ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2001, p. 660–663 Vol. 45, No. 3
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.3.660–663.2001

GUEST COMMENTARY

Standard Numbering Scheme for Class B β-Lactamases

MORENO GALLENI,[1]* JOSETTE LAMOTTE-BRASSEUR,[1] GIAN MARIA ROSSOLINI,[2] JIM SPENCER,[3] OTTO DIDEBERG,[4] JEAN-MARIE FRÈRE,[1] AND THE METALLO-β-LACTAMASE WORKING GROUP†

Centre d'Ingénierie des Protéines, Université de Liège, B-4000 Liège, Belgium[1]; Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, I-53100 Siena, Italy[2]; Division of Protein Structure, NIMR, London NW7 1AA, United Kingdom[3]; and Laboratoire de Cristallographie Macromoleculaire, Institut de Biologie Structurale Jean-Pierre Ebel (CNRS-CEA), F-38027 Grenoble, France[4]

Metallo-β-lactamases constitute the molecular class B of Ambler (1) and group 3 according to the Bush-Jacoby-Medeiros functional classification (6). In recent years, many new enzymes of this class have been described and the sequences of the corresponding genes have been determined. Their clinical importance is highlighted by the fact that they hydrolyze carbapenems, compounds which most often escape the activity of active-site serine β-lactamases. Moreover, most metallo-β-lactamases are broad-spectrum enzymes which also hydrolyze a variety of penicillins and cephalosporins (13, 21, 22, 26). On the basis of the known sequences, three different lineages, identified as subclasses B1, B2, and B3, can be characterized. Subclass B1 contains most known metallo-β-lactamases, including the β-lactamase II (BcII) proteins from *Bacillus cereus* or other *Bacillus* spp. (15, 16, 19) and *Bacillus* sp. strain 170 (16), the CcrA (24) (also named CfiA [29]) proteins of *Bacteroides fragilis*, the BlaB proteins from *Chryseobacterium meningosepticum* (2, 26, 34), the IND-1 enzyme from *Chryseobacterium indologenes* (3), the IMP proteins found in some clinical isolates of *Pseudomonas aeruginosa* (17, 28), *Serratia marcescens* (21), *Klebsiella pneumoniae* (GenBank EMBL accession no. D29636), and *Acinetobacter baumannii* (25), and the VIM proteins found in some *P. aeruginosa* clinical isolates (18, 22). Subclass B2 includes the enzymes produced by various species of *Aeromonas* (CphA [20], ImiS [33], and CphA2 [23]) and the Sfh-I β-lactamase (GenBank accession no. AF197943) from *Serratia fonticola*. Finally, subclass B3 includes the L1 proteins from *Stenotrophomonas maltophilia* (27, 32), the GOB proteins from *C. meningosepticum* (2), the FEZ-1 enzyme from *Legionella gormanii* (5), and the THIN-B β-lactamase produced by *Janthinobacterium lividum* (25a).

The three-dimensional structures of several B1 (BcII [7, 9, 12], CcrA [8, 10], and IMP-1 [11]) enzymes and one B3 (L1 [31]) enzyme have been solved by X-ray crystallography. Despite a very low degree of sequence similarity between the two subclasses, the general structures and the relative positions of the secondary structure elements are similar. Surprisingly, the L1 enzyme is a tetramer (4, 31), whereas the B1, B2, and other B3 (FEZ-1 [5; P. S. Mercuri, F. Bouillenne, L. Boschi, J. Lamotte-Brasseur, G. Amicosante, B. Devreese, J. van Beeumen, J. M. Frère, G. M. Rossolini, and M. Galleni, unpublished data] and GOB-1 [2]) β-lactamases so far studied are monomers.

There are, however, no doubts that the proteins are homologous and the sequences of representatives of the three subclasses can be easily aligned. Indeed, in addition to the expected differences at the N and C termini, several insertions and deletions are necessary to allow the alignment of the few conserved residues acting, for instance, as ligands of the two zinc ions which can bind at the active site. Thus, homologous residues from the different class B sequences which are known to play a relevant role in the structure and function often differ in their numbering, even within each subclass.

In order to facilitate the comparative analysis of the structures and of the catalytic mechanisms, we would like to propose a standard numbering scheme for the class B β-lactamases, the BBL numbering, by analogy with the ABL numbering which has been widely accepted for class A β-lactamases. For the class B enzymes, the task was complicated by insertions and deletions and by the generally low degree of similarity but facilitated by the availability of some three-dimensional structures, which allowed the identification of homologous secondary structure elements, even when the sequence similarity was not obvious.

Figure 1 shows the proposed alignment and the derived numbering. The observed (B1 and B3) and expected (B2) secondary structure elements are indicated.

The following comments can be made. (i) Not all the known sequences are shown. When variants of an enzyme are known and the amino acid alignment exhibits more than 80% sequence identity, only the first described sequence is included in the alignment.

(ii) Alignments at the N and C termini are rather uncertain, due to a high variability even within each subclass. As is done for the class A enzymes, residue no. 1 is the first residue of the leader peptide sequence of the *S. maltophilia* L1 protein (32). Since they are highly divergent and irrelevant to the functional structure, the other leader sequences have not been included

* Corresponding author. Mailing address: Centre for Protein Engineering, B6 Sart Tilman, University of Liège, B4000 Liège, Belgium. Phone: 32-043663419. Fax: 32-043663364. E-mail: mgalleni@ulg.ac.be.

† Members are listed in the Appendix.

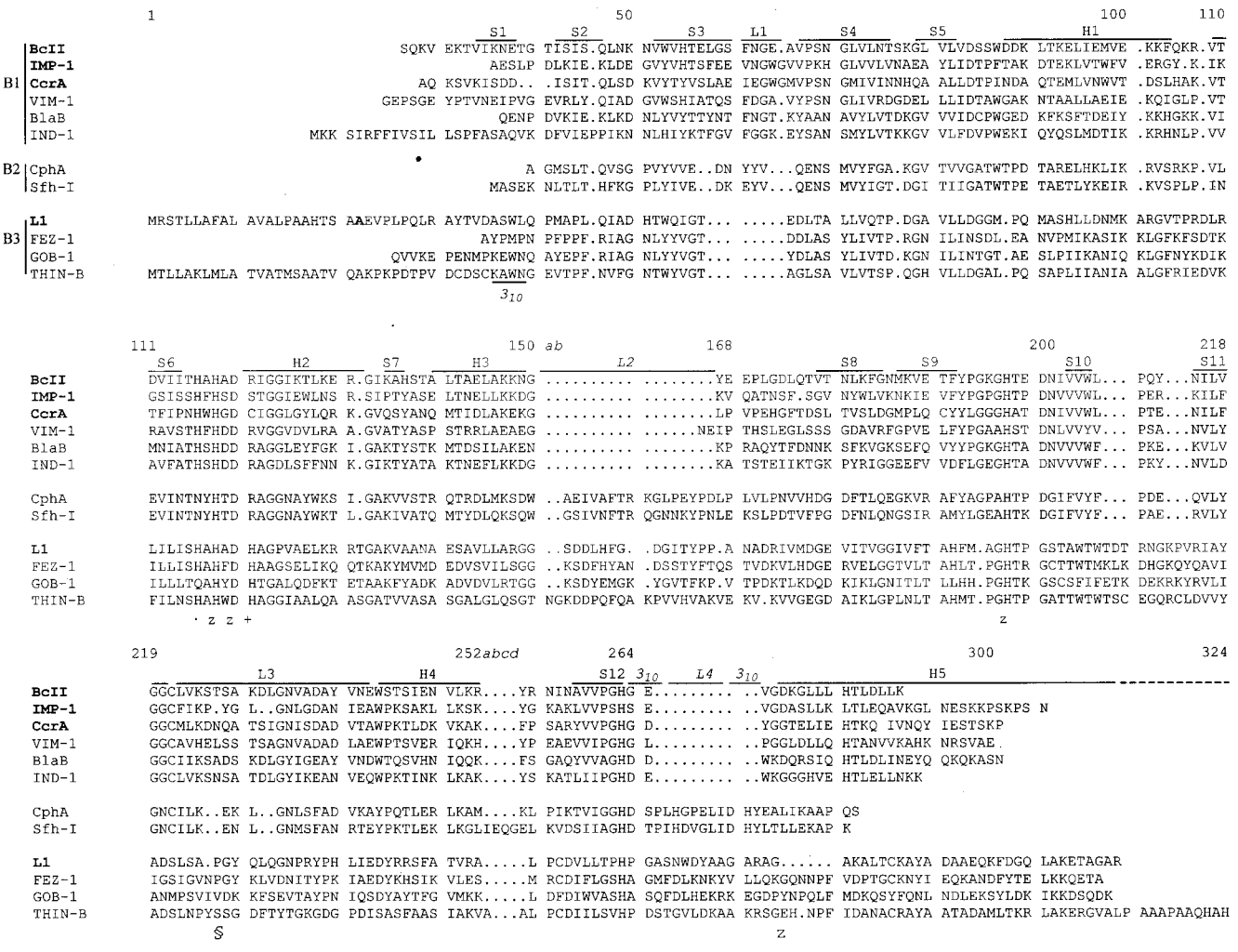

FIG. 1. Alignment of 12 class B β-lactamases numbered according to the BBL scheme. The sequences are referred to by their familiar names. BcII, *Bacillus cereus* 569H (15); IMP-1, *Pseudomonas aeruginosa* 101/477 (17); CcrA, *Bacteroides fragilis* TAL3636 (24); VIM-1, *Pseudomonas aeruginosa* VR-143/97 (18); BlaB, *Chryseobacterium meningosepticum* NCTC10585 (26); IND-1, *Chryseobacterium indologenes* 001 (3); CphA, *Aeromonas hydrophila* AE036 (20); Sfh-I, *Serratia fonticola* UTAD54 (GenBank accession no. AF197943); L1, *Stenotrophomonas maltophilia* IID1275 (32); FEZ-1, *Legionella gormanii* ATCC33297^T (5); GOB-1, *Chryseobacterium meningosepticum* PINT (2); and THIN-B, *Janthinobacterium lividum* JAC1 (25a). The names written in bold refer to the enzymes for which the three-dimensional structure is known. The amino acid in bold (Ala 22 of L1) represents the first amino acid of the mature β-lactamase. Conserved secondary structure elements of subclasses B1 and B3 are indicated above the sequences: 3_{10}, 3_{10} helix; S, β strand; H, helix. Secondary structure elements specific to subclasses B1 and B3 are highlighted by italic characters above and under the sequences, respectively. Amino acid insertions in newly sequenced enzymes are represented by small letters. The residues acting as zinc ligands in at least one subclass are characterized as follows: z, conserved residues in the three subclasses; ·, conserved residues in subclass B1 and some enzymes of subclass B3; +, conserved residue in subclass B3; §, conserved residues in subclasses B1 and B2.

unless the site of action of the signal peptidase has not been verified (Sfh-I [GenBank accession no. AF197943], IND-1 [3], and THIN-B [25a]).

(iii) This is only a numbering scheme. The fact that residues in different proteins have been assigned the same number does not imply that they occupy exactly the same relative spatial position. Indeed, if the Zn ions and their ligands are superimposed, the $G_{232}N_{233}$ dyad of BcII is more than 3 Å away from the corresponding residues in the *S. maltophilia* enzyme.

(iv) The loop which can close the active site of B1 enzymes extends between residues BBL 61 and 65 (11, 14, 30). It is absent in subclass B3 (31) and probably in B2.

(v) Any insert in a newly discovered enzyme can be characterized by small letters following the number of the last residue of the consensus sequence. Accordingly, residues $N_{140}G_{141}$ of THIN-B are defined as BBL 150a and -b and residues $I_{198}EQG_{201}$ of Sfh-I are defined as BBL 252a, -b, -c and -d, respectively.

(vi) Table 1 shows a cross-reference of the BBL numbering of the residues identified as or suspected to be the Zn1 and Zn2 ligands and that used for the individual enzymes up to the present time. Note that in subgroup B3, one of the Zn2 ligands (H121) originates with a very different part of the polypeptide chain compared to subgroup B1. Similarly, in subclass B2 and for the B3 GOB-1 enzyme, the sequence alignments unambiguously point to residues H118, H196, and N116 (B2) or Q116

TABLE 1. Numbering of the important class B residues[a]

β-Lactamase	Zn1 ligands			Zn2 ligands		
Subclass B1						
Consensus BBL	**His116**	**His118**	**His196**	**Asp120**	**Cys221**	**His263**
BcII	His86	His88	His149	Asp90	Cys168	His210
IMP-1	His77	His79	His139	Asp81	Cys158	His197
CcrA	His99	His101	His162	Asp 103	Cys181	His223
VIM-1	His88	His90	His153	Asp92	Cys172	His214
BlaB	His76	His78	His139	Asp80	Cys158	His200
IND-1	His96	His98	His159	Asp100	Cys178	His220
Subclass B2						
Consensus BBL	**Asn116**	**His118**	**His196**	**Asp120**	**Cys221**	**His263**
CphA	Asn69	His71	His148	Asp73	Cys167	His205
Sfh-I	Asn72	His74	His151	Asp76	Cys170	His212
Subclass B3						
Consensus BBL	**His/Gln116**	**His118**	**His196**	**Asp120**	**His121**	**His263**
L1	His84	His86	His160	Asp88	His89	His225
FEZ-1	His71	His73	His149	Asp75	His76	His215
GOB-1	Gln80	His82	His157	Asp84	His85	His213
THIN-B	His105	His107	His185	Asp109	His110	His253

[a] For CcrA, the numbering is reported in references 10 and 24. When not confirmed by a three-dimensional structure, the ligands are underlined. The consensus (bold) and putative consensus (bold and underlined) ligand numbers are given for each subgroup.

(B3), but such a function is rather unusual for asparagine and glutamine side chains.

APPENDIX

The metallo-β-lactamase group also includes the following: G. Amicosante and N. Franceschini, Dipartimento di Scienze e Tecnologie Biomediche, Università di L'Aquila, I-67100 Coppito, L'Aquila, Italy; K. Bush, The R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08869; N. O. Concha, Department of Structural Biology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406; O. Herzberg, Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, MD 20850; D. M. Livermore, Antibiotic Resistance Monitoring and Reference Laboratory, Central Public Health Laboratory, London NW9 5HT, United Kingdom; P. Nordmann, Service de Bactériologie-Virologie, Hopital de Bicêtre, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre, France; B. A. Rasmussen, Wyeth-Ayerst Research, Pearl River, NY 10965; J. Rodrigues and M. J. Saavedra, Department of Animal Health, University of Trás-os-Montes e Alto Douro, 5000-911 Vila Real, Portugal; B. Sutton and S. M. Fabiane, The Randall Centre, King's College London, London SE1 1UL, United Kingdom; and J. H. Toney, Department of Biochemistry, Merck Research Laboratories, Rahway, NJ 07065-0900.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the European Union (grant ERB3512-IC15-CT98-0914) as part of the training and mobility of researchers program and by the Belgian Program Pôles d'Attraction Interuniversitaire initiated by the Belgian state, prime minister's office, Services Fédéraux des Affaires Economiques, Techniques et Culturelles (PAI P4/03).

REFERENCES

1. **Ambler, R. P.** 1980. The structure of beta-lactamase. Philos. Trans. R. Soc. B Biol. Sci. **289:**321–331.
2. **Bellais, S., D. Aubert, T. Naas, and P. Nordmann.** 2000. Molecular and biochemical heterogeneity of class B carbapenem-hydrolyzing β-lactamases in *Chryseobacterium meningosepticum*. Antimicrob. Agents Chemother. **44:** 1878–1886.
3. **Bellais, S., S. Leotard, L. Poirel, T. Naas, and P. Nordmann.** 1999. Molecular characterization of a carbapenem-hydrolyzing beta-lactamase from *Chryseobacterium (Flavobacterium) indologenes*. FEMS Microbiol. Lett. **171:** 127–132.
4. **Bicknell, R., E. L. Emanuel, J. Gagnon, and S. G. Waley.** 1985. The production and molecular properties of the zinc beta-lactamase of *Pseudomonas maltophilia* IID 1275. Biochem J. **229:**791–797.
5. **Boschi, L., P. S. Mercuri, M. L. Riccio, G. Amicosante, M. Galleni, J.-M. Frère, and G. M. Rossolini.** 2000. The *Legionella (Fluoribacter) gormanii* metallo-β-lactamase: a new member of the highly divergent lineage of molecular-subclass B3 β-lactamase. Antimicrob. Agents Chemother. **44:**1538–1543.
6. **Bush, K., G. Jacoby, and A. A. Medeiros.** 1995. A functional classification for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
7. **Carfi, A., E. Duée, M. Galleni, J. M. Frére, and O. Dideberg.** 1998. 1.85 Å resolution structure of the zinc (II) β-lactamase from *B. cereus*. Acta Crystallogr. Sect. D **54:**313–323.
8. **Carfi, A., E. Duée, R. Paul-Soto, M. Galleni, J. M. Frère, and O. Dideberg.** 1998. X-ray structure of the ZnII beta-lactamase from Bacteroides fragilis in an orthorhombic crystal form. Acta Crystallogr. Sect. D **54:**45–57.
9. **Carfi, A., S. Pares, E. Duée, M. Galleni, C. Duez, J. M. Frère, and O. Dideberg.** 1995. The 3D structure of a zinc metallo-β-lactamase from *Bacillus cereus* reveals a new type of protein fold. EMBO J. **14:**4914–4921.
10. **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.
11. **Concha, N. O., C. A. Janson, P. Rowling, S. Pearson, C. A. Cheever, B. P. Clarke, C. Lewis, M. Galleni, J. M. Frere, D. J. Payne, J. H. Bateson, and S. S. Abdel-Meguid.** 2000. Crystal structure of the IMP-1 metallo beta-lactamase from *Pseudomonas aeruginosa* and its complex with a mercaptocarboxylate inhibitor: binding determinants of a potent, broad-spectrum inhibitor. Biochemistry **39:**4288–4298.
12. **Fabiane, S. M., M. K. Sohi, T. Wan, D. J. Payne, J. H. Bateson, T. Mitchell, and B. J. Sutton.** 1998. Crystal structure of the zinc-dependent beta-lactamase from *Bacillus cereus* at 1.9 Å resolution: binuclear active site with features of a mononuclear enzyme. Biochemistry **37:**12404–12411.
13. **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.
14. **Fitzgerald, P. M., J. K. Wu, and J. H. Toney.** 1998. Unanticipated inhibition of the metallo-beta-lactamase from *Bacteroides fragilis* by 4-morpholineethanesulfonic acid (MES): a crystallographic study at 1.85 Å resolution. Biochemistry **37:**6791–6800.
15. **Hussain, M., A. Carlino, M. J. Madonna, and O. Lampen.** 1985. Cloning and sequencing of the metallothioprotein β-lactamase II gene of *Bacillus cereus* 569H in *Escherichia coli*. J. Bacteriol. **164:**223–229.
16. **Kato, C., T. Kudo, K. Watanabe, and K. Horikoshi.** 1985. Nucleotide sequence of the beta-lactamase gene of alkalophilic Bacillus sp. strain 170. J. Gen. Microbiol. **131:**3317–3324.
17. **Laraki, N., M. Galleni, I. Thamm, M. L. Riccio, G. Amicosante, J. M. Frère, and G. M. Rossolini.** 1999. Structure of In31, a blaIMP-containing *Pseudomonas aeruginosa* integron phyletically related to In5, which carries an unusual array of gene cassettes. Antimicrob. Agents Chemother. **43:**890–901.

18. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla_{VIM}, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1584–1590.
19. **Lim, H. M., J. J. Pene, and R. W. Shaw.** 1988. Cloning, nucleotide sequence, and expression of the *Bacillus cereus* 5/B/6 beta-lactamase II structural gene. J. Bacteriol. **170:**2873–2878.
20. **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.
21. **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.
22. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
23. **Rasmussen, B. A., and K. Bush.** 1997. Carbapenem-hydrolyzing β-lactamases. Antimicrob. Agents Chemother. **41:**223–232.
24. **Rasmussen, B. A., Y. Gluzman, and F. P. Tally.** 1990. Cloning and sequencing of the class B beta-lactamase gene (*ccrA*) from *Bacteroides fragilis* TAL3636. Antimicrob. Agents Chemother. **34:**1590–1592.
25. **Riccio, M. L., N. Franceschini, L. Boschi, B. Caravelli, G. Cornaglia, R. Fontana, G. Amicosante, and G. M. Rossolini.** 2000. Characterization of the metallo-beta-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla(IMP) allele variants carried by gene cassettes of different phylogeny. Antimicrob. Agents Chemother. **44:**1229–1235.

25a. **Rossolini, G. M., A. Condemi, F. Pantanella, J.-D. Docquier, G. Amicosante, and M. C. Thaller.** 2001. Metallo-β-lactamase producers in environmental microbiota: new molecular class B enzyme in *Janthinobacterium lividum*. Antimicrob. Agents Chemother. **45**:836–843.

26. **Rossolini, G. M., N. Franceschini, M. L. Riccio, P. S. Mercuri, M. Perilli, M. Galleni, J. M. Frère, and G. Amicosante.** 1998. Characterization and sequence of the *Chryseobacterium* (*Flavobacterium*) *meningosepticum* carbapenemase: a new molecular class B beta-lactamase showing a broad substrate profile. Biochem. J. **332:**145–152.
27. **Sanchagrin, F., J. Dufresne, and R. C. Levesque.** 1998. Molecular heterogeneity of the L-1 metallo-β-lactamase family from *Stenotrophomonas maltophilia*. Antimicrob. Agents Chemother. **42:**1245–1248.
28. **Senda, K., Y. Arakawa, K. Nakashima, H. Ito, S. Ichiyama, K. Shimokata, N. Kato, and M. Ohta.** 1996. Multifocal outbreaks of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta-lactams, including carbapenems. Antimicrob. Agents Chemother. **40:**349–353.
29. **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* beta-lactamase II. J. Bacteriol. **172:**2584–2593.
30. **Toney, J. H., P. M. Fitzgerald, N. Grover-Sharma, S. H. Olson, W. J. May, J. G. Sundelof, D. E. Vanderwall, K. A. Cleary, S. K. Grant, J. K. Wu, J. W. Kozarich, D. L. Pompliano, and G. G. Hammond.** 1998. Antibiotic sensitization using biphenyl tetrazoles as potent inhibitors of *Bacteroides fragilis* metallo-beta-lactamase. Chem. Biol. **5:**185–196.
31. **Ullah, J. H., T. R. Walsh, I. A. Taylor, D. C. Emery, C. S. Verma, S. J. Gamblin, and J. Spencer.** 1998. The crystal structure of the L1 metallo-β-lactamase from *Stenotrophomonas maltophilia* at 1.7 Å resolution. J. Mol. Biol. **284:**125–136.
32. **Walsh, T. R., L. Hall, S. J. Assinda, W. W. Nichols, S. J. Cartwright, A. P. MacGowan, and P. M. Bennett.** 1994. Sequence and analysis of the L1 metallo-β-lactamase from *Xanthomonas maltophilia*. Biochim. Biophys. Acta **1218:**199–201.
33. **Walsh, T. R., W. A. Neville, M. H. Haran, D. Tolson, D. J. Payne, J. H. Bateson, A. P. MacGowan, and P. M. Bennett.** 1998. Nucleotide and amino acid sequences of the metallo-beta-lactamase, ImiS, from *Aeromonas veronii* bv. *sobria*. Antimicrob. Agents Chemother. **42:**436–439.
34. **Woodford, N., M.-F. I. Palepou, G. S. Babini, B. Holmes, and D. M. Livermore.** 2000. Carbapenemases of *Chryseobacterium (Flavobacterium) meningosepticum*: distribution of *blaB* and characterization of a novel metallo-β-lactamase gene, *blaB3*, in the type strain, NCTC 10016. Antimicrob. Agents Chemother. **44:**1448–1452.