1	pRMOX1	*Klebsiella pneumoniae*	NU2936	100	ND	ND	40	ND	ND	ND	ND	ND	200	1.5	80	ND
	Chr	*Morganella morganii*	GN5407[x]	100	46	16	<0.01	<0.01	<0.01	ND	<0.01	ND	<0.01	ND	ND	ND
	Chr	*Morganella morganii*	1510	100	37	8.2	0.55	<0.1[g]	<0.1[g]	ND	0.034	ND	ND	ND	ND	ND
CEP-1	R22K	*Proteus mirabilis*	22	100[v]	160	35	1.0	0.28	0.21	<0.1	ND	ND	ND	ND	ND	ND
	Chr	*Proteus rettgeri*	GN4430	100	85	3.3	0.70	0.1	0.1	ND	0.1	ND	0.10	ND	ND	ND
S&A	Chr	*Pseudomonas aeruginosa*	NCTC 8203[y]	100[d, g]	140	33	2	0.63	<0.3	ND	0.5	ND	<1	<1	ND	ND
AmpC	Chr	*Pseudomonas aeruginosa*	PAO1	100	ND	ND	70	ND	ND	ND	ND	ND	0.45	ND	ND	ND
	Chr	*Pseudomonas aeruginosa*	GN10362	100	140	29[d]	<1	<1	<1	ND	<1	ND	<1	ND	ND	<1
	ND	*Pseudomonas aeruginosa*	GN918	100[d]	7	13	1	<0.5	<0.5	<0.5	ND	ND	ND	ND	ND	ND
	ND	*Rhodobacter sphaeroides*	Y-1	100[h]	3400	100[d, z]	6[d, z]	<6[d, z]	<6[d, z]	ND	ND	ND	ND	ND	ND	ND
	Chr	*Serratia marcescens*	SC 8247[aa]	100	100	6.8	0.04	<0.1	<0.1	ND	0.001	110	0.16	<0.1	<0.01	<0.01
S2	Chr	*Serratia marcescens*	SC 9782	100	ND	ND	0.03	ND	ND	ND	ND	ND	0.05	ND	0.04	ND
	ND	*Serratia marcescens*	921/79	100[ab]	540	24	2.9	ND	ND	ND	NDet	ND	0.37	<0.05	<0.01	<0.01

[a] Abbreviations: LOR, cephaloridine; LOT, cephalothin; PEN, benzylpenicillin; AMP, ampicillin; CARB, carbenicillin; CLOX, cloxacillin; OXA, oxacillin; FOX, cefoxitin; NCF, nitrocefin; TAX, cefotaxime; TAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; CA, clavulanic acid; SUL, sulbactam; TZB, tazobactam; pCMB, *p*-chloromercuribenzoate; Chr, chromosomal; P, plasmid; Nuc, nucleotide sequence; IC_{50}, 50% inhibitory concentration.

[b] ND, not determined.

[c] K_i.

[d] Iodometric assay.

[e] Hydrolysis rate relative to that of cephalothin.

[f] K_m.

[g] Acidimetric assay.

[h] Relative rate of hydrolysis at a fixed substrate concentration (1.2 mM).

[i] NDet, not detected.

[j] Cephalosporinases with similar properties have been reported from *Citrobacter freundii* GN7391 (92, 115, 264, 296) and SR19 (196).

[k] K_i values for cephalosporinase from *Citrobacter freundii* 2732 (92).

[l] Seeberg et al. (275) divided *Enterobacter cloacae* cephalosporinases into types A and B on the basis of the pI. Type A strains had similar kinetic properties and were found in the following *Enterobacter cloacae* strains: 149M, 208, M6300 and 5822M2, whose enzymes have pIs of 8.8 (99, 103, 134, 275, 299); GN7471, whose enzyme has a pI of 8.4 (103, 192); SC 12629, whose enzyme has a pI of >9.0 (53). The kinetic data presented here are for enzymes produced by strains 208 and SC 12629.

[m] Type B *Enterobacter cloacae* cephalosporinase (275). *Enterobacter cloacae* 5 and 352M (275), 363 (269, 273), and 908R (99, 299) produced enzymes with similar characteristics.

[n] pIs of 8.3, 8.25, and 8.95 have also been reported.

[o] Published IC_{50} values are erroneously reported in nanomolar instead of micromolar in references 217 and 311 (217a).

[p] Other *Escherichia coli* strains that produce AmpC-like cephalosporinases include strain SOL, enzyme with a pI of 9.3 (149); strain 255 (269, 273, 297); and strains 214 T and 419 (69).

[q] Relative rate of hydrolysis at fixed substrate concentration (500 μM).

[r] Relative (V_{max}/K_m).

[s] High degree of homology with AmpC cephalosporinase of *Citrobacter freundii* OS60 (161) and *Citrobacter freundii* GN346 (308), as reported by Fosberry et al. (89).

[t] Strain produces two variants. Apparent molecular sizes of 37 and 35 kDa were reported for the pI 6.8 and pI 7.2 enzymes, respectively.

[u] High degree of homology with AmpC cephalosporinase of *Citrobacter freundii* OS60 (310).

[v] Hydroxylamine assay.

[w] Partial sequence has 90% homology with *E. cloacae ampC* gene.

[x] The cephalosporinases from *Morganella morganii* M3, with a pI of 7.6 (332), and that from strain SC 10986, with a pI of 7.5 (43), have similar kinetic properties.

[y] Cephalosporinases from *Pseudomonas aeruginosa* 174K (191), V31 (127), and 18SH (97, 98) have similar kinetic properties.

[z] Relative hydrolysis rates. In spectrophotometric assays, rates for cephalosporins are normalized to that of cephaloridine; in microiodometric assays, rates for penicillins are relative to that of benzylpenicillin. Microbiological data indicate a strong cephalosporinase activity.

[aa] A cloxacillin-inhibitable cephalosporinase from *Serratia marcescens* T-26E1 had similar hydrolysis properties (269). Other *Serratia marcescens* strains that produce AmpC-like cephalosporinases include S7 (334), SC15071 (47), SR50 (202), TN81 (127), and GN7647 (294).

[ab] Relative hydrolysis rates at a fixed substrate concentration (100 μM).

[ac] $(k_3/k_2)K$.

TABLE 2—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
>100[c]	200[c]	ND	12[c]	ND	−	−	38	9.9	ND	ND	113
ND	ND	ND	ND	ND	−	−	30	ND	ND	ND	195
>250	0.12	ND	2	0.074[c]	−	−	38, 41	9.3	ND	ND	33
>40	ND	ND	0.3[f]	0.26	±	−	43	7.0	ND	ND	124
42	1.6	15	ND	ND	ND	ND	41	6.4	Nuc	C	124, 245
>10	>10	ND	ND	>10	ND	ND	ND	ND	ND	ND	295
ND	ND	ND	ND	<3	−	ND	ND	ND	ND	ND	84
ND	ND	ND	0.046[f]	0.007[f]	ND	ND	34	8.9	Nuc	C	206, 269, 273, 306–308, 328, 329
59[k]	3.8[k]	ND	0.0014[f]	0.005[f]	−	ND	40	8.6	Nuc	C	92, 97, 98, 161, 296
ND	>100	ND	0.0012[c]	0.0005[f]	ND	ND	32	8.8	Nuc	C	43, 53, 97, 98, 103, 275
>100	5.6	0.009	0.0024[c]	0.0004[f]	ND	ND	39	8.2, 7.8[n]	Nuc	C	48, 49, 53, 97–99, 103, 134, 275
710[o]	ND	ND	0.2[o]	0.5[o]	ND	ND	ND	8.5	Nuc	C	311
190	ND	ND	0.0012[f]	0.0005[f]	−	−	39.6	9.2	Nuc	C	36, 97, 98, 132, 143, 148, 162
360[o]	ND	19[o]	ND	ND	ND	ND	ND	8.5	ND	ND	217
>100	>100	ND	ND	0.007[c]	−	ND	39	8.7	ND	ND	192
360	18	3.2	ND	ND	ND	ND	37	8.8	Nuc[s]	C	89, 224
>100	<100	100	0.020	0.024	−	ND	37, 35[t]	6.8, 7.2[t]	Nuc	C	101
800[o]	ND	ND	0.2[o]	1.0[o]	ND	ND	ND	9.4	Nuc[u]	C	310, 311
210[o]	ND	8.3[v]	0.4[o]	5.0[o]	ND	ND	ND	8.4	Nuc[w]	C	217
5.6[c]	ND	ND	40[f]	0.35[c]	ND	−	38	8.9	Nuc	C[x]	117, 118
>100	>100	ND	ND	0.001[c]	−	ND	41	8.7	ND	ND	303
1,100[c]	8.9[c]	ND	ND	0.0004[c]	ND	ND	38–40	7.2	ND	ND	95, 269, 271–273, 332
ND	ND	ND	ND	100	−	ND	37.5	ND	ND	ND	35, 36, 145
>10	>10	ND	ND	0.30[f]	+	ND	42	8.7	ND	ND	177
ND	ND	ND	ND	0.013	+	ND	29	7.7	ND	ND	28, 236, 258, 293
ND	ND	ND	ND	ND	ND	ND	ND	ND	Nuc	C	117, 168
>1,000	8	ND	ND	0.006[c]	−	ND	34	8.7	ND	ND	197
MD	ND	ND	ND	0.023[c]	++	ND	34	8.7	ND	ND	326
ND	ND	ND	ND	<0.01	+	ND	39	4.3	ND	ND	24
ND	ND	ND	<0.01	ND	ND	ND	37	>9	Nuc	C	45, 97, 98, 133
51	5.2	6.0	33	ND	ND	ND	ND	7.1	ND	ND	47, 49
ND	ND	ND	0.012[ac]	ND	ND	ND	ND	>9.0	ND	ND	108

strates or inhibitors (45, 46, 129, 184; this minireview). Because of the diversity of enzymatic characteristics of the β-lactamases, many attempts have been made to categorize these enzymes by using their biochemical attributes.

HISTORICAL CLASSIFICATION SCHEMES

Classification of β-lactamases on the basis of function began when cephalosporinases, β-lactamases with high hydrolysis rates for cephalosporins, were differentiated from penicillinases, enzymes with good penicillin-hydrolyzing activity (88). Functional classification schemes that have enjoyed acceptance among β-lactamase researchers include (i) the classification of Sawai et al. (270) in 1968, describing penicillinases and cephalosporinases by using the response to antisera as an additional discriminator; (ii) the Richmond and Sykes (253) scheme in 1973 that included all of the β-lactamases from gram-negative bacteria described at that time, classifying the enzymes into five major groups on the basis of substrate profile; (iii) the extension of the Richmond and Sykes scheme by Sykes and Matthew (292) in 1976, emphasizing the plasmid-mediated β-lactamases that could be differentiated by isoelectric focusing; (iv) the scheme proposed by Mitsuhashi and Inoue (194) in 1981 in which the category "cefuroxime-hydrolyzing β-lactamase" was added to the "penicillinase and cephalosporinase" classification; and (v) the groupings proposed by Bush (44–46) in 1989 that included enzymes from all bacterial sources and that was the first scheme to try to correlate substrate and inhibitory properties with molecular structure.

Molecular structure classifications were first proposed by Ambler (2) in 1980 when only four amino acid sequences of β-lactamases were known. At that time a single class of serine enzyme was designated, the class A β-lactamases that included the *Staphylococcus aureus* PC1 penicillinase, in contrast to the class B metallo-β-lactamase from *Bacillus cereus*. The class C cephalosporinases were described by Jaurin and Grundstrom (132) in 1981, and class D oxacillin-hydrolyzing enzymes were segregated from the other serine β-lactamases in the late 1980s (121, 215). Eventually, as a result of more easily attainable sequence data, sequences of all important β-lactamases will become available, and an inclusive phylogenetic tree can be constructed correlating the relationships among the molecular and functional classes.

BUSH-JACOBY-MEDEIROS CLASSIFICATION

In this minireview an updated version of the Bush scheme is presented, together with a dendrogram based on the currently available β-lactamase sequences. Table 1 shows the correlations between the proposed classification and other frequently cited schemes. As in the 1989 system, four groups of β-lactamases are designated: group 1 cephalosporinases that are not well inhibited by clavulanic acid (Table 2), group 2 β-lactamases that are generally inhibited by active site-directed β-lactamase inhibitors and that belong to molecular classes A or D (Tables 3 to 10), group 3 metallo-β-lactamases that are poorly inhibited by all classical β-lactamase inhibitors except EDTA and *p*-chloromercuribenzoate (pCMB) (Table 11), and group 4 penicillinases that are not inhibited by clavulanic acid (Table 12). Attempts were made to conserve the major groupings in

TABLE 3. Group 2a: penicillin-hydrolyzing enzymes inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis													IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP	CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
I	Chr	*Bacillus cereus*	569	100	100	22	2.0	10	<0.1	<0.1	NDet[b]	11	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	27.8	8.6	AA, Nuc	A	2, 62, 64, 73, 146, 167, 317
III	Chr	*Bacillus cereus*	569/H/9	100	71	ND	0.3	ND	12	ND	ND	85[d]	ND	ND	ND	ND	ND	ND	ND	ND	>1,000	ND	ND	31.5	6.8	ND	A	64
	Chr	*Bacillus licheniformis*	749/C	100	68	18	0.38	0.45	29	2.2	ND	21	0.30	0.68	ND	ND	+	ND	ND	ND	ND	ND	ND	23.0	ND	AA, Nuc	A	63, 175, 183, 237
	ND	*Citrobacter amalonaticus*[e]	VAN	100[f]	28	53	ND	7.1	5.0	14	ND	ND	ND	ND	ND	ND	<4	<10	ND	ND	−	ND	ND	22	4.8	ND	ND	232
MJ-2	ND	*Citrobacter amalonaticus*	HB29	100	22	13[f,g]	<0.2[f,g]	8.5	3.5	18	ND	ND	22	NDet	ND	ND	+	ND	ND	ND	+	+	ND	25	5.55, 5.4	ND	ND	40, 75
	ND	*Eikenella corrodens*	EC-38	100	170	15	<0.03[f]	ND	32	10	ND	44	0.1	ND	<0.05[g]	<0.01[g]	0.12[h]	0.61[h]	ND	>400[h]	25[h]	−	±[i]	29	5.5	ND	ND	155
	ND	*Fusobacterium nucleatum*	F21	100	420	50	ND	ND	4.9	0.25	ND	110	ND	ND	ND	0.03	<10[j]	800[h]	ND	ND	ND	−	ND	26.0	4.8	ND	ND	309
LEN-1	Chr	*Klebsiella pneumoniae*	LEN-1	ND	100[k]	ND	ND	ND	27	ND	ND	ND	NDet	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Nuc	A	10, 11
	Chr	*Klebsiella pneumoniae*	SJ22[l]	100[m]	120	8.5	ND	ND	8.5	0.80	NDet	ND	1.9	NDet	NDet	ND	0.07	ND	ND	710[h]	ND	ND	ND	ND	8.1	ND	ND	228
	ND	*Klebsiella pneumoniae*	L164	100[g]	110	6	ND	ND	3	2	NDet	ND	NDet	NDet	NDet	ND	0.05	ND	ND	ND	ND	ND	ND	ND	8.1	ND	ND	27
	Chr	*Lysobacter enzymogenes*	UASM495	100	210	38	<1[f]	<1[f]	16	35	ND	ND	7.7[f]	ND	ND	ND	0.28	ND	ND	ND	ND	+	−	28	>9.6	Nuc	A	39, 318
	ND	*Nocardia farcinia*	ATCC3318	100	150	27	ND	ND	5.1	0.80	ND	180	0.40	ND	ND	ND	0.13	130	13	ND	380	ND	ND	ND	4.49, 4.56	ND	ND	290
NPS-1	pMLH50	*Pseudomonas aeruginosa*	M302	100	220	18	ND	40	3.0	ND	ND	ND	<1	<0.1	<0.1	<1	ND	ND	ND	ND	>100	−	ND	25	6.5	ND	ND	164
	Chr	*Rhodopseudomonas capsulata*	sp108	100[n]	27	25	NDet	ND	4.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.5	Nuc	A	54
PC1 (A)	P	*Staphylococcus aureus*	PC1	100	180	ND	ND	ND	1.1	0.01	ND	14	ND	ND	0.01	ND	0.03	0.08	0.03	350	ND	ND	ND	26.8	10.1	Nuc[o]	A	1, 2, 45, 49, 79, 338
B	ND	*Staphylococcus aureus*	22260	100	260	ND	ND	ND	4.3	0.06	ND	11	ND	ND	ND	ND	0.41[p]	ND	12[q]	ND	ND	ND	ND	ND	10.1	ND	ND	38, 79, 338
C	ND	*Staphylococcus aureus*	V137	100	170	ND	ND	ND	2.7	0.05	ND	6.5	ND	ND	ND	ND	0.62	ND	122	ND	ND	ND	ND	ND	10.1	Nuc[q]	A	38, 79, 338
D	ND	*Staphylococcus aureus*	FAR10	100	290	ND	ND	ND	2.7	0.02	ND	57	ND	ND	ND	ND	0.40	ND	25	ND	ND	ND	ND	ND	9.7	Nuc[r]	A	38, 79, 338
Exo	ND	*Streptomyces albus*	G	100	140	>36	6.8	9.6	7.1	9.3	ND	89	>0.04	NDet	>0.02	0.04	<20	ND	ND	ND	250	ND	−	30.5	6.0–6.5	Nuc	A	75, 137, 174, 175, 190
	ND	*Streptomyces cellulosae*	KCC-0127	100[f,m]	37	3.7	7.3	ND	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	100	+	−	27	9.5	Nuc	A	208, 209

[a] Abbreviations: AA, amino acid; the other abbreviations are defined in footnote *a* to Table 2.
[b] NDet, not detected.
[c] ND, not determined.
[d] Relative k_{cat}/K_m.
[e] Originally, *Levinea malonatica*.
[f] Relative hydrolysis rate at a fixed substrate concentration.
[g] Microacidometric assays.
[h] K_i.
[i] K_i of 270 μM.
[j] K_i of 220 μM without preincubation of enzyme and clavulanic acid.
[k] Ampicillin as reference.
[l] The following three strains of *Klebsiella pneumoniae* produced penicillinases with very similar profiles: SJ22, SJ23m, and ST403m.
[m] Microiodometric assays.
[n] Relative rates of hydrolysis with no methodology given.
[o] Nonidentical genes for penicillinases of immunological type A sequenced from strains PC1, S1, and 1071 (79). The most abundant biochemical information available is for the PC1 enzyme, for which an amino acid sequence was determined separately (1).
[p] Data for type B from strain PC1116 (38).
[q] Nonidentical genes for penicillinases of immunological type C from strains 3796 and 3804 have been partially sequenced (79).
[r] Gene for penicillinases of immunological type D was sequenced from strain FAR4 (79).

the 1989 Bush outline. However, three changes are noted. Because the number of TEM- and SHV-derived β-lactamases continues to increase, it was decided to classify derivatives of these enzymes in groups that retain the "2b" prefix. In place of the former group 2b′ designation, the extended-spectrum β-lactamases have been placed into a 2be group (Table 5), to show that these are enzymes are derived from the group 2b enzymes and have an extended spectrum of activity. Likewise, the β-lactamases structurally derived from group 2b with reduced affinity for β-lactamase inhibitors have been placed into a new group, group 2br (Table 6). It is anticipated that a similar nomenclature could be used in the future to describe closely related β-lactamases derived from enzymes in other groups. The third group of enzymes added to the scheme are the group 2f β-lactamases (Table 10), carbapenem-hydrolyzing enzymes that are weakly inhibited by clavulanic acid and that are now known to contain an active-site serine.

In the current scheme only β-lactamases from naturally occurring bacterial isolates were added to the tables. The 1989 classification included representative enzymes for each genus and for each grouping of β-lactamase. The additions to the 1989 tables have been more comprehensive, including a large number of novel enzymes characterized in the past 5 years. Also, some older enzymes reevaluated by using substrates or inhibitors not available when the first data were reported for those β-lactamases. As noted below, some of these recent kinetic evaluations have caused selected enzymes to be reclassified.

CLASSIFICATION STRATEGIES

Representative β-lactamases belonging to all molecular classes are described in Tables 2 to 12, with separation into groups based primarily on published functional characteristics. The strategy used for classifying the enzymes was similar to that used previously (44). Enzymes were first separated according to their inhibition characteristics with the metal chelator EDTA. β-Lactamases that were inhibited by EDTA were assigned to group 3, a group comprising only a small number of β-lactamases.

After the metalloenzymes were isolated from other β-lactamases, enzymes were grouped according to substrate profile. Priorities were assigned according to the following considerations. First, relative hydrolysis rates for benzylpenicillin and cephaloridine were evaluated to determine whether an enzyme would be classified as a penicillinase or a cephalosporinase. If an enzyme hydrolyzed one of these substrates at a relative rate approximately 30% less than that observed for the other β-lactam, then the enzyme was assigned to either a penicillinase or a cephalosporinase category. It should be noted that occasional cephalosporinases hydrolyzed benzylpenicillin but no other penicillins; on the basis of this activity and the differential microbiological responses of the producing organism to penicillins and cephalosporins, an assignment to group 1 was made. Broad-spectrum enzymes were those that hydrolyzed the two substrates at approximately equivalent rates (Table 4).

Subgroups of enzymes were further defined by examining rates of hydrolysis of carbenicillin or cloxacillin (oxacillin) by penicillinases. If cloxacillin or oxacillin was hydrolyzed at a rate >50% that for benzylpenicillin, the enzyme was placed in group 2d, a group that may also include enzymes that hydrolyze carbenicillin (Table 8). These enzymes are generally not as well inhibited by clavulanic acid as are most group 2 β-lactamases. If carbenicillin was hydrolyzed at a rate >60% that for benzylpenicillin and cloxacillin or oxacillin was hydrolyzed at a rate <50% that for benzylpenicillin, the enzyme was placed in group 2c (Table 7).

If hydrolysis rates for the extended-spectrum β-lactam antibiotics, ceftazidime, cefotaxime, or aztreonam, were >10% that for benzylpenicillin, the enzyme was assigned to group 2be (Table 5), the extended-spectrum β-lactamases. This group was originally designated "extended-broad-spectrum β-lactamases" (45), to reflect the broad-spectrum penicillin and cephalosporin activities also exhibited by the enzymes within this class. Cephalosporinases that hydrolyzed cefotaxime well but that lacked good penicillin-hydrolyzing activity and that were inhibited by clavulanic acid were assigned to group 2e (Table 9). Other exceptions were made for assignment to the 2be group. The decision was made to include β-lactamases such as TEM-7 and TEM-12, enzymes derived as a result of point mutations in the TEM-2 and TEM-1 genes, respectively; even though the hydrolysis criteria were not met rigorously, large increases in hydrolysis rates for ceftazidime were noted compared with those of the parent enzymes, resulting in increased MICs of that cephalosporin for TEM-producing organisms.

Inhibition characteristics were then examined. Inhibition by EDTA automatically defined an enzyme as a group 3 metallo-β-lactamase. Inhibition by the suicide inactivator clavulanic acid was an essential characteristic required for assignment of most of the enzymes and, for the cephalosporinases, could often be inversely correlated with inhibition by cloxacillin and the monobactam aztreonam. For example, cephalosporinases were grouped either into group 1 (Table 2) or group 2e. Group 1 enzymes were not well inhibited by clavulanic acid, but were often inhibited by a low concentration of aztreonam or cloxacillin. Group 2e cephalosporinases that were inhibited by clavulanic acid did not have a high affinity for the monobactam.

Penicillinases that were not well inhibited by clavulanic acid were assigned to group 4 (Table 12). Although all but two of the enzymes in group 4 had hydrolysis rates for cloxacillin that would qualify the enzymes for assignment to group 2d, the resistance to inhibition by clavulanic acid was higher than that seen for most group 2d enzymes. Therefore, these enzymes will remain in group 4 until additional information, e.g., sequence data, would indicate a more suitable assignment.

PARAMETERS IN TABLES

The parameters used in the tables are equivalent to those described in the 1989 scheme (45), with additional substrate and inhibition data included. Hydrolysis of oxacillin, cefoxitin, and nitrocefin were added to the substrate profiles, and inhibition by tazobactam was added. Hydrolysis of methicillin was included for the enzymes in group 2d. It is noteworthy that many of the substrate hydrolysis data now being provided in published reports include V_{max} or relative V_{max} data. Comparison of V_{max} values is usually a better indication of enzymatic characteristics than the relative hydrolysis rates obtained at a single substrate concentration, data that were frequently reported in earlier literature. Because of the prevalence of V_{max} data obtained spectrophotometrically, it will be assumed that the data in the tables were reported as such unless indicated otherwise.

It has been noted that use of the parameter V_{max}/K_m rather than V_{max} is a more informative measure of the hydrolysis capacity of an enzyme (52, 175), especially at low substrate concentrations. On the basis of V_{max}/K_m data, the differences between penicillinases and cephalosporinases may become indistinct, because many cephalosporinases are found to have high catalytic efficiencies for penicillin hydrolysis because of low K_m values (high affinities) for penicillins (97, 144). How-

TABLE 4. Group 2b: broad-spectrum β-lactamases inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis											
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP
CEP-2	PLQ3	*Achromobacter* sp.	MULB 906	100[b]	NDet[c]	48	NDet	NDet	110	110	NDet	ND[d]	ND	ND	ND	ND
	Chr	*Alcaligenes denitrificans*, subsp. *xylosoxydans*	Adx 89/2	100[b]	15	5[e]	<1	ND	100	80	ND	ND	1.5	1.0	ND	<1
Form I	Chr	*Citrobacter diversus*	ULA27	100	21	10	0.01	36	160	11	ND	ND	ND	ND	NDet	0.003
OHIO-1	pDS075	*Enterobacter cloacae*[h]														
OHIO-1	pDS076	*Serratia marcescens*[h]	75	100[b]	140	11	<0.5	<0.5	79	8.0	ND	ND	<1	<1	<1	<1
SHV-1 (PIT-2)	p453	*Escherichia coli*	P453	100	150	6.3	0.80	<0.5	48	6.5	NDet	ND	0.18	0.02	0.38	<0.01
TLE-1	pMG204b	*Escherichia coli*	7604	100[b]	67	13	6	4	52	15	2	ND	6	ND	ND	ND
ROB-1	R_{Rob}	*Haemophilus influenzae*	F990	100[b]	110	19	<0.2	ND	37	4.5	<1	ND	<1	ND	ND	ND
LXA-1	pMG219	*Klebsiella oxytoca*	F177	100[j]	160	40	<1	<1	120	45	ND	ND	<1	ND	ND	ND
TLE-2	pUK702[k]	*Klebsiella pneumoniae*	175	100	140	13	ND	ND	ND	ND	ND	99	ND	ND	ND	ND
	Chr	*Klebsiella pneumoniae*	ST53	100[b]	120	8.5	ND	ND	69	6.2	NDet	ND	NDet	NDet	NDet	ND
	(Chr?)	*Mycobacterium fortuitum*	D316[m]	100	107	19	ND	0.46	110	150	ND	850	5.6	ND	ND	ND
	ND	*Mycobacterium smegmatis*	NCTC 8158	100	ND	ND	<1[n]	ND	77	22	ND	ND	ND	ND	ND	ND
HMS-1	R997	*Proteus mirabilis*		100[o]	250	14	2.0	<2	180	3	ND	ND	ND	ND	ND	ND
TEM-2	RP1	*Pseudomonas aeruginosa*	1822	100	100	6.0	3.8	ND	120	9.4	NDet	ND	0.08	<0.01	0.4	<0.01
TEM-1	R1[p]	*Salmonella paratyphi*	R7268	100	110	10	<0.2	4	140	20	ND	ND	0.07	0.01	0.3	<0.01

[a] Abbreviations are defined in footnotes *a* to Tables 2 and 3.
[b] Microacidimetric assays.
[c] NDet, not detected.
[d] ND, not determined.
[e] Ticarcillin.
[f] K_i.
[g] K_m.
[h] Both strains were identified simultaneously.
[i] Inhibited with cephaloridine as the substrate; not inhibited when benzylpenicillin was the substrate (181).
[j] Substrate of 10 mM; relative hydrolysis rates.
[k] Also codes for TEM-1 and SHV-1 β-lactamases.
[l] Inhibited with nitrocefin as the substrate; not inhibited when benzylpenicillin was the substrate.
[m] Mutant from *Mycobacterium fortuitum* ATCC 19542 after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.
[n] Dicloxacillin as substrate.
[o] Iodometric assays; 5.0 mM substrate.
[p] Originally plasmid R6K (RTEM) was identified as producing TEM-1 (180). However, by 1978 a strain described as carrying the R6K plasmid produced TEM-2 as determined by amino acid sequencing (3), suggesting a mix-up of strains.

ever, because fewer K_m data than hydrolysis data are available, especially for some of the older enzymes, classification on the basis of hydrolysis rates is being retained as the discriminating factor among groups. This approach can be especially justified for those β-lactams with low K_m (<10 μM) as well as low V_{max} values; at physiologically attainable substrate concentrations (>10 μg/ml, approximately 20 μM), V_{max} would be the major determinant of relative hydrolytic abilities.

Assay methodology has been indicated for each of the hydrolysis profile tables. Unless noted otherwise, the assays were conducted spectrophotometrically. For many substrates, data obtained by different assay procedures can be compared directly. However, hydrolysis rates obtained for the extended-spectrum cephalosporins are consistently lower when spectrophotometric assays are used for kinetic evaluations than when microacidimetric assays are used to obtain the data. Comparative data from both sets of assays have been included for representative enzymes in group 2be in which these differences may be most significant.

Since 1989 a number of novel β-lactamases have been described, and they are included in the present groups. A set of AmpC-like cephalosporinases that have moved from the chromosome to plasmids has been described more frequently. Note that the designation "AmpC" refers to a family of related enzymes, not to the same protein produced in a variety of members of the family *Enterobacteriaceae*. These plasmid-mediated enzymes have been added to group 1, because it was not felt to be necessary to discriminate between chromosomal and plasmid-encoded enzymes. The extended-spectrum β-lactamases, whose numbers have increased significantly, represent one of the largest groups of novel enzymes, with extensive biochemical and molecular information being made available. Included among the recently described β-lactamases are the mutant TEM enzymes with decreased susceptibilities to the active site-directed β-lactamase inhibitors, now assigned to the new group 2br. Additional metallo-β-lactamases have appeared, most notably the plasmid-mediated enzymes from *Pseudomonas aeruginosa* and *Bacteroides fragilis* that have appeared in Japan. Although the β-lactamase in *Pseudomonas aeruginosa* appears to be uncommon, the plasmid-mediated metalloenzyme in *Bacteroides fragilis* may be a more serious problem (16). A last notable addition to the β-lactamase family is the set of enzymes in group 2f, the carbapenem-hydrolyzing molecular class A β-lactamases. Previously, the only β-lactamases with significant rates of hydrolysis for carbapenems were the class B metallo-β-lactamases.

DENDROGRAM OF β-LACTAMASES

The complete nucleotide or amino acid sequence of many β-lactamases has now been determined. A dendrogram expressing the molecular relationship among 88 enzymes classified in Tables 2 to 11 was constructed by the progressive alignment method (86) by using the Pileup Multiple Sequence Analysis Program in the software package of the University of Wisconsin Genetics Computer Group (76). Comparisons were

TABLE 4—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
ND	ND	ND	ND	>100	−	ND	36	8.1	ND	ND	159
<10	ND	ND	>1,000	9,000	ND	ND	ND	9.5	ND	ND	74
<80	ND	ND	4.2[f]	<100[g]	+	−	29	6.8	Nuc	A	5–7, 227
<1	≤75	ND	>1,200	13,000	ND	ND	22	7.0	Nuc	A	280, 316
0.03	17	0.14	2,500[g]	4.0	±[i]	ND	28.8	7.6	Nuc	A	19, 104, 148, 181, 222, 230
0.11	5.5	0.05	ND	100	ND	ND	20	5.55	ND	ND	185, 222
<0.01	<1	ND	ND	<100	ND	ND	ND	8.1	Nuc	A	14, 61, 136, 189, 256, 257
<100	ND	ND	ND	<100	ND	ND	24.0	6.7	ND	ND	331
0.08	ND	ND	ND	90.0	±[l]	ND	19.0	6.5	ND	ND	249
0.03	ND	ND	ND	ND	ND	ND	ND	8.1	ND	ND	228
ND	ND	ND	ND	ND	ND	ND	29.0	4.9	AA	A	4, 302
ND	ND	ND	ND	50.0	ND	ND	ND	ND	ND	ND	193
ND	ND	ND	ND	<100	+	ND	21.0	5.2	ND	ND	181
0.18	8.7	0.05	2,900	ND	−	−	28.9	5.6	AA, Nuc	A	3, 45, 51, 52, 87, 109, 179, 181, 222
0.09	6.1	0.04	5,400	1,000[g]	−	−	28.9	5.4	Nuc	A	43, 45, 71, 72, 109, 110, 128, 181, 222, 291, 311

made without the signal sequence whenever that information was available. The configuration of such a dendrogram is a function of the method used for its construction (77). The alignments are also based on entire amino acid sequences rather than critical motifs (100). Somewhat different trees have been published previously on the basis of 18 (139), 31 (66), or 47 (207) β-lactamase sequences.

Figure 1 shows the dendrogram representing the clustering relationships. Enzymes differing in only a few amino acids, such as the many TEM and SHV derivatives, are joined to the right of the figure. Vertical branch lengths extending to the left are inversely proportional to the similarity between sequences, but the dendrogram is not an exact phylogenetic alignment. Furthermore, the program aligns all sequences supplied, whether or not they are related. Nonetheless, there is a close correlation between structural clustering and functional classification. Sequenced group 1 cephalosporinases belong to molecular class C. Group 2 enzymes with sequence information are either in class A or in class D for the group 2d cloxacillin-hydrolyzing enzymes. Group 3 metallo-β-lactamases are all class B enzymes. On the dendrogram group 1, group 2d, and group 3 enzymes are clustered on independent branches, while the remaining group 2 enzymes form a complex pattern in which enzymes assigned to different subgroups are intermingled.

Because of the small size of group 4, it is possible that the enzymes assigned to it may fall more readily into other groups as their characteristics are further evaluated. For example, the LCR-1 β-lactamase was assigned to group 4 in the 1989 scheme (46), but it was recently sequenced and found to be closely related to the class D OXA enzymes (66). Upon reexamination of the hydrolytic properties of a highly purified LCR-1 preparation, hydrolysis of oxacillin was shown to proceed rapidly (330a) so that the enzyme has been reassigned to group 2d (Table 8).

DISCUSSION

Classification of a novel β-lactamase ideally should include all of the parameters discussed above. However, realistically, this is not always possible, nor is it necessary. Minimal requirements should include substrate profiles for benzylpenicillin and cephaloridine or cephalothin as reference substrates. The choice of additional substrates will vary according to the characteristics of each enzyme. Often, the substrate profile of a novel enzyme is suggested by the resistance phenotype of the producing organism, provided that only a single enzyme is present. Thus, if a member of the family *Enterobacteriaceae* is resistant to expanded-spectrum cephalosporins but susceptible to β-lactamase–inhibitor combinations, an extended-spectrum β-lactamase is probably present and the substrate profile should include cefotaxime, ceftazidime, and aztreonam as discriminating substrates. At present, with the ease of obtaining sequence data, it is often possible that the molecular class of an enzyme will be known before a complete biochemical characterization is available. If a class D penicillinase is identified, substrates should include oxacillin and cloxacillin. Inhibitor profiles should include clavulanic acid as a minimal requirement. Other inhibitors should be added to describe the character of the enzyme more completely. For carbapenem-hydrolyzing enzymes, possible inhibition by EDTA and pCMB should be determined. For known class A or class C β-lactamases, the latter two inhibitors may be omitted.

Although this functional grouping of β-lactamases is probably the most comprehensive that is available, no functional classification will ever be completely satisfactory. All groupings must assume a somewhat artificial set of constraints, because β-lactamases are known to encompass a great deal of diversity in the number of amino acid substitutions that can be tolerated with the retention of β-lactam-hydrolyzing activity (216, 274). As noted by Matagne et al. (175), there is a certain fluidity between the various enzyme groups, depending on which enzymatic parameters are used and which substrates are used for comparison. For example, the classical penicillinase from *Actinomadura* sp. strain R39, formerly classified in group 2a (45), was first reclassified as a group 2be enzyme on the basis of hydrolysis of cefotaxime, a substrate not available when the enzyme was initially characterized. When V_{max} values for both cloxacillin and oxacillin were included, the penicillinases from both *Actinomadura* sp. strain 39 and *Streptomyces cacaoi* KCC-0352 were moved to group 2d, although the enzymes seem to be more closely related on a molecular level to the class A β-lactamases. Similar situations are certain to arise in the future with enzymes that have not been examined by using the

TABLE 5. Group 2be: extended-spectrum β-lactamases inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP
TEM-3 (CTX-1)	pCFF04	*Klebsiella pneumoniae*	CF104	100	110	35	0.97	5	120	31, 110[b]	<1	ND[c]	170, 450[b]	8.3	0.36	0.01
TEM-4	pUD16	*Escherichia coli*	CB-134	100[b]	50[e]	12	9	13	230	ND	<1	ND	300	10	<1	<1
TEM-5 (CAZ-1)	pCFF14	*Klebsiella pneumoniae*	CF504	100[b]	78[e]	60	ND	ND	ND	380	ND	ND	150	490	120	<0.1
TEM-5	pCFF14	*Escherichia coli*	CF604	100	50	27	<10	ND	300	48	ND	ND	29	100	45	0.9
TEM-6	pMG226	*Escherichia coli*	(several)	100[b]	37	19	6	25	200	51	<1	ND	12	55	11	<1
TEM-7	pIF100	*Citrobacter freundii*	M2	100[f]	93	20	5.7	12	120	16	ND	ND	1.9	1.7	ND	ND
TEM-8 (CAZ-2)	pCFF34	*Klebsiella pneumoniae*	CF704	100[b]	240[e]	75	ND	ND	ND	170	ND	ND	640	260	210	<0.1
TEM-9	pMG228	*Klebsiella pneumoniae*	2639E[h]	100	51	19	8.7	ND	67	33	<0.05	ND	12	35	40	1.2
TEM-10 (MGH-1)	pJPQ100	*Klebsiella pneumoniae*	KC2	100	130	36	16	ND	77	18	<0.05	ND	1.6	68	10	<0.02
TEM-11 (CAZ-lo)	P	*Klebsiella pneumoniae*	2326	ND	ND	ND	ND	ND	100[f,j]	ND	<0.5	ND	2.5	0.9	<0.5	ND
TEM-12 (YOU-2) (CAZ-3)	Chr/pUD27[l]	*Escherichia coli*	MG32	100	14[f]	ND	<1[b]	ND	57	22[f]	ND	120[f]	2.4	3.8	6.1	<1[b]
TEM-16 (CAZ-7)	pCFF84	*Klebsiella pneumoniae*	CF1304	100[b]	ND	ND	ND	ND	ND	ND	ND	ND	9.8	98	28	ND
TEM-20	pUD30	*Klebsiella pneumoniae*	A268	100[b]	150	12[m]	2	ND	150	ND	ND	ND	250	<1	<1	<1
TEM-21	pUD22	*Klebsiella pneumoniae*	D660	100[b]	66	13[m]	1	ND	290	ND	ND	ND	493	57	<1	<1
TEM-22	pSLH52	*Klebsiella pneumoniae*	SLK52	100[b]	97[e]	16	1	2	410	ND	<0.5	ND	130	10	<0.05	<0.5
TEM-24 (CAZ-6)	pCFF74	*Klebsiella pneumoniae*	CF1104	100[b]	ND	ND	ND	ND	ND	ND	ND	ND	208	848	134	ND
TEM-25 (CTX-2)	P	*Salmonella mbandaka*	CF1509	100[b]	36[e]	17[m]	ND	ND	ND	98	<0.5	ND	140	<0.5	<0.5	ND
TEM-26 (YOU-1)	pJPQ101	*Klebsiella pneumoniae*	KPS1	100	ND	32	18	ND	120	ND	ND	ND	7.5	170	49	ND
SHV-2	pBP60	*Klebsiella ozaenae*	2180	100	150[f,p]	19[b]	ND	18[b]	330[b]	110[b]	<1	ND	4[f], 70[b]	6.5[b]	1.0[b]	<1[b]
SHV-3	pUD21	*Klebsiella pneumoniae*	86-4	100[b]	153	21	<1	ND	250	ND	ND	ND	37	<1	<1	<1
SHV-4 (CAZ-5)	p210-2	*Klebsiella pneumoniae*	Kp 210-2	100[b]	195	35[m]	ND	ND	320[b]	200	ND	ND	115	52	4	<1
SHV-5 (CAZ-4)	pAFF1, pCFF54	*Klebsiella pneumoniae*	160 (CF3104)	100	242	31[b]	9[b]	10[f]	140[f]	180[b], 43[f]	ND	ND	134[b], 25[f]	49[b], 11[f]	2	<1
SHV-6[s]	pSLH47	*Klebsiella pneumoniae*	SKL-47	100[b]	52	8[m]	<1	ND	80	ND	ND	ND	1	0.09	0.3	ND
	ND	*Capnocytophaga* spp.	Van1	ND	32[b]	ND	ND	ND	100[b,j]	ND	ND	ND	11	1.3	ND	ND
B1	ND	*Citrobacter amalonaticus*	A2370H	100[b]	19	11	ND	94	190	66	ND	ND	35	NDet[t]	ND	ND
B2	ND	*Citrobacter amolonaticus*	A2370H	100[b]	12	9	ND	37	180	64	ND	ND	29	NDet	ND	ND
MJ-2	ND	*Citrobacter amalonaticus*	HB29	100[b]	8[w]	13[w]	<0.2	8.5	3.5	18	ND	ND	22	NDet	ND	ND
MEN-1	P	*Escherichia coli*	MEN	100[b]	60[e]	8.2[m]	ND	ND	ND	1,300	ND	ND	170	1	6.5	ND
CTX-ase-M-1	pMVP-3	*Escherichia coli*	GRI	ND	ND	ND	ND	ND	100[j]	ND	ND	ND	13	0.02	ND	ND
K1	Chr	*Klebsiella aerogenes*[x]	K1082E	100	100[y]	9.5	14[y]	ND	59	32	ND	ND	ND	ND	14[y]	ND
K1	Chr	*Klebsiella oxytoca*[aa]	SC10436	100	61	20	10	ND	36	16	ND	35	1.8	0.01	15	<0.01
	ND	*Klebsiella oxytoca*[ab]	D488	100[b]	95	ND	ND	ND	140	91	NDet	ND	7.0	NDet	8.9	ND
MJ-1	ND	*Klebsiella oxytoca*	IV4	100[b,w]	72	14	15	32	95	80	ND	ND	19	ND	ND	ND
PER-1	Chr	*Pseudomonas aeruginosa*	RNL-1	100	170[e]	ND	<0.5	ND	360	470	<0.5	ND	1500	2500	1	<0.5
	Chr	*Pseudomonas cepacia*	GN11164	100	200	22	ND	ND	62	200	<1	ND	110	ND	ND	ND
	Ind[ac]	*Pseudomonas pseudomallei*	HK21	100	32	20	<1	ND	160	470	<1	ND	250	<1	ND	<1
	ND	*Pseudomonas stutzeri*		100	300	6.5	3.0	2.4	140	120	0.14	220	420	120	27	0.1
CTX-ase-M-2	pMVP-4	*Salmonella typhimurium*	CAS-5	ND	ND	ND	ND	ND	100[j]	ND	ND	ND	14	0.04	ND	ND

[a] Abbreviations are defined in footnotes *a* to Tables 2 and 3.
[b] Microacidimetric assay.
[c] ND, not determined.
[d] K_m.
[e] Amoxicillin.
[f] Substrate of 100 μM.
[g] Inhibitor restored cephalosporin or penicillin activity in microbiological assays.
[h] Enzyme for hydrolysis was purified from transconjugant *Escherichia coli* 2639E (50).
[i] Identical amino acid sequences were reported for enzymes designated MGH-1 from *Klebsiella pneumoniae* (251) and TEM-23 from *Escherichia coli* F2 (315). At least two nucleotide sequences have been identified (241).
[j] Cephaloridine was the reference substrate.
[k] The molecular class was identified by oligotyping.
[l] Also found on transposon Tn*841* (111). Two nucleotide sequences have been identified (41, 58, 251).
[m] Ticarcillin.
[n] K_i.
[o] Two nucleotide sequences have been reported (112, 200, 251, 313).
[p] Microiodometric assays.
[q] Inhibited with cephaloridine as the substrate; not inhibited when benzylpenicillin was the substrate.
[r] Multiple sequences have been reported for the SHV-2 β-lactamase.
[s] Not yet proven by sequence to be unique.
[t] NDet, not detected.
[u] Small effects of inhibitor were seen on the activities of cephalosporins in microbiological assays.
[v] B2 apparently derived from B1 on storage.
[w] Substrate of 240 μM for penicillin assays and 300 μM for cephalosporin assays.
[x] Most probably a *Klebsiella oxytoca* strain by current nomenclature.
[y] Substrate of 10 mM; relative hydrolysis rates.
[z] Amino acid sequences of active-site peptides of K1 enzymes from 1082E and SC10436 differed only at the residue preceding the active site serine: asparagine in strain 1082E and cysteine in strain SC10436. Substitutions were compatible with differential susceptibilities to thiol group reagents (82, 135).
[aa] Originally designated *Klebsiella pneumoniae.*
[ab] Other β-lactamases described from *Klebsiella oxytoca* with similar substrate profiles are from strain E23004, enzyme with a pI of 7.4, Class A sequence (11); strain GN10650, enzyme with a pI of 5.3 (125); strain KH111, enzyme with a pI of 5.2 (325); and strain 5445 (TEM-E2 on plasmid pUK721), enzyme with a pI of 5.3 (223).
[ac] Inducible enzyme activity was assumed to be chromosomal.
[ad] An isoform with a pI of 5.2 was identified in the purified protein preparation.

TABLE 5—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
0.03	0.03	0.01	18[d]	<100	−	ND	29	6.3	Nuc	A	45, 140, 148, 221, 222, 283, 284, 286
<1	<1	ND	ND	<100	+	ND	24	5.9	Nuc	A	221
0.03	1.2	0.28	100[d]	ND	+	−	29	5.55	Nuc	A	59, 148, 222, 229, 284
0.01	0.12	ND	270[d]	ND	+	−	29	5.6	Nuc	A	50
0.12	0.45	0.17	ND	ND	ND	ND	29	5.9	Nuc	A	22, 169, 217, 221, 222
0.10	0.62	0.18	ND	ND	ND	ND	29	5.4	Nuc	A	105, 222
+[g]	+[g]	+[g]	62[d]	ND	ND	ND	29	6.0	Nuc	A	56, 57, 59, 169, 170, 282
0.29	0.90	0.34	ND	ND	+	−	29	5.59	Nuc	A	50, 130, 170, 222, 287
0.03	0.34	0.08	30[d]	ND	+	−	29	5.57	Nuc	A[i]	222, 240, 241, 251
+[g]	+[g]	ND	ND	ND	ND	ND	29	5.6	ND	A[k]	169, 319
0.012	0.085	0.013	870[d]	<1000	ND	ND	29	5.25	Nuc	A	41, 58, 169, 251, 252, 315, 324
+	+	+	31[d]	ND	ND	ND	ND	6.3	Nuc	A	57, 60
<5	+	ND	ND	<1000	ND	ND	ND	5.4	ND	A[k]	26
<50	+	ND	ND	<1000	ND	ND	ND	6.4	ND	A[k]	26
<0.05	>1	ND	38	<100	ND	ND	29	6.3	Nuc	A	13
+	+	+	29[d]	ND	ND	ND	29	6.50	Nuc	A	57, 60
+[g]	ND	ND	92[n]	ND	ND	ND	ND	5.3	Nuc	A	58, 238
0.01	0.35	0.08	89[d]	30[d]	ND	ND	29	5.58	Nuc[o]	A	200, 251, 252, 313
0.05	2.8	0.13	10[d]	<100	±[q]	ND	29	7.6	AA, Nuc[r]	A	20, 120, 131, 141, 142, 148, 222
0.04	2.7	0.10	ND	>1000	ND	ND	29	7.0	Nuc	A	131, 201, 316
0.03[n]	0.14[n]	+[g]	1.1[d]	ND	ND	ND	29	7.8	AA	A	12, 152, 225, 282
0.01	0.63	0.08	0.02[n]	ND	ND	ND	ND	8.2	Nuc	A	12, 23, 31, 104, 222, 282
<1	1	ND	ND	>1000	ND	ND	ND	7.6	ND	ND	12
+[g]	ND	ND	ND	ND	ND	ND	ND	5.6	ND	ND	255
±[u]	±[u]	ND	ND	ND	ND	ND	ND	6.05	ND	ND	40
±[u]	±[u]	ND	ND	ND	ND	ND	ND	5.5[v]	ND	ND	40
+	ND	ND	ND	ND	+	ND	25	5.55, 5.4	ND	ND	40, 75
0.50	ND	ND	ND	ND	ND	ND	ND	8.4	AA	A	18, 29
0.08	0.55	0.02	ND	ND	ND	ND	30	8.9	ND	ND	21
ND	ND	ND	ND	ND	−	−	26.5	ND	AA[z]	A	82, 166, 172
0.007	1.6	ND	800[d]	390[d]	ND	ND	27	6.5	AA[z]	A	45, 48, 135, 290
0.2[f]	ND	ND	1,350[g]	ND	ND	ND	ND	ND	AA	A	18, 250
0.09	40	0.43	ND	+	−	ND	25	5.35	ND	ND	75, 222
+	+	ND	ND	+	ND	−	29	5.4	Nuc	A	204, 205
1.7[n]	1.8[n]	ND	ND	3.4[d]	+	ND	22	9.3	ND	ND	114
<10	ND	ND	ND	10	ND	−	30	7.7	ND	ND	163
0.32[d]	3.0[d]	ND	10[d]	0.94[d]	+	−	29	5.4[ad]	ND	ND	91
0.20	2.10	0.02	ND	ND	ND	ND	30	7.9	ND	ND	21

same profiles as those used for a specific classification scheme. Resolution of other discrepancies between classification by structure and function may, as a result, elucidate critical regions of particular enzymes contributing to their biochemical properties. In spite of the anomalies mentioned above, however, the proposed scheme appears to be a workable, and potentially useful, compilation of β-lactamase characteristics.

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TABLE 6. Group 2br: broad-spectrum β-lactamases with reduced binding of clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
				PEN	AMP	CARB	CLOX	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP	CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
TEM-30 (IRT-2)[b]	P	*Escherichia coli*	GUER[c]	100[d]	150	ND[e]	ND	5	1.5	ND	ND	<1	<1	<1	<1	4[f]	81[f]	2.3[f]	ND	>100	+	ND	24	5.2	Nuc	A[g]	25a, 314, 337
TEM-31 (IRT-1)[h]	P	*Escherichia coli*	SAL	100[d]	250	ND	ND	13	<1	ND	ND	<1	<1	<1	<1	9.4	260	2.9	ND	>100	−	ND	24	5.2	Nuc	A	25a, 314, 337
TEM-32 (IRT-3)	pHM3408	*Escherichia coli*	3408	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	12	160	5	ND	ND	ND	ND	ND	5.4	ND	ND	32
TEM 33	P	*Escherichia coli*	59904	100	160	ND	ND	9	ND	ND	ND	ND	ND	ND	ND	4	36	0.4	ND	ND	ND	ND	ND	5.4	Nuc	A	337
TEM-34	P	*Escherichia coli*	92741	100	150	ND	ND	36	ND	ND	ND	ND	ND	ND	ND	2	16	0.5	ND	ND	ND	ND	ND	5.4	Nuc	A	337
TEM-35 (IRT-4)	P	*Escherichia coli*	98041	100	150	3.0[d,i]	ND	31	13[d]	ND	ND	ND	ND	ND	ND	17	62	0.7	ND	ND	ND	ND	ND	5.2	Nuc	A	42a, 337
TEM-36	ND	*Escherichia coli*	86325	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.9	20	1.2	ND	ND	ND	ND	ND	5.2	Nuc	A	337
TRC-1	pUK901	*Escherichia coli*	307	100	120	0.94	ND	ND	ND	ND	ND	0.33	0.25	ND	ND	50	ND	ND	ND	ND	ND	ND	25	5.25	ND	A[j]	301
	ND	*Nocardia brasiliensis*	NB-361-2	<1	ND	ND	ND	100[k]	17	ND	550	ND	ND	ND	ND	11	64	1.7	ND	13	ND	ND	ND	5.04	ND	ND	289

[a] Abbreviations are defined in footnote *a* to Table 2.
[b] Also designated E-GUER and TRI-2.
[c] Enzyme was also identified in *Escherichia coli* 92734, 86947, and 10476.
[d] Microacidimetric assays.
[e] ND, not determined.
[f] Average values for enzymes from *Escherichia coli* 92734, 86947, and 10476. IC_{50} values for IRT-2 were 9.4 μM (clavulanic acid), 260 μM (sulbactam), and 2.9 μM (tazobactam) (314).
[g] The gene from *Escherichia coli* GUER was sequenced. Genes from other strains were identified by oligotyping.
[h] Also designated E-SAL and TRI-1.
[i] Ticarcillin.
[j] Hybridization with an intragenic TEM-1 probe.
[k] Cephaloridine as 100.

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TABLE 7. Group 2c: Carbenicillin-hydrolyzing β-lactamases inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP
CARB-5	Chr?	*Acinetobacter calcoaceticus* var. *anitratus*	A85-145	100[b]	80	61	2.0	3.0	8.0	4.0	ND[c]	ND	<0.5	ND	ND	<0.5
AER-1	Chr	*Aeromonas hydrophila*	VL7711	100[b]	38	98	NDet[d]	0.9	26	77	17	47	20	ND	ND	ND
Type B	Chr?	*Alcaligenes denitrificans* subsp. *xylosoxydans*	Adx 40	100[b]	110	100[e]	<1	ND	31	3.0	ND	ND	<1	<1	ND	<1
	ND	*Clostridium butyricum*	NBL 3	100	160	180	ND	ND	18	0.3	ND	41	0.03	ND	ND	0.001
	P1	*Corynebacterium pseudodiphtheriticum*	C56	100	130	90	9	ND	3.0	ND	ND	ND	ND	ND	ND	ND
BRO-1	ND	*Moraxella catarrhalis*	Ravisio	100	100	95	13	ND	13	12	1.0	370	8.0	ND	<1	<1
BRO-2	ND	*Moraxella catarrhalis*	Multiple	100	78	ND	21	ND	14	11	ND	570	ND	ND	ND	ND
	Chr	*Proteus mirabilis*	GN79	100[j]	140	100	<2	ND	3	ND	ND	ND	ND	ND	ND	ND
	pCS229	*Proteus mirabilis*[k]	N-29	100[j]	120	130	<2	ND	6	ND	ND	ND	ND	ND	ND	ND
PSE-1	RPL11	*Pseudomonas aeruginosa*	RPL11	100[j]	110[b]	110[b]	2[b]	9[b]	18[b]	5[b]	2[b]	31[j]	0.13	0.05	0.09	0.09
PSE-3	Rms149	*Pseudomonas aeruginosa*	Ps142	100[j]	100[b]	250[b]	3[b]	ND	10[b]	ND	ND	ND	16	0.91	4.0	0.67
PSE-4	pMG19	*Pseudomonas aeruginosa*	Dalgleish	100[b]	88[j]	150[j]	0.4[j]	8.3[j]	40[j]	4[j]	ND	ND	0.02	0.02	0.10	0.01
CARB-3	ND	*Pseudomonas aeruginosa*	Cilote	100[b]	100	150	0.5	13	44	0.5	ND	ND	ND	ND	ND	ND
CARB-4	pUD12	*Pseudomonas aeruginosa*	P83 372	100[j]	130	79	<1	1	18	3	ND	ND	ND	ND	ND	ND
SAR-1	pUK657	*Vibrio cholerae*	DT136	100	63	120	ND	ND	21	ND	ND	89	ND	ND	ND	ND

[a] Abbreviations are defined in footnote *a* to Table 2.
[b] Acidimetric assays.
[c] ND, not determined.
[d] NDet, not detected.
[e] Data for ticarcillin; enzyme described as a carbenicillin-hydrolyzing β-lactamase (231).
[f] K_{ir}
[g] K_i.
[h] Multiple pI values have been reported: 5.6 with satellite bands at 4.4, 5.0, and 6.2 (80); 5.13, 5.24, 5.49, and 6.10 from a single isolate (288). A membrane-bound enzyme with a pI of 6.20 was also observed; it had an inhibition profile similar to that of BRO-1 (288). After cell-bound enzyme was solubilized with papain, BRO-1 had a pI of 6.5 (81). An unnamed enzyme from *Branhamella catarrhalis* NNBR-8303 with a pI of 5.4 had very similar enzymatic properties (335).
[i] Multiple pI values have been reported: 5.24, 5.49, 6.10, and 6.55 from a single isolate (288). After cell-bound enzyme was solubilized with papain, BRO-2 had a pI of 6.9 (81). A membrane-bound enzyme with a pI of 6.20 was observed; it had an inhibition profile similar to that of BRO-2 (288). Evidence suggests that BRO-1 and BRO-2 are closely related.
[j] Iodometric assays.
[k] High-producing *Proteus mirabilis* N-29 and low-producing *Proteus mirabilis* N-3 and β-lactamases with pIs of 6.9 and 6.0, respectively, and enzymatic properties similar to those of the PSE-1 enzyme. β-Lactamase activity from *Proteus mirabilis* N-29 and N-3 and *Pseudomonas aeruginosa* strains with RPL11 (PSE-1) and pMG19 (PSE-4) were neutralized by anti-N-29 penicillinase serum. Enzyme activity in strain GN79, which differs structurally (Fig. 1), was not neutralized (298).
[l] The nucleotide sequence is unpublished. The GenBank nucleotide sequence accession number is D13210 (Y. Sakurai, K. Tsukamoto, H. Sugiyama, Y. Takeuchi, and T. Sawai).

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TABLE 7—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
<1	<1	ND	ND	<1,000	+	ND	28	6.35	ND	ND	220
ND	ND	ND	ND	ND	ND	ND	22	5.9	ND	ND	110
<10	ND	ND	ND	>1,000	ND	ND	ND	5.7	ND	ND	74, 231
≤0.04	<20	≤0.4	ND	4,200[f]	+	ND	32	4.4	ND	ND	138
33[g]	40[g]	ND	ND	74[f]	−	ND	14	6.74	ND	ND	126
<0.01	<0.01	<0.01	85[f]	1.4[f]	ND	ND	ND	5.45[h]	ND	ND	80, 81, 83, 222, 288, 335
<0.01	<0.01	<0.01	ND	1.5[f]	ND	ND	ND	Multiple[i]	ND	ND	81, 288
ND	ND	ND	ND	120	ND	ND	27.0	6.6	Nuc	A	262, 270, 298
ND	ND	ND	ND	86	ND	ND	22.0	6.9	Nuc[j]	A	298
ND	ND	ND	260[e]	>100	+	ND	28.5	5.7	Nuc	A	46, 110, 122, 182, 187
ND	ND	ND	ND	>1,000	−	ND	?	6.9, 7.05	Nuc	A	46, 54, 188, 268
0.15	3.7	0.10	230[f]	50[f]	−	−	32.0	5.3	Nuc	A	37, 46, 96, 222
ND	ND	ND	ND	ND	ND	ND	31.0	5.75	Nuc	A	150, 154
<4	4	ND	ND	>100	+	ND	22	4.3	Nuc	A	235
0.005	ND	ND	ND	7	−	ND	34.0	4.9	ND	ND	248

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TABLE 8. Group 2d: cloxacillin-hydrolyzing β-lactamases[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												
				PEN	AMP	CARB	CLOX	OXA	MET	LOR	LOT	FOX	TAX	TAZ	ATM	IMP
OXA-1	RGN238	*Escherichia coli*	K10-35	100[b]	380	63[c]	75[c]	180[c]	390	30[b]	ND[d]	ND	ND	ND	ND	ND
OXA-2	R46	*Salmonella typhimurium*	Type 1a	100	140	2.3[e]	48[e]	710	31	37[b]	3.8[e]	2[f]	0.40	0.02[e]	3.6	ND
OXA-3	R57b	*Klebsiella pneumoniae*		100[b]	180	10	350	340	ND	44	10	ND	ND	ND	ND	ND
OXA-4	pMG203	*Escherichia coli*	7529	100[f]	440	39	64	220	710	190	83	<0.2	64	ND	ND	ND
OXA-5	pMG54	*Pseudomonas aeruginosa*	76072601	100[f]	190	40	260	210	110	89	180	10	49	ND	ND	ND
OXA-6	pMG39	*Pseudomonas aeruginosa*	Ming	100[f]	600	46	300	1,000	590	150	24	<0.2	28	ND	ND	ND
OXA-7	pMG202	*Escherichia coli*	7181	100[f]	540	48	490	700	420	140	51	4	31	ND	ND	ND
OXA-9	pJHCMW1	*Klebsiella pneumoniae*	JHCK1	100	110	200	ND	81	ND	ND	ND	ND	ND	ND	ND	ND
OXA-10 (PSE-2)	R151	*Pseudomonas aeruginosa*	POW151[h]	100[i]	270[i]	28[j]	230[j]	430[j]	230	32[i]	<2[i]	<0.01[i]	1[i]	0.12	6.1	0.05
OXA-11	pMLH52	*Pseudomonas aeruginosa*	ABD	100	72	3.8	ND	530	ND	0.6	1.7	<0.1	1.0	0.6	ND	<0.1
OXA-12 (AsbB1)	Chr	*Aeromonas sobria*	AER 14M	100	ND	160	190	210	ND	14	ND	ND	ND	≤2	ND	≤1
	Chr	*Actinomadura* sp.	R39	100	510	59	41	250	120	54	<0.01	160	76	>3.5	5.4	<0.01
Type A (OXA)	Ind[l]	*Alcaligenes denitrificans* subsp. *xylosoxydans*	Adx 53	ND	ND	ND	470	ND	ND	100[f,m]	63	ND	<2	<2	ND	<2
	ND	*Bacteroides fragilis*	GN11499	100	360	43	270	ND	ND	89	59	<1	ND	ND	ND	ND
	Ind	*Clostridium clostridioforme*		100[f]	ND	ND	490	ND	ND	27	ND	<1	51	ND	ND	ND
LCR-1	pMG76	*Pseudomonas aeruginosa*	2293E	100[i]	150[i]	4[i]	≤8	63	20	55[i]	24[i,n]	ND	ND	ND	9.0[i]	ND
M-OXA	Chr	*Pseudomonas*	C	100[o]	120	53	240	250	130	15	NDet[p]	NDet	ND	ND	ND	ND
	ND	*Streptomyces cacaoi*	KCC-S0352	100	30	88	60	190	25	1.0	ND	100	>0.05	>0.3	16	ND

[a] Abbreviations are defined in footnote *a* to Table 2. MET, methicillin. No enzymes in this group had reported hydrolysis rates for nitrocefin.
[b] Relative hydrolysis rates determined by hydroxylamine assay with substrate at 5 mM.
[c] Steady-state rate for biphasic hydrolysis. Burst rates were as follows: carbenicillin, 110; cloxacillin, 250; oxacillin, 260 (157).
[d] ND, not determined.
[e] Steady-state rate for biphasic hydrolysis. Burst rates were as follows: carbenicillin, 36; cloxacillin, 160; cephalothin, 5.2; tazobactam, 0.08 (156, 157).
[f] Relative hydrolysis rates were determined titrimetrically.
[g] Unpublished nucleotide sequence. The GenBank nucleotide accession number is X75562 (E. Scoulica, A. Aransay, and Y. Tselentis).
[h] The sequence was determined from plasmid pMON234. PSE-2 was also produced from plasmid R140 identified in *Escherichia coli* R140, *Klebsiella pneumoniae* R156, *Providencia stuartii* R178, and *Enterobacter cloacae* R248.
[i] Relative hydrolysis rates were determined iodometrically.
[j] PSE-2 from plasmid pMON234 showed biphasic kinetics. Steady-state rates are reported. Burst rates were as follows: carbenicillin, 120; cloxacillin, 1,400; oxacillin, 500 (157).
[k] K_m.
[l] Inducible enzyme activity was assumed to be chromosomal.
[m] Cephaloridine as 100.
[n] Relative hydrolysis rate for nitrocefin was 31. No other group 2d enzyme was tested with nitrocefin.
[o] Microiodometric or colorimetric assays.
[p] NDet, not detected.

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TABLE 8—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
1.8	4.7	1.4	>100	ND	+	ND	23.3	7.4	Nuc	D	46, 70, 109, 151, 157, 181, 222, 327
1.4	0.14	0.01	1,400	>100	−	ND	29.6	8.65 or 7.7	Nuc	D	8, 46, 68, 70, 109, 116, 121, 151, 156, 157, 181, 185, 222
ND	ND	ND	ND	ND	−	ND	42.8	7.1	ND	ND	70, 109, 181
8.4	16	5.6	ND	>100	ND	ND	23.0	7.5	Nuc	D	34, 185, 222, 234
3.1	18	0.25	ND	>100	ND	ND	27.0	7.62	Nuc	D	66, 185, 222
1.6	51	1.7	ND	<100	ND	ND	40.0	7.68	ND	ND	185, 222
0.36	40	0.61	ND	>100	ND	ND	25.3	7.65	Nuc[g]	D	185, 222
<20,000	ND	ND	ND	<10,000	+	−	ND	6.9	Nuc	D	304, 305
0.81	37	0.94	>1,000	>100	+	ND	27.5	6.1	Nuc	D	46, 106, 121, 157, 165, 178, 179, 222, 233
4.5	ND	0.5	ND	>100	ND	ND	27	6.4	Nuc	D	106
0.009	0.24	0.03	ND	480[k]	ND	−	28.6	8.6	Nuc	D	245
+	ND	ND	ND	420	ND	−	31	5.00	Nuc	A	78, 119, 137, 174, 175
3.0	ND	ND	>1,000	9.0	ND	ND	ND	7.4	ND	ND	74, 231
<0.1	<0.1	ND	ND	ND	+	ND	42	6.9	ND	ND	267
3.6	59	7.8	ND	57[k]	+	−	ND	4.2	ND	ND	9
100	ND	ND	ND	<100	−	ND	44	5.85 or 6.5	Nuc	D	66, 188, 281, 330a
>50	ND	ND	ND	ND	−	−	30	5.5	ND	ND	263
0.11	0.62	ND	ND	88	−	−	34	4.7	Nuc	A	158, 171, 174, 175, 190, 210, 211, 312

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TABLE 9. Group 2e: cephalosporinases inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis												
				LOR	LOT	PEN	AMP	CARB	CLOX	OXA	FOX	NCF	TAX	TAZ	ATM	IMP
	ND[b]	*Bacteroides fragilis*	G-242[c]	100	40	1.9	ND	ND	ND	ND	ND	ND	4.0	ND	ND	ND
CepA	Chr	*Bacteroides fragilis*	CS30	100[f]	ND	1.0	ND	ND	ND	ND	ND	19	ND	ND	ND	ND
	pBFKW1	*Bacteroides fragilis*	GAI-10150	100[f]	ND	6.8	25	ND	ND	ND	0.3	ND	33	ND	ND	ND
CblA	Chr	*Bacteroides uniformis*	WAL-7088	100	ND	10	ND	ND	ND	ND	ND	250	ND	ND	ND	ND
CfxA	Chr	*Bacteroides vulgatus*	CLA341	100[f]	68	11	7.2	ND	ND	ND	<0.01	290	1.0	ND	ND	ND
	ND	*Capnocytophaga* sp.	Van2	100	ND	ND	3.9[h]	ND	ND	ND	ND	ND	2.7	0.35	ND	ND
	ND	*Capnocytophaga* sp.	IC 5/21	100	53	NDet[j]	NDet	NDet	ND	ND	ND	ND	46	ND	ND	ND
Form II	Chr	*Citrobacter diversus*	ULA-27	100	5.9	14	5.9	3.1	<0.01	11	ND	ND	ND	ND	NDet	0.01
FEC-1	pFCX1	*Escherichia coli*	FP1546	100[f]	200	ND	17	ND	ND	ND	NDet	ND	23	0.13	ND	ND
FUR	P	*Klebsiella pneumoniae*	1510	100[m]	ND	ND	ND	ND	ND	ND	<0.5	ND	5.8	<0.5	<0.5	ND
	ND	*Nocardia brasiliensis*	Nb-361-1	100	21	<1	ND	ND	ND	ND	ND	51	ND	ND	ND	ND
FPM-1	pPM1	*Proteus mirabilis*	6003	100	240	ND	29	8.2	ND	ND	ND	ND	20	0.26	ND	ND
	Ind[o]	*Proteus penneri*	Wy 1001	100	50	3.4	8.5	<1	ND	ND	NDet	ND	48	<1	<1	ND
	ND	*Proteus vulgaris*	GN76/C-1[p]	100[f]	120	14	15	2.0	<0.1	ND	<0.1	ND	ND	ND	ND	0.01
	Ind	*Proteus vulgaris*	SC 10950	100	ND	9.6	24	ND	ND	ND	ND	ND	87	<0.1	0.83	0.05
	Chr	*Proteus vulgaris*	V3-con[q]	100	ND	24	51	3.3	ND	ND	0.07	ND	22	ND	ND	(+)[r]
	Chr	*Proteus vulgaris*	RO104	100	120	3.3	3.4[h]	ND	ND	ND	NDet	ND	13	0.17	ND	ND
L2	ND	*Xanthomonas maltophilia*	IID1275, GN12873	100	7.0	32	26	3.0	4.0	ND	0.001	ND	2.0	ND	12.0	25
BlaI	Chr	*Yersinia enterocolitica*	Y56	100	250	38	32	12	ND	ND	NDet	ND	NDet	ND	ND	ND

[a] Abbreviations are defined in footnotes *a* to Tables 2 and 3.
[b] ND, not determined.
[c] β-Lactamases from multiple strains of *Bacteroides* spp. with similar hydrolysis profiles were reported by Britz and Wilkinson (42), Olsson-Liljequist et al. (213), Sato et al. (266), and Tajima et al. (295). Other strains such as *Bacteroides fragilis* GN11477 produce a cephalosporin-hydrolyzing enzyme with an undetermined inhibition profile (266). See Rasmussen et al. (242) for a more complete compilation of *Bacteroides* β-lactamase characteristics (242).
[d] K_i.
[e] pI values for similar enzymes have been reported as 4.9 (213), 5.2 (266), 5.3 (213), and 5.6 (213).
[f] A single substrate concentration of 100 μM was assayed.
[g] Addition of clavulanic acid to amoxicillin lowered the MIC from 1,600 to 6.25 μg/ml.
[h] Amoxicillin.
[i] Addition of clavulanic acid to amoxicillin with Van-2-producing strains lowered the MIC from >64 to 0.25 μg/ml.
[j] NDet, not detected.
[k] K_m.
[l] Acidimetric assay.
[m] Substrate at 100 μM.
[n] Inhibitor restored cephalosporin activities in microbiological assays.
[o] Inducible enzyme activity was assumed to be chromosomal.
[p] Cephalosporinases from *Morganella morganii*, *Proteus inconstans*, and *Proteus rettgeri* have been described by Sawai et al. (270). Other *Proteus vulgaris* cephalosporinases have similar substrate profiles but slightly different molecular sizes and isoelectric points: strain TN1945, pI 8.8; molecular mass, 28 kDa; strain GN4413, pI 8.2; molecular mass, 27.5 kDa; strain GN4818, pI 6.9; molecular mass, 27 kDa (212).
[q] A β-lactamase with a substrate profile similar to that of V3-con but a pI of 7.8 was also described from *Proteus vulgaris* Va1-con. Both were high-level β-lactamase-producing ("stably derepressed") strains that were selected with cefotaxime from parent strains with an inducible cephalosporinase (332).
[r] Hydrolysis followed biphasic kinetics.

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TABLE 9—*Continued*

IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
<1.0	<1.0	ND	ND	0.6[d]	+	ND	32	4.7[e]	ND	ND	336
<1.0	ND	ND	ND	ND	ND	ND	31.5	4.9	Nuc	A	254
(+)[g]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	330
<1	ND	ND	ND	ND	ND	ND	33.5	4.6	Nuc	A	285
1.0	<1	ND	ND	ND	+	−	35.4	ND	Nuc	A	218
(+)[i]	ND	ND	ND	ND	ND	ND	ND	5.6	ND	ND	255
<6	ND	ND	ND	<2,000	+	ND	38	3.6	ND	ND	90
<80	ND	ND	6.7	<100[k]	+	−	29	6.2	ND	ND	5–7
0.01	0.02	ND	ND	ND	ND	ND	48	8.2	ND	ND	176
+[n]	±[n]	ND	ND	ND	ND	ND	ND	7.5	ND	ND	319
0.01	1.5	0.17	ND	0.03	+	ND	ND	5.1	ND	ND	289
0.15	ND	ND	520	44	ND	ND	26	7.2	ND	ND	322
1.2[d]	2.4[d]	ND	5,400[k]	ND	ND	ND	30	6.8	ND	ND	102
0.35[d]	2.1[d]	ND	ND	0.54[d]	ND	ND	30	8.7	ND	ND	269–271
0.04	ND	ND	26	ND	ND	ND	ND	7.4	ND	ND	46
ND	ND	ND	ND	ND	ND	ND	32	8.9	ND	ND	332
0.35[d]	0.23[d]	ND	ND	ND	ND	ND	28	8.3	AA	A	226
0.58[d]	1.9[d]	ND	26[k]	24[k]	+	−	27	8.4	ND	ND	30, 55, 260
ND	ND	ND	ND	ND	ND	ND	28	ND	Nuc	A	277, 278

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TABLE 10. Group 2f: carbapenem-hydrolyzing nonmetallo-β-lactamases[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis										IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
				PEN	AMP	CARB	CLOX	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP	CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
IMI-1	Ind[b]	*Enterobacter cloacae*	1413B	100	540	ND[c]	ND	5,600	340	ND	380	9.7	0.019	140	250	0.28	1.8	0.030	93[d]	ND	ND	–	ND	7.0	ND[e]	A	186, 246
NMC-A	Chr	*Enterobacter cloacae*	NOR-1	100[f]	305	ND	NDet[g]	ND	1,300	NDet	ND	100	0.72	190	200	0.32	10	2.0	260[d]	120	ND	–	30	6.9	Nuc	A	198, 203
Sme-1	Chr	*Serratia marcescens*	S6	100	1,300	27	ND	1,200	ND	21	ND	18	ND	16	310	14	3.3	3.0	62[d]	ND	ND	–[h]	29.3	9.7, 9.85[i]	Nuc	A	49, 198, 334

[a] Abbreviations are defined in footnote *a* to Table 2.
[b] Ind, inducible. Assumed to be chromosomal.
[c] ND, not determined.
[d] K_m.
[e] Approximately 95% sequence homology with NMC-A (246).
[f] Microacidimetric assays.
[g] NDet, not detected.
[h] Initially reported to be inhibited by EDTA (334). Later reported as not inhibitable, with the first results being due to pH effects (198).
[i] Computer-predicted pI value.

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TABLE 11. Group 3: metallo-β-lactamases not inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis													IC_{50} for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP	CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
CphA/A2	Chr[b]	*Aeromonas hydrophila*	AE036	100	>9,600	300	ND[c]	14	2.3	ND	Inac[d]	6.0	1.3	ND	<0.01	3,200	ND	37[e]	ND	>1,000	25[e,f]	+	+	28	8.0	Nuc	B	85, 173, 276
A2h	Chr[b]	*Aeromonas hydrophila*	AER 19M	100	ND	ND	0.65	ND	<0.01	1.1	ND	59	0.29	0.18	<2	40	>40[g]	ND	ND	51[e]	>50	±	+	28	8.0	ND	ND	124
	Ind[b]	*Aeromonas hydrophila*	872	100	1,700	100	ND	ND	1,200	ND	ND	ND	<10	ND	ND	9,200	ND	ND	ND	ND	ND	ND	ND	ND	7.1	ND	ND	279
II	Chr	*Bacillus cereus*	5/B/6	100	160	110	92	48	3.7	ND	0.03	6.6	8.8	ND	<0.01	>15	ND	5,200[e]	ND	>500	1,800[e,f]	ND	+	25	ND	Nuc	B[h]	85, 160
II	Chr	*Bacillus cereus*	569[i]	100	67	ND	ND	89	42	81	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2,300[e]	ND	+	24.9	8.3	AA, Nuc	B[h]	2, 15, 123, 146, 147, 259
CcrA	Chr	*Bacteroides fragilis*	QMCN3	100	ND	ND	ND	ND	78	46	17	170	39	11	ND	79	ND	ND	1,200	ND	ND	+	+	26	ND	Nuc	B	244, 247
CcrA	Chr	*Bacteroides fragilis*	QMCN4	100	ND	ND	ND	ND	38	39	9.3	460	26	14	ND	150	ND	ND	1,400	ND	ND	+	+	26	ND	Nuc	B	244, 247
CcrA	Chr	*Bacteroides fragilis*	TAL3636	100	98	98	360	ND	22	15	5.3	100	51	68	<0.01	100	>500	>500	400	>500	110[e]	+	+	26	5.2	Nuc[j]	B	17, 67, 243, 300, 333
	Chr[b]	*Flavobacterium odoratum*	GN14053	100	220	71	ND	ND	48	330	39	ND	520	<1	<1	500	>100	>100	ND	>100	ND	+	+	26.0	5.8	ND	ND	265
	Ind	*Legionella gormanii*	ATCC 33297	100	990	270	240	ND	1,400	2,600	140	ND	640	ND	<10	71	>100	>100	ND	ND	17[e]	+	+	25.0	10.5	ND	ND	94
	pMS350	*Pseudomonas aeruginosa*	GN17203	100	30	55	ND	ND	14	16	7.1	ND	3.1	ND	<0.1	23	>100	>100	ND	>100	ND	+	+	28.0	9.0	ND	ND	321
PCM-1	Ind	*Pseudomonas cepacia*	5JIV	100	49	96	<40	ND	310	ND	ND	ND	ND	26	<80	1,300	<100	ND	<100	<1,000	<1,000	+	+	ND	8.5	ND	ND	25
IMP-1	Chr	*Serratia marcescens*	TN9106	ND	100[k]	ND	ND	ND	30	ND	ND	ND	ND	16	0.08	6.9	>10	ND	ND	4.0[e]	>10	ND	+	30	>9.5	Nuc	B	214
L-1	Ind	*Xanthomonas maltophilia*	GN12873[l]	100	58	46	42	ND	6.0	5.0	ND	ND	7.0	ND	ND	24	>400[m]	>400[m]	>400[m]	ND	230[e]	−	+	118.0	6.9	Nuc[n]	B	49, 261, 320
L-1	Ind	*Xanthomonas maltophilia*	ULA-511	100	16	25	ND	26	2.5	ND	0.1	1.8	6.0	ND	<0.01	5.9	ND	76[e]	ND	>500	27[e,f]	ND	ND	ND	ND	ND	B[o]	85

[a] Abbreviations are defined in footnotes *a* to Tables 2 and 3.
[b] Inducible.
[c] ND, not determined.
[d] Inac, inactivation.
[e] K_m.
[f] Oxacillin.
[g] Imipenem was the substrate. The 50% inhibitory concentration was 0.40 μM with nitrocefin and benzylpenicillin as substrates.
[h] Sequence of gene from strain 569H differs from metallo-β-lactamase gene from strain 5/B/6 by 24 amino acids.
[i] Strain 569/H was used to produce β-lactamase for kinetics.
[j] Identical nucleotide sequences reported for genes *cfiA* from strain 2480 (300) and *ccrA* from strain 3636 (243). A closely related enzyme from *B. fragilis* is plasmid mediated (17).
[k] Ampicillin as 100.
[l] An apparent tetrameric metallo-β-lactamase with a pI of 6.8 and similar kinetic properties was reported from *Xanthomonas maltophilia* 5B105 (219).
[m] Inhibition data for strain *Xanthomonas maltophilia* 1712.
[n] Sequence determined for *Xanthomonas maltophilia* IID 1275.
[o] Assumed to be homologous to metallo-β-lactamase from *Xanthomonas maltophilia* IID 1275.

TABLE 12. Group 4: penicillinases not well inhibited by clavulanic acid[a]

Enzyme	Production	Original host	Strain	Relative rate of hydrolysis													IC$_{50}$ for inhibition (μM)					Inhibited by:		Molecular mass (kDa)	pI	Sequence	Molecular class	Reference(s)
				PEN	AMP	CARB	CLOX	OXA	LOR	LOT	FOX	NCF	TAX	TAZ	ATM	IMP	CA	SUL	TZB	ATM	CLOX	pCMB	EDTA					
	Chr	*Alcaligenes faecalis*	GN14061	100	94	64	69	ND[b]	<1	<1	<1	ND	<1	ND	<1	1	>100	>100	ND	>100[c]	154[d]	ND	+[e]	29	5.9	ND	ND	93
	Chr	*Bacteroides fragilis*	G-237	100	120	130	120	ND	38	42	20	ND	31	ND	ND	180	>100	>100	ND	ND	46[d]	+	ND	26	4.8	ND	ND	336
	ND	*Campylobacter jejuni*	52	100[f]	125[f]	ND	59[f]	ND	NDet[g]	NDet	NDet	93	NDet	ND	ND	ND	45	59	1.5	ND	ND	−	−	ND	8.8	ND	ND	153
	Ind[h], Chr	*Clostridium butyricum*		100[f]	91	97	8.0	ND	20	ND	ND	ND	ND	ND	ND	ND	>40	ND	ND	ND	>1,000	+	ND	85	4.4–4.5	ND	ND	107
SAR-2	pUK734	*Escherichia coli*	146	100[i]	100	48	ND	64	27	ND	ND	ND	20	ND	ND	ND	>100	ND	ND	ND	<0.001	−	ND	36	8.3	ND	ND	199
	Chr	*Pseudomonas cepacia*	249	100	ND	83	ND	54	3.9	<0.1	<0.1	ND	ND	<0.1	<0.1	<0.1	>50	>400	>400	ND	>100[j]	−	ND	33.5	ND	ND	ND	239
	Chr	*Pseudomonas paucimobilis*		100[k]	62	46	15	ND	3.9	ND	ND	0.04	ND	<0.1	1.6	<0.1	19	<50	4.0	ND	2,300	−	−	30	4.6	ND	ND	65

[a] Abbreviations are defined in footnote *a* to Table 2.
[b] ND, not determined.
[c] K_i.
[d] K_m.
[e] Inhibited 78% by 3 mM EDTA. Enzyme activity was regained after dialysis against distilled water.
[f] Iodometric assays.
[g] NDet, not detected.
[h] Inducible only by cephalothin.
[i] Relative rate at a fixed substrate concentration of 100 μM.
[j] Dicloxacillin.
[k] Relative rate at a fixed substrate concentration of 50 μg/ml; HPLC assays.

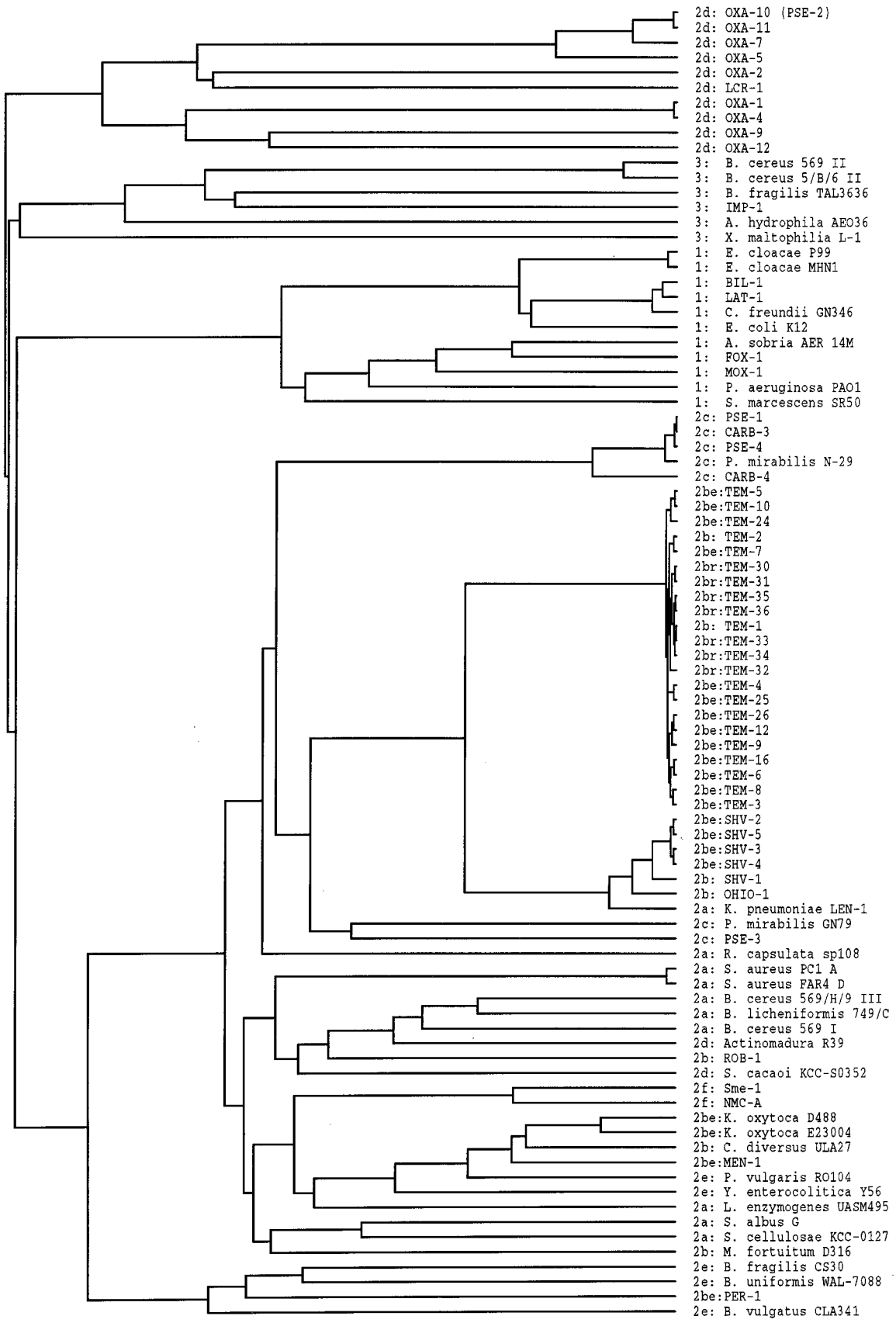

FIG. 1. Dendrogram showing relationships among β-lactamases clustered on the basis of structural similarities and their functional classification.

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