

# A Structure-Based Classification of Class A $\beta$ -Lactamases, a Broadly Diverse Family of Enzymes

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## SUMMARY

For medical biologists, sequencing has become a commonplace technique to support diagnosis. Rapid changes in this field have led to the generation of large amounts of data, which are not always correctly listed in databases. This is particularly true for data concerning class A  $\beta$ -lactamases, a group of key antibiotic resistance enzymes produced by bacteria. Many genomes have been reported to contain putative  $\beta$ -lactamase genes, which can be compared with representative types. We analyzed several hundred amino acid sequences of class A  $\beta$ -lactamase enzymes for phylogenetic relationships, the presence of specific residues, and cluster patterns. A clear distinction was first made between DD-peptidases and class A enzymes based on a small number of residues (S70, K73, P107, 130SDN132, G144, E166, 234K/R, 235T/S, and 236G [Ambler numbering]). Other residues clearly separated two main branches, which we named subclasses A1 and A2. Various clusters were identified on the major branch (subclass A1) on the basis of signature residues associated with catalytic properties (e.g., limited-spectrum  $\beta$ -lactamases, extended-spectrum  $\beta$ -lactamases, and carbapenemases). For subclass A2 enzymes (e.g., CfxA, CIA-1, CME-1, PER-1, and VEB-1), 43 conserved residues were characterized, and several significant insertions were detected. This diversity in the amino acid sequences of  $\beta$ -lactamases must be taken into account to ensure that new enzymes are accurately identified. However, with the exception of PER types, this diversity is poorly represented in existing X-ray crystallographic data.

## INTRODUCTION

Natural and acquired resistance to  $\beta$ -lactam compounds, a major family of antibiotics, can result from the synthesis of one or more  $\beta$ -lactamases, which inactivate these drugs (EC 3.5.2.6).

The tremendous diversity of these enzymes and their major impact on medicine led to several attempts to classify them by as early as 1970 (1, 2). By 1995, >190 unique bacterial proteins had been described, together with their abilities to interact with various  $\beta$ -lactams, serving as the substrates or inhibitors (3). The diverse enzymatic properties of  $\beta$ -lactamases led to many attempts to categorize them on the basis of their biochemical attributes (4). The classification by Bush et al., based on the functional characteristics of  $\beta$ -lactamases, was proposed in 1995. This classification included three major groups, defined on the basis of their substrate and inhibitor profiles, molecular masses, and isoelectric points. This classification scheme was updated in 2010, with the addition of peptide sequences to the proposed list of attributes describing new  $\beta$ -lactamases (5).

An alternative classification, based on primary structure, was first proposed by Ambler in 1980 (6). At that time, the identification criterion used, which was based on peptide sequencing, was clearly limited to a small number of laboratories. There were four classes in this system: classes A, B, C, and D. More than 30 years later, this approach is still relevant. The class A, C, and D proteins are serine enzymes, with no significant structural similarities between classes, whereas those of class B, which is currently divided into three subclasses (subclasses B1, B2, and B3), are metalloenzymes containing one or two zinc ions (7). Functional group 2

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molecular class A  $\beta$ -lactamases are the most abundant, with >550 enzymes, including numerous variants (8). As a support to an updated classification, a large number of sequences have been obtained in the last decade. On 18 September 2014, we queried the GenBank nucleotide sequence database with the keyword “ $\beta$ -lactamase.” This query identified 338,691 entries, suggesting a large number of duplicate entries. It became clear that the molecular classification of these enzymes needed to be reconsidered, and the functional scheme did not include a number of naturally produced enzymes originating from various Gram-positive and Gram-negative species. In addition, several new enzymes have been identified in clinical studies. Finally, the sequences of many genomes have been reported to contain “putative” or “provisional”  $\beta$ -lactamase genes, which can be compared with well-known representative enzymes (<http://www.ncbi.nlm.nih.gov/> and <http://www.uniprot.org/>).

The large number of sequences deposited in databases raises questions about the relevance of the molecular definition of class A  $\beta$ -lactamases and their diversity. Better knowledge of sequences should improve interpretation by medical biologists and, thus, the information that they provide to clinicians. Among emerging technologies for a clinical microbiology laboratory, microarray methods (chips) are more and more attractive for the detection of resistance genes (9).

#### PHYLOGENY ANALYSIS

Hall and Barlow determined a new phylogeny for class A serine  $\beta$ -lactamases based on protein structure (10). Their analysis of 83 nucleotide/peptide sequences from class A  $\beta$ -lactamases clearly separated two groups of enzymes. One major group included widespread types such as TEM-1, PSE-1, SHV-1, and CTX-M. The second one is the CFB group, for *Cytophagales-Flavobacteriales-Bacteroidales*. Otherwise, there was probable confusion regarding class A  $\beta$ -lactamases for the NPS-1 enzyme and for some enzymes produced by species such as *Deinococcus radiodurans*, *Fusobacterium nucleatum*, or *Thermosynechococcus elongatus*. After sequencing, NPS-1, formerly identified as a class A type enzyme, was finally classified as a class D enzyme (5). The *Thermosynechococcus elongatus* enzyme was identified as penicillin-binding protein A (PBP-A) (11). As early as 1980, Ambler proposed that the following motifs are characteristic for class A  $\beta$ -lactamases: 70SerxxLys (where x's represent variable amino acids), 130SerAspAsn (the “SDN” motif), and 234LysThr/SerGly (the “KTG” motif) (6). The Glu166 residue in the  $\Omega$ -loop was found to be critical for the fast hydrolysis of penicillins and for distinguishing between class A  $\beta$ -lactamases and other serine proteins such as DD-peptidases or PBPs (11, 12). Moreover, it was recently confirmed that the 166AspxxLysAsn motif (the “ExxLN” motif) is crucial for the definition of this molecular class (13).

Figure 1 illustrates a phylogenetic analysis of 285  $\beta$ -lactamases, while Table 1 lists the 114  $\beta$ -lactamases identified by classical methods, with a single representative per group of enzymes (so the TEM-type, e.g., is reported only once) (4, 14–88). The 174 putative enzymes were selected by searching the NCBI database (<http://www.ncbi.nlm.nih.gov/>) using the keywords “class A  $\beta$ -lactamase” or “ $\beta$ -lactamase” (89–91). Considerable diversity was found among class A  $\beta$ -lactamases, which formed at least two different subclasses, which we propose to name subclasses A1 and A2, widely distributed among several bacterial phyla: *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria*,

including *Alpha*-, *Beta*-, and, in particular, *Gammaproteobacteria*, as previously reported (10, 92). Several clusters (clusters 8 to 17) were obtained, including a Gram-positive bacterial clade containing the genus *Streptomyces* and several genera from the *Firmicutes*: *Bacillus*, *Clostridium*, *Mycobacterium*, *Nocardia*, *Nocardiopsis*, *Staphylococcus*, and *Streptomyces*. Two  $\beta$ -lactamase sequences, encoding ROB-1 and ACI-1, products of the Gram-negative bacteria (GNB) *Haemophilus influenzae* and *Acidaminococcus fermentans*, respectively, were found among the 88 sequences from Gram-positive bacteria in the phylogenetic tree. Sequence analyses have already suggested that genes encoding  $\beta$ -lactamases can be transferred from Gram-positive to Gram-negative bacteria (*trans*-Gram transfer) (93).

The Gram-negative *Proteobacteria*, a major group of bacteria producing various representative chromosomal and plasmid-encoded  $\beta$ -lactamases, is represented principally by the *Gammaproteobacteria* and contains diverse pathogens from several phyla, including *Enterobacteriales*, *Pseudomonadales*, and *Vibrionales*. Several clusters were obtained, corresponding to *Burkholderia* (cluster 5), LSBLs (limited-spectrum  $\beta$ -lactamases) (clusters 26 to 29), ESBLs (extended-spectrum  $\beta$ -lactamases) (clusters 4, 6, 7, and 25), *Francisella* (cluster 3), *Xanthomonas* (cluster 23), and *Yersinia* (cluster 7) (Fig. 1). Some of the clusters obtained correspond to functional groups from the classification by Bush et al., such as penicillinases (groups 2a and 2b), carbenicillinases (group 2c), extended-spectrum  $\beta$ -lactamases (group 2be), and carbapenemases (group 2f). The branch corresponding to functional groups 2a, 2b, 2c, and 2ce was named the “LSBL” group. This major branch split off early from the root of the subclass A1 branch and may encompass four phyla. In contrast, other enzymes, such as ESBL, were found in several clusters (clusters 4, 6, 7, and 25).

Another significant group of enzymes formed a clear cluster, which we termed subclass A2, comprising three large orders of Gram-negative, non-spore-forming, aerobic, microaerophilic or anaerobic, and rod-shaped bacteria (originally named the CFB group). The emergence of this phylum predates the divergence of Gram-positive and Gram-negative bacteria (10, 94). Again, horizontal transfer events seem to have occurred in this group, as suggested by the presence of PER-1 in *Pseudomonas aeruginosa* and the presence of VEB-1 and TLA-1 in several species of enterobacteria (*Gammaproteobacteria*) (10, 95).

Finally, the branch containing several species of *Deinococcus*, *Fusobacterium*, and *Acidobacteria* was defined on the basis of only three motifs, SxxK, SDN, and KT/SG. However, no representative  $\beta$ -lactamase was identified in this branch, suggesting that more residues (e.g., Glu166) may be required for the correct identification of a class A  $\beta$ -lactamase from this branch (see Annotation in Data Banks, below).

#### PRIMARY STRUCTURE/SEQUENCE ANALYSIS

In terms of molecular structure, the largest group of class A  $\beta$ -lactamases was originally characterized on the basis of 26 strictly conserved residues (Table 2) (6). Molecular comparisons of the various class A enzymes were facilitated by the use of a standard numbering scheme, as indicated by the label “ABL” (for class A  $\beta$ -lactamase) (96). Several years later, Matagne et al. confirmed these findings and updated the list of residues that are involved in the catalytic mechanism and/or in substrate binding (12). Such

characteristic residues have also recently been identified in 67 putative  $\beta$ -lactamases (97).

In an alignment of 268 sequences for representative and putative class A  $\beta$ -lactamases from subclasses A1 and A2, it was confirmed that strictly (100%) and highly conserved (between 90 and 99%) residues, such as Gly45, Ser70, Lys73, Leu81, Pro107, Ser130, Asp131, Asn132, Ala134, Gly144, Gly156, Glu166, Lys/Arg234, Thr/Ser235, and Gly236, differentiated between subclasses (Table 2).

The enzymes of subclass A1 were principally described in studies by Ambler et al. (96), Matagne et al. (12), and Risso et al. (97), whereas those of subclass A2 were discovered more recently. The two subgroups were found to contain very different conserved residues, which clearly distinguished between the two subclasses. These residues included Asn (subclass A1) or Asp (subclass A2) at position 136, Asp or Asn at position 179, Thr or Trp/Tyr at position 180, and Asp or His/Arg at position 233 (Table 2 and Fig. 2).

Finally, other newly characterized conserved residues (Fig. 2) were found to be specific to subclass A1 (Glu37, Arg/Lys61, Arg65, Ala125, Asp157, Trp210, and Trp229) or to subclass A2 (Lys40, Asn61, His/Lys65, Val71, Tyr125, Cys135, Tyr177, Met211, Leu252, Val263, Phe264, and Val265). The composition of the  $\Omega$ -loop (residues 161 to 179) differed considerably between subclass A1 and subclass A2 enzymes. Finally, as for PER-1 and PER-2, an alignment of subclass A2  $\beta$ -lactamase sequences revealed the presence of several insertions (98, 99).

An examination of the overall amino acid composition of  $\beta$ -lactamases revealed that representative enzymes from subclass A2 had small numbers of arginine residues ( $8.2 \pm 3.9$  residues on average) and large numbers of lysine residues ( $29.0 \pm 5.5$  residues). For other groups or clusters (subclass A1) corresponding to Gram-positive bacteria and LSBL enzymes, for example, there were  $20.1 \pm 7.6$  and  $23.3 \pm 2.6$  arginine and  $28.6 \pm 10.8$  and  $9.6 \pm 3.1$  lysine residues, respectively.

## MOLECULAR CHARACTERISTICS OF SUBCLASS A1 $\beta$ -LACTAMASES

### Enzymes Produced by Gram-Positive Bacteria

Diverse representative class A  $\beta$ -lactamases have been studied for various genera, including *Bacillus*, *Clostridium*, *Nocardia*, *Nocardiodopsis*, *Staphylococcus-Enterococcus*, and *Streptomyces* (Table 1) (4, 100–108). Among these genera, the production of  $\beta$ -lactamases is of limited relevance, but these are of historical interest, particularly because they also produce other hydrolytic enzymes (4). Most of these penicillin-hydrolyzing enzymes, displaying inhibition by clavulanic acid, have been classified as group 2a enzymes on a functional basis (4). Amino acid consensus sequences were determined for each cluster, with the identification of several important motifs (Fig. 3).

$\beta$ -Lactamases have returned to the spotlight in recent years due to the use of treatments combining penicillins with clavulanic acid for infections caused by mycobacteria, which are acid-fast, rod-shaped bacteria (109–112). Many enzymatic studies have been carried out on *Mycobacterium tuberculosis* (BlaC), *Mycobacterium abscessus* (MAB-1), and *Mycobacterium fortuitum* (BlaF/MFO-1) (Table 1) (4, 22, 23, 113). The enzymes produced by these species have a broader spectrum of activity, also degrading cephalosporins, for example, and they are less sensitive to clavulanic acid; nevertheless, they are classified as functional group 2b enzymes.

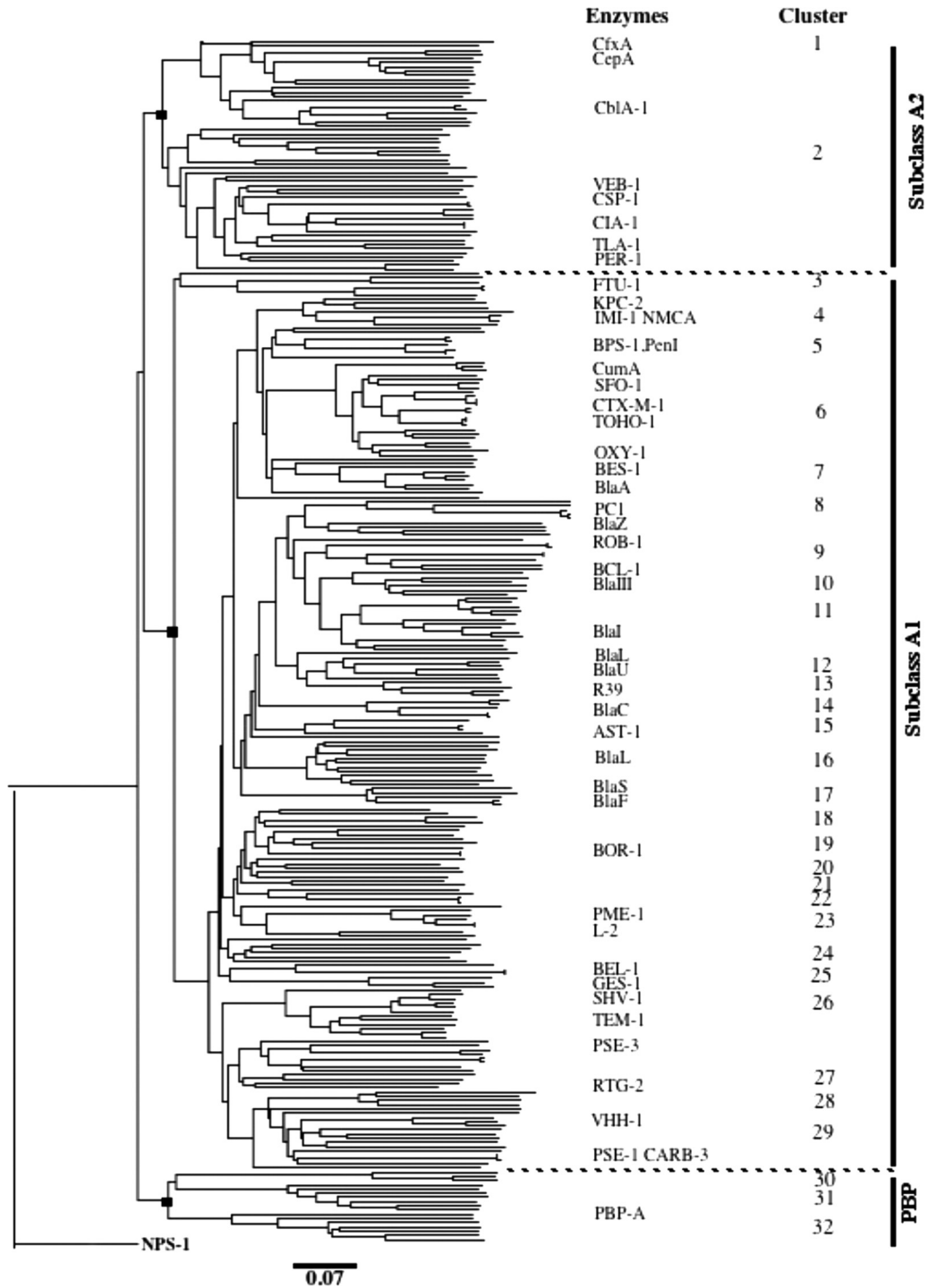
Multiple sequences from the genera described above have been included in sequence databases. Various clusters were identified among these sequences (Fig. 1). In addition to the highly and strictly conserved residues defining class A  $\beta$ -lactamases (Table 3), various other conserved residues were identified: Leu36, Ala42, Ala48, Met117, Ala125, Gly143, Pro174, Ser/Thr181, Thr/Ser182, Pro183, Ala185, Asn214, Thr/Ser216, Arg/Lys222, Gly224, Pro226, Tyr241, Gly242, Asn245, Asp246, Pro258, and Ser/Thr265. Table 3 also provides information about the principal amino acid substitutions described and the organisms in which they are found. Consensus sequences were established for ABL enzymes, and comparisons of the sequences of these enzymes evidenced multiple clusters (Fig. 3).

Several molecular characteristics can be used to distinguish between  $\beta$ -lactamases produced by various mycobacteria. The Asn132Gly substitution decreases penicillinase activity because hydrogen bonds to the substrate are lost (23). Another key difference identified on the basis of amino acid sequence alignments was the 4-residue insertion at Gly146, named residues “146a, -b, -c, and -d,” in BlaC from *M. tuberculosis* and *Mycobacterium canettii*. This insertion is also present in *M. bovis* and *M. africanum*; it may increase the size of the active site (23). Other features of *Mycobacterium* enzymes are the Ser237 and Arg276 or Arg220 residues, which favor cephalosporinase activity and may account for a broader substrate spectrum. In contrast, all the enzymes of functional groups 2a and 2c have an Arg residue at position 244. The 234KTG236 triad was observed in *Mycobacterium* enzymes but is modified to 234KSG236 in several other  $\beta$ -lactamases. This second motif was also observed in TEM-1 produced by Gram-negative bacteria (96). Given the narrow spectrum of activity of such enzymes, no amino acid substitution for expanded-spectrum resistance or resistance to inhibitors could be identified (12, 114, 115). Finally, the deletion of two residues between Ser218 and Ile221, in enzymes produced by several mycobacteria such as *M. fortuitum*, was found to be unique to the class A  $\beta$ -lactamases of Gram-positive bacteria (Fig. 3) (23–91, 93–113).

### Enzymes Produced by Gram-Negative Bacteria

The amino acid sequences of 145 representative and putative class A  $\beta$ -lactamases from subclass A1 (*Proteobacteria*) identified in Gram-negative bacteria (GNB) were compared. ABL consensus sequences were obtained for 11 clusters (Fig. 4). As previously documented for Gram-positive bacteria, 26 strictly or highly conserved amino acids were clearly identified. Some of these amino acids were essential for catalytic processes and/or substrate binding (underlined) in the various types of subclass A1  $\beta$ -lactamases produced by GNB (Table 4 and Fig. 5) (12, 96): Glu37, Gly45, Phe66, Ser70, Lys73, Leu81, Pro107, Ser130, Asp131, Asn132, Ala134, Asn136, Gly144, Gly156, Asp157, Arg/His164, Glu166, Leu169, Asp179, Thr180, Leu199, Leu207, Asp/Glu233, Lys/Arg234, Thr/Ser235, and Gly236. Additional highly conserved residues from this GNB group included Arg/Lys43, Arg/Lys61, Arg65, Thr/Ser71, Ala125, Gly143, Thr149, Arg/Lys153, Arg/Lys161, Leu162, Asp163, Asn170, Gly175, Asp/Glu176, Arg178, Thr/Ser181, Thr/Ser182, Pro183, Ala185, Thr210, Pro226, Thr229, and Tyr264.

Various ABL consensus sequences were specified according to the clusters highlighted (Fig. 4). A group of naturally or originally limited-spectrum  $\beta$ -lactamases (LSBLs) was isolated, which included various types of predominantly chromosomal enzymes but also a num-



**FIG 1** Rooted phylogram for 285 representative and putative class A “β-lactamases.” The protein sequences of representative enzymes are listed in Table 1. Putative enzymes referenced by a GI number or by a UniProt or GenBank accession number are listed but contain the following conserved motifs common to class A β-lactamases: 70SxxK, 130SDN, and 234K/RT/SG. All sequences were aligned by using Clustal X, and the tree was constructed by the neighbor-joining method (89–91). The tree was rooted by using NPS-1 (class D type) (5). Compositions of clusters are as follows. Cluster 1 contains CfxA, *Bacteroides plebeius* (GI:494836881), *Bacteroides dorei* (GI:495118154), *Bacteroides vulgatus* (GI:492440614), CepA, *Bacteroides thetaiotaomicron* (GI:499421831), *Bacteroides finegoldii* (GI:495040696), *Bacteroides xylanisolvens* (GI:505345436), *Bacteroides caccae* (GI:547310572), *Paraprevotella clara* (GI:547244659), *Alistipes putredinis* (GI:548241566), *Odoribacter splanchnicus* (GI:547746400), *Coprobacter fastidiosus* (GI:550265095), *Bacteroides salyersiae* (GI:492712252), *Parabacteroides johnsonii* (GI:495433020), *Bacteroides uniformis* (GI:492414506), CblA-1, *Bacteroides clarus* (GI:496412045), *Bacteroides stercoris* (GI:547517529), *Bacteroides cellulolyticus* (GI:494410911), and *Odoribacter laneus* (GI:547316755). Cluster 2 contains *Koribacter versatilis* (GI:499841677), *Fibrella aestuarina* (GI:505147229), *Solibacter usitatus* (GI:500003326), *Fischerella muscicola* (GI:515346414), *Geitlerinema* sp. (GI:504984817), *Oscillatoria nigro-viridis* (GI:504988346), *Microcoleus vaginatus* (GI:493685886), *Terriglobus saanensis* (GI:503334310), *Granulicella mallensis* (GI:504031923), *Oxalobacter formigenes* (GI:492543941), *Leadbetterella byssophila* (GI:503175515), *Aequorivita sublithicola* (GI:504594895), *Flavobacterium rivuli* (GI:519057907), *Microscilla marina* (GI:488788845),-

ber of plasmid-encoded or transposable-element-encoded enzymes (TEM-1, SHV-1, GIL-1, LAP-1, LEN-1, OHIO-1, OKP-A, ORN-1, PLA-1, and TER-1; cluster LSBL1). Sequence comparisons identified another group of three clusters. The first of these clusters, cluster LSBL2, was characterized by the plasmid-encoded or, preferentially, transposable-element-encoded enzymes, such as PSE/CARB-type enzymes. However, it also included chromosome-encoded enzymes such as VHH-1, VHW-1, and VAK-3. The second cluster (cluster LSBL3) included BlaP (RTG-1), CARB-5 (RTG-2), and SCO-1, and the third, small cluster (cluster LSBL4) included CKO-1/MAL-1, AER-1, PSE-3, and HER-1. These enzymes were classified according to their penicillinase activity (functional group 2a or 2b) or their capacity to hydrolyze benzylpenicillin, ampicillin, and carbenicillin (carbenicillinase; functional group 2c) (Table 1) (4). Most of these  $\beta$ -lactamases are chromosome encoded and species specific. This was the case for SHV and OKP from *Klebsiella pneumoniae*, LEN from *Klebsiella variicola*, GIL-1 from *Citrobacter gillenii*, and PLA from *Raoultella* (formerly *Klebsiella*) *planticola* (Table 1). The same is

true for a second heterogeneous group, containing VHH and VHW from *Vibrio harveyi*, PLES-1 from *Plesiomonas shigelloides*, CKO-1 from *Citrobacter koseri*, and HER-1 from *Escherichia hermannii* (Table 1). Chromosomal enzymes generally confer a low level of resistance to penicillins, with strong synergy between penicillins and inhibitors such as clavulanate being observed (116). Some of these enzymes have been transferred between species (SHV and PSE types) and were thus identified in diverse species. Another example is that of PSE-1, a  $\beta$ -lactamase usually observed in *Pseudomonas* isolates (Table 1), the gene for which has been found on the chromosome of several serovars of *Salmonella*, including the pandemic *Salmonella enterica* serovar Typhimurium strain DT104 (117).

Interestingly, a psychrophilic marine bacterium, *Moritella marina* (formerly *Vibrio marinus*), produces a  $\beta$ -lactamase that is ~50% identical to CARB/PSE-type enzymes. It is also 52 to 55% identical to VHH-1, VHW-1, and VAK-3, which are produced by several *Vibrio* species (56).

*Runella slithyiformis* (GI:338212617), *Spirochaeta smaragdinae* (GI:302338706), SPU-1, CSP-1, CME-1, *Elizabethkingia anophelis* (GI:496376198), CIA-1, CGA-1, *Chryseobacterium gleum* (GI:489068003), a *Flavobacteriaceae* bacterium (GenBank accession number [ACU07378](#)), TLA-2, TLA-1, *Dysgonomonas gadei* (GI:493853886), *Dysgonomonas mossii* (GI:493895874), *Mucilaginibacter paludis* (GI:495788773), *Chitinophaga pinensis* (GI:502446270), *Solitalea canadensis* (GI:504491830), PER-1, and *Rheinheimera* sp. (GI:496172849). Cluster 3 contains BRO-1, FTU-1, FPH-1, *Francisella novicida* (GI:489124215), *Francisella tularensis* (GI:56707736), and *Francisella philomiragia* (GI:490415072). Cluster 4 contains KPC-2, SFC-1, BIC-1, FRI-1 (GenBank accession number [KT192551](#)), IMI-1, NMC-A, and SME-1. Cluster 5 contains *Janthinobacterium lividum* (GI:722541454), MIN-1, PenA-1, PenB, BURTH, BPS-1, PenI, and LUT-1. Cluster 6 contains HugA, BlaP, CumA, SMO-1, RAHN-1, SFO-1, FONA-1, KLUC-1, FEC-1, CTX-M-1, MEN-1, KLUG-1, CTX-M-8, CTX-M-2, TOHO-1 (CTX-M-44), KLUA-1, CdiA, *Citrobacter rodentium* (GI:283784953), SED-1, K1, OXY-1, RIC-1, and GRI-1. Cluster 7 contains BES-1, *Desulfovibrio fructosivorans* (GI:302491851), *Photorhabdus temperata* (GI:572732591), BlaA, *Yersinia intermedia* (GI:491326176), *Yersinia enterocolitica* (GI:386308434), *Yersinia rohdei* (GI:490853828), *Yersinia frederiksenii* (GI:490849647), ERP-1, and DES-1. Cluster 8 contains *Staphylococcus saprophyticus* (GI:73663495), *Staphylococcus lentus* (GI:515566961), PC1, BlaZ, *Staphylococcus capitis* (GI:488367486), *Listeria weihenstephanensis* (GI:163862487), *Listeria rocourtia* (GI:577782107), CAD-1, and *Carnobacterium maltaromaticum* (GI:508605778). Cluster 9 contains *Acidaminococcus intestini* (GI:352684689), ACI-1, *Clostridium boltea* (GI:488630754), *Clostridium clostridioforme* (GI:488638901), *Clostridium kluyveri* (GI:153955520), *Clostridium botulinum* (GI:387816964), and *Clostridium butyricum* (GI:488645596). Cluster 10 contains BCL-1, BlaIII, *Bacillus mycolides* (GI:238631946), *Bacillus megaterium* (GI:384046004), BlaP, and *Bacillus sonorensis* (GenBank accession number [WP\\_006636516](#)). Cluster 11 contains *Bacillus siamensis* (GenBank accession number [WP\\_016936501](#)), *Bacillus amyloliquefaciens* (GI:384159812), *Bacillus vallismortis* (GenBank accession number [WP\\_010330579](#)), *Bacillus atrophaeus* (GenBank accession number [WP\\_010788750](#)), *Bacillus subtilis* (GI:430758242), *Paenibacillus alvei* (GI:528203625), *Paenibacillus dendritiformis* (GI:493726732), *Bacillus anthracis* (GI:16586918), *Bacillus thuringiensis* (GI:228921220), *Brevibacillus brevis* (GI:226310791), *Paenibacillus elgii* (GI:498185650), and *Paenibacillus vortex* (GI:493231110). Cluster 12 contains BlaL, BlaU, *Streptomyces globisporus* (GI:40252999), *Streptomyces badius* (GI:543894), *Streptomyces rapamycinicus* (GI:521362049), *Streptomyces violaceusniger* (GI:345012931), and *Streptomyces albulus* (GenBank accession number [AIA01176](#)). Cluster 13 contains R39, *Nocardiopsis halotolerans* (GI:516138136), *Nocardiopsis synnemataformans* (GI:516136495), and *Nocardiopsis dassonvillei* (GI:297561341). Cluster 14 contains *Mycobacterium ulcerans* (GI:118617810), *Mycobacterium marinum* (GI:522804997), *Mycobacterium kansasii* (GI:556580243), BlaC, and *Mycobacterium canettii* (GI:433635127). Cluster 15 contains FAR-1, AST-1, *Nocardia cyriacigeorgica* (GI:379708460), and *Nocardia brasiliensis* (GI:407644603). Cluster 16 contains *Streptomyces rimosus* (GI:490078826), *Streptomyces clavuligerus* (UniProt accession number Z54190), *Streptomyces prunicolor* (GI:517893908), *Streptomyces svceus* (GenBank accession number [EDY57556](#)), *Streptomyces lavendulae* (GI:460976), BlaL, BlaF, *Streptomyces afghaniensis* (GI:519330093), *Streptomyces aureofaciens* (GI:115040), *Streptomyces avermitilis* (GI:298300955), and *Streptomyces scabiei* (GI:290958323). Cluster 17 contains *Mycobacterium smegmatis* (GI:441478338), *Mycobacterium rhodesiae* (GI:353180119), BlaS, and BlaF. Cluster 18 contains *Rhodopseudomonas palustris* (GI:39933439), *Nitrobacter winogradskyi* (GI:75674549), *Rhizobium leguminosarum* (GI:209548458), *Rhizobium etli* (GI:86356860), and *Chelativorans* sp. (GI:110634564). Cluster 19 contains *Oligotropha carboxidovorans* (GI:209885828), *Afipia* sp. (GI:496699757), *Pseudomonas stutzeri* (GI:489382757), *Hahella chejuensis* (GI:83645112), *Halomonas anticariensis* (GI:654477376), BOR-1, and *Bordetella parapertussis* (GI:33597187). Cluster 20 contains *Hydrogenophaga intermedia* (GI:633892804), *Collimonas fungivorans* (GI:340786568), *Polaromonas* sp. (GI:495145711), *Acidovorax citrullii* (GI:120611221), and *Roseomonas cervicalis* (GI:296263154). Cluster 21 contains *Caulobacter vibrioides* (GI:490759186) and *Caulobacter segnis* (GI:295690758). Cluster 22 contains *Comamonas aquatica* (GI:594555005), *Comamonas testosteroni* (GI:299532614), *Delftia acidovorans* (GI:160365590), and *Delftia tsuruhatensis* (GI:160365590). Cluster 23 contains PME-1, XCC-1, *Xanthomonas vasicola* (GI:498052867), *Xanthomonas axonopodis* (GI:515739148), *Xanthomonas oryzae* (GI:384420274), *Xanthomonas campestris* (GI:289667228), L2, and *Stenotrophomonas maltophilia* (GI:344208573). Cluster 24 contains *Nitrosomonas europaea* (GI:114331058), *Glaciicola arctica* (GI:494893065), *Hydrogenovibrio marinus* (GI:737659509), *Geobacter lovleyi* (GI:501447176), *Marinobacter nanhaiticus* (GI:478771237), and *Bacillus ambifaria* (GI:493811254). Cluster 25 contains BEL-1, IBC-1, GES-1, *Sphingomonas paucimobilis* (GI:612114377), SGM-1, and *Sphingobium* sp. (GI:494981436). Cluster 26 contains LAP-1, OHIO-1, SHV-1, *Klebsiella variicola* (GI:674224895), LEN-1, OKP-A, GIL-1, TEM-1, "Ca. Hamiltonella defensa" (GI:238898184), TER-1, PLA-1, and ORN-1. Cluster 27 contains HER-1, PSE-3, *Pseudomonas putida* (GI:430799226), AER-1, MAL-1, CKO-1, *Cronobacter pulveris* (GI:658501069), and *Kosakonia radicinicans* (GI:635194415). Cluster 28 contains SCO-1, *Alcaligenes faecalis* (GI:651371260), RTG-2 (CARB-5), and *Thalassospira xiamenensis* (GI:745811510). Cluster 29 contains PAL-1, *Acinetobacter gyllenbergii* (UniProt accession number V2WAG0), *Acinetobacter beijerinckii* (UniProt accession number N9EC93), *Xenorhabdus bovienii* (GI:666614305), *Vibrio caribbeanicus* (GI:309368641), *Photobacterium leiognathi* (GI:86160920), *Vibrio parahaemolyticus* (GI:28900332), VHH-1, VHW-1, AmpC, MP-1, *Listonella damsela* (UniProt accession number H1A9J3), *Oleispira antarctica* (GI:508730878), *Vibrio splendidus* (GI:84385766), PLES-1, CARB-3, PSE-1, *Endozooecomonas lysicola* (GI:658302966), and VAK-3. Cluster 30 contains *Fusobacterium periodonticum* (GI:492792583), *Fusobacterium nucleatum* (GI:296155167), and *Fusobacterium hwasookii* (GI:657691008). Cluster 31 contains *Deinococcus peraridilitoris* (GI:505047536), *Deinococcus maricopensis* (GI:503321584), *Deinococcus proteolyticus* (GI:325284092), *Deinococcus deserti* (GI:502016181), *Deinococcus radiodurans* (GI:499189538), *Deinococcus phoenicis* (GI:736332987), and *Deinococcus geothermali* (GI:94554705). Cluster 32 contains *Synechococcus elongatus* (GI:81169293), *Richelia intracellularis* (GI:739354188), *Nodularia spumigena* (GI:493207907), *Nostoc punctiforme* (GI:501377316), *Cylindrospermum stagnale* (GI:505018675), *Anabaena cylindrica* (GI:505029987), and *Trichormus azollae* (GI:502957652).

TABLE 1 Representative class A  $\beta$ -lactamases examined in the UniProt database<sup>f</sup>

Class A $\beta$ -lactamase	Origin of name	UniProt accession no. <sup>a</sup>	Location(s) of gene <sup>b</sup>	Organism	Group(s) <sup>c</sup>	No. of aa <sup>d</sup>	Reference
ACI-1	<i>Acidaminococcus</i>	Q9XBM2	?	<i>Acidaminococcus fermentans</i>	2be	284	14
AER-1	<i>Aeromonas</i>	Q44056	Tn	<i>Aeromonas hydrophila</i>	2c	304	4
AmpC <sup>e</sup>	Ampicillin resistance class C	Q6T3Q5	Chr	<i>Vibrio fischeri</i>	2b	283	15
AST-1	<i>Nocardia asteroides</i>	Q9EZQ7	Chr	<i>Nocardia asteroides</i>	2a	310	16
BCL-1	<i>Bacillus clausii</i>	A8RR46	Chr	<i>Bacillus clausii</i>	2a	307	17
BEL-1	Belgium extended-spectrum $\beta$ -lactamase	Q3SAW3	P	<i>Pseudomonas aeruginosa</i>	2be	283	18, 19
BES-1	Brazil extended-spectrum $\beta$ -lactamase	Q9L6I3	P	<i>Serratia marcescens</i>	2be	292	20
BIC-1	Bicêtre carbapenemase	D2WFL1	Chr	<i>Pseudomonas fluorescens</i>	2f	294	21
BlaA	$\beta$ -Lactamase	Q01166	Chr	<i>Yersinia enterocolitica</i>	2e	294	4, 87
BlaC	$\beta$ -Lactamase	A5U493	Chr	<i>Mycobacterium tuberculosis</i>	2b	307	22, 23
BlaF	$\beta$ -Lactamase	Q59517	Chr	<i>Mycobacterium fortuitum</i>	2b	294	4
BlaL	$\beta$ -Lactamase	Q03680	Chr	<i>Streptomyces cacaoi</i>	2d	325	4
BlaP	$\beta$ -Lactamase	P00808	Chr	<i>Bacillus licheniformis</i>	2a	307	4
BlaP	$\beta$ -Lactamase	P30897	Chr	<i>Proteus mirabilis</i>	2c	270	4
BlaS	$\beta$ -Lactamase	Q7WVE1	Chr	<i>Mycobacterium smegmatis</i>	2be	293	4
BlaU	$\beta$ -Lactamase	P14560	Chr	<i>Streptomyces cacaoi</i>	2a	314	4
BlaY	$\beta$ -Lactamase	P00809	Chr	<i>Bacillus cereus</i>	2a	306	4
BlaZ	$\beta$ -Lactamase	P00807	Chr	<i>Staphylococcus aureus</i>	2a	281	4
BlaIII	$\beta$ -Lactamase type III	P06548	Chr	<i>Bacillus cereus</i>	2a	316	4
BOR-1	<i>Bordetella</i>	Q7WKQ6	Chr	<i>Bordetella bronchiseptica</i>	2a	305	24
BPS-1	<i>Burkholderia pseudomallei</i>	Q9AGU2	Chr	<i>Burkholderia pseudomallei</i>	2be	295	4, 25
BRO-1	<i>Branhamella (Moraxella)</i>	Q59514	P	<i>Moraxella catarrhalis</i>	2c	313	4
BURTH	<i>Burkholderia thailandensis</i>	Q2T5A3	Chr	<i>Burkholderia thailandensis</i>	2be	322	26, 27
CAD-1	<i>Carnobacterium divergens</i>	Q4QXY0	Chr	<i>Carnobacterium divergens</i>	2a	304	28
CARB-3	Carbenicillin resistance	P37322	P	<i>Pseudomonas aeruginosa</i>	2c	288	4
CblA	Chromosomal $\beta$ -lactamase of class A	P30898	Chr	<i>Bacteroides uniformis</i>	2e	296	4
CdiA	<i>Citrobacter diversus</i>	P22390	Chr	<i>Citrobacter amalonaticus</i>	2e	294	4
CepA	Cephalosporinase of class A	Q57150	Chr	<i>Bacteroides fragilis</i>	2e	300	4
CfxA	Cefoxitin resistance class A	P30899	Chr, Tn	<i>Bacteroides vulgatus</i>	2e	321	4
CGA-1	<i>Chryseobacterium gleum</i> class A	Q8VT49	Chr	<i>Chryseobacterium gleum</i>	2be	292	29
CIA-1	<i>Chryseobacterium indologenes</i> class A	G9M9P7	Chr	<i>Chryseobacterium indologenes</i>	2be	292	30
CKO-1	<i>Citrobacter koseri</i>	Q8RNV0	Chr	<i>Citrobacter koseri</i>	2b	300	31
CME-1	<i>Chryseobacterium meningosepticum</i>	Q9RAZ9	Chr	<i>Elizabethkingia meningoseptica</i>	2be	295	32
CSP-1	<i>Capnocytophaga sputigena</i>	D5HKL4	Chr	<i>Capnocytophaga sputigena</i>	2be?	305	33
CTX-M-1	Cefotaxime Munich	Q7AVW6	P	<i>Escherichia coli</i>	2be	291	4
CTX-M-2	Cefotaxime Munich	P74841	P	<i>Salmonella Typhimurium</i>	2be	291	4, 34
CTX-M-8	Cefotaxime Munich	Q9RMT4	Chr	<i>Citrobacter amalonaticus</i>	2be	291	35
CumA	Cefuroxime class A	P52664	Chr	<i>Proteus vulgaris</i>	2e	300	4
DES-1	<i>Desulfovibrio desulfuricans</i>	Q8KVT3	Chr	<i>Desulfovibrio desulfuricans</i>	2be	324	36
ERP-1	<i>Erwinia persicina</i>	Q8L1Z4	Chr	<i>Erwinia persicina</i>	2be	293	37
FAR-1	<i>Nocardia farcinica</i>	Q5YXD6	Chr	<i>Nocardia farcinica</i>	2a	313	38
FEC-1	Fecal <i>Escherichia coli</i>	Q8G9E9	P	<i>Escherichia coli</i>	2e, 2be	291	4
FONA-1	<i>Serratia fonticola</i> class A	Q9RIR3	Chr	<i>Serratia fonticola</i>	2be	295	39
FPH-1	<i>Francisella philomiragia</i>	YP_001676751*	Chr	<i>Francisella philomiragia</i>	2b	294	40
FRI-1	French imipenemase	KT192551*	P	<i>Enterobacter cloacae</i>	2f	294	88
FTU-1	<i>Francisella tularensis</i>	CAJ79318*	Chr	<i>Francisella tularensis</i>	2a	294	41
GES-1	Guiana extended spectrum	Q9KJY7	P	<i>Klebsiella pneumoniae</i>	2be	287	42
GIL-1	<i>Citrobacter gillenii</i>	A4KCT8	Chr	<i>Citrobacter gillenii</i>	2b?	286	43
GRI-1	<i>Leminorella grimontii</i>	A4FRA6	Chr	<i>Leminorella grimontii</i>	2be	294	
HER-1	<i>Escherichia hermannii</i>	Q93FN7	Chr	<i>Escherichia hermannii</i>	2b?	290	44
HugA	Hôpital Universitaire Genève class A	Q8VTN0	Chr	<i>Proteus penneri</i>	2be	298	45
IBC-1	Integron-borne cephalosporinase	Q83ZP8	In	<i>Enterobacter cloacae</i>	2be	287	46
IMI-1	Imipenem hydrolyzing	Q46991	Chr	<i>Enterobacter cloacae</i>	2f	292	47
K1	First resistant <i>Klebsiella</i> isolate (aztreonam)	Q938A8	Chr	<i>Klebsiella oxytoca (K. aerogenes)</i>	2be	290	4
KLUA-1	<i>Kluyvera ascorbata</i>	Q9RLX4	Chr	<i>Kluyvera ascorbata</i>	2be	291	48
KLUC-1	<i>Kluyvera cryocrescens</i>	Q8VVP3	Chr	<i>Kluyvera cryocrescens</i>	2be	291	49
KLUG-1	<i>Kluyvera georgiana</i>	Q8GNP9	Chr	<i>Kluyvera georgiana</i>	2be	291	50
KPC-2	<i>Klebsiella pneumoniae</i> carbapenemase	Q93LQ9	P	<i>Klebsiella pneumoniae</i>	2be	293	51
L2	Second labile enzyme	Q9RBQ1	Chr	<i>Stenotrophomonas maltophilia</i>	2e	303	4
LAP-1	Initials of one of the authors	A0SVI2	P	<i>Enterobacter cloacae</i>	2b	285	52
LEN-1	Name of strain	P05192	Chr	<i>Klebsiella pneumoniae</i>	2a	279	4
LUT-1	<i>Pseudomonas luteola</i>	Q670S6	Chr	<i>Pseudomonas luteola</i>	2e	296	53
MAB-1	<i>Mycobacterium abscessus</i>	B1MCL3	Chr	<i>Mycobacterium abscessus</i>	2be	289	54
MAL-1	<i>Levinea malonatica</i>	Q9AL74	Chr	<i>Citrobacter koseri</i>	2a	300	4
MEN-1	Named after patient	P28585	P	<i>Escherichia coli</i>	2be	291	4
MIN-1	<i>Minibacterium massiliensis</i>	A6SVG3	Chr	<i>Minibacterium massiliensis</i>	2be	299	55
MP-1	Name of strain	Q9RA17	Chr	<i>Moritella marina</i>		287	56
NMC-A	Not metallo-carbapenemase class A	P52663	Chr, In	<i>Enterobacter cloacae</i>	2f	292	4, 57
OHIO-1	Discovered in the state of Ohio	P18251	P	<i>Enterobacter cloacae</i>	2b	286	4
OKP-A	Other <i>Klebsiella pneumoniae</i>	Q2YHZ5	Chr	<i>Klebsiella pneumoniae</i>	2b	286	58
ORN-1	<i>Raoultella ornithinolytica</i>	Q6W7F0	Chr	<i>Raoultella ornithinolytica</i>	2b	291	59
OXY-1	<i>Klebsiella oxytoca</i>	P22391	Chr	<i>Klebsiella oxytoca</i>	2be	291	60
PCI	Strain PCI	M25252	Chr	<i>Staphylococcus aureus</i>	2a	281	4

(Continued on following page)

TABLE 1 (Continued)

Class A $\beta$ -lactamase	Origin of name	UniProt accession no. <sup>a</sup>	Location(s) of gene <sup>b</sup>	Organism	Group(s) <sup>c</sup>	No. of aa <sup>d</sup>	Reference
PenA-1	Penicillin resistance class <u>A</u>	G7HSV5	Chr	<i>Burkholderia cenocepacia</i>	2be	312	61
PenA	Penicillin resistance class <u>A</u>	O08350	Chr	<i>Burkholderia cepacia</i>	2be	302	4, 62
PenA	Penicillin resistance class <u>A</u>	B9BS30	Chr	<i>Burkholderia multivorans</i>	2be	302	63
PenI	Penicillin resistance	H7C785	Chr	<i>Burkholderia pseudomallei</i>	2be	295	63
PER-1	<i>Pseudomonas</i> extended resistant	P37321	P In	<i>Pseudomonas aeruginosa</i>	2be	308	4
PLES-1	<i>Plesiomonas shigelloides</i>	R8AQR8	Chr	<i>Plesiomonas shigelloides</i>	2b	299	
PLA-1	<i>Raoultella planticola</i>	Q6W7F0	Chr	<i>Raoultella planticola</i>	2b	291	59
PME-1	<i>Pseudomonas aeruginosa</i> ESBL	E9N9H5	P	<i>Pseudomonas aeruginosa</i>	2be	309	64
PSE-1	<i>Pseudomonas</i> -specific enzyme	Q03170	P	<i>Pseudomonas aeruginosa</i>	2c	287	4
PSE-3	<i>Pseudomonas</i> -specific enzyme	AJ877225	P	<i>Pseudomonas aeruginosa</i>	2c	293	4
R39	Resistant strain no.	Q60225	Chr	<i>Actinomadura</i> sp.	2a, 2d, 2be	304	10
RAHN-1	<i>Rahnella aquatilis</i>	Q93ET5	Chr	<i>Rahnella aquatilis</i>	2be	295	65
RIC-1	<i>Leminorella richardii</i>	A4FRA8	Chr	<i>Leminorella richardii</i>	2be	295	
ROB-1	Named after patient	P67918	P	<i>Haemophilus influenzae</i>	2b	305	4
RTG-2	Triad 234ArgThrGly236 (RTG)	Q9JP71	Chr	<i>Acinetobacter calcoaceticus</i>	2c	298	4, 66
SCO-1	Author's name (S. Corvec)	A5Y0S3	P	<i>Acinetobacter baumannii</i>	2b	288	67, 68
SED-1	<i>Citrobacter sedlakii</i>	Q93PQ0	Chr	<i>Citrobacter sedlakii</i>	2be?	295	69
SFC-1	<i>Serratia fonticola</i>	Q6JP75	Chr	<i>Serratia fonticola</i>	2be	309	70
SFO-1	<i>Serratia fonticola</i>	Q9XE09	P	<i>Enterobacter cloacae</i>	2be	295	71
SGM-1	<i>Sphingobium</i>	G2IJJ9	Chr	<i>Sphingobium</i> sp.	2be	316	72
SHV-1	Sulphydryl reagent variable	P0AD64	Chr, P	<i>Klebsiella pneumoniae</i>	2b	286	4
SMO-1	Smolensk, Russia	R4V074	Chr	<i>Ewingella</i> sp.	2be	295	73
SME-1	<i>Serratia marcescens</i> enzyme	P52682	Chr	<i>Serratia marcescens</i>	2f	294	4, 74
SPU-1	<i>Capnocytophaga sputigena</i>	E2D9D5	Chr	<i>Capnocytophaga sputigena</i>	2be	293	75, 76
STRAL	<i>Streptomyces albus</i>	P14559	Chr	<i>Streptomyces albus</i>	2a	314	4
STRCE	<i>Streptomyces cellulosae</i>	Q06650	Chr	<i>Streptomyces cellulosae</i>	2a	311	4
STRFR	<i>Streptomyces fradiae</i>	P35392	Chr	<i>Streptomyces fradiae</i>	2a	306	4
STRLA	<i>Streptomyces lavendulae</i>	P35393	Chr	<i>Streptomyces lavendulae</i>	2b	305	77, 78
TEM-1	Named after patient	U48775	P	<i>Shigella flexneri</i>	2b	286	4
TER-1	<i>Raoultella terrigena</i>	D2D0D6	Chr	<i>Raoultella terrigena</i>	2b	284	79
TLA-1	Named after an Inca tribe (Tlahuicas)	Q9X6W1	P, In	<i>Escherichia coli</i>	2be	314	80
TLA-2	Named after an Inca tribe (Tlahuicas)	Q5W3A6-1	P	Uncultured bacteria	2be	304	81
TOHO-1	Japanese school of medicine (Toho)	Q47066	P	<i>Escherichia coli</i>	2be	291	82
VAK-3	<i>Vibrio alginolyticus</i> KV3 isolate	H9BW95	Chr	<i>Vibrio alginolyticus</i>	2b	283	83
VEB-1	Vietnam extended-spectrum $\beta$ -lactamase	O87489	P	<i>Escherichia coli</i>	2be	287	84
VHH-1	<i>Vibrio harveyi</i> strain HB3	Q9REJ2	Chr	<i>Vibrio harveyi</i>	2c	283	85
VHW-1	<i>Vibrio harveyi</i> strain W3B	Q9REJ3	Chr	<i>Vibrio harveyi</i>	2c	290	85
XCC-1	<i>Xanthomonas campestris</i> pv. <i>campestris</i>	O87643	Chr	<i>Xanthomonas campestris</i>	2e	295	86

<sup>a</sup> Asterisks indicate a NCBI GenBank database accession number (<http://www.ncbi.nlm.nih.gov/>).

<sup>b</sup> Chr, chromosome; In, integron; P, plasmid; Tn, transposon.

<sup>c</sup> Classified by functional group (4, 5).

<sup>d</sup> aa, amino acids.

<sup>e</sup> Such denomination is usually reserved for naming of a class C enzyme (4).

<sup>f</sup> See <http://www.uniprot.org/>.

Only a few residues distinguish these enzymes from others such as those of the ESBL1 cluster or carbapenemases (Fig. 4 and 5): Cys77 and Cys123 with a disulfide bridge (67, 118), Glu171, Gly236, Ala237, Gly238, and Arg244. The Asn245 and Asp246 residues are absent from these enzymes. As previously reported, when Arg is present at position 244, a different residue is found at position 220 or 276 of the corresponding enzyme, Leu and Asn, respectively, in LSBL1, for example (12).

These clusters have a key motif in common: the segment spanning positions 231 to 238 has the sequences IADKTGAG in LSBL1 enzymes (with the exception of TEM types, which have a KSG triad rather than KTG), IADRSAG in LSBL2 enzymes, and VADRTGAG in LSBL3 enzymes. Carbenicillinases (LSBL2 and LSBL3) are unique among class A  $\beta$ -lactamases in possessing an arginine residue at position 234, whereas the corresponding residue is a lysine in LSBL1 and LSBL4 enzymes. Kinetic characterization of an R234K PSE-4 mutant revealed that this mutation decreased the  $k_{cat}/K_m$  ratio by a factor of 50, confirming the importance of Arg234 for the carbenicillinase activity (119). Residue 234 is located within the binding pocket, close to nucleophilic residue Ser70. Furthermore, TEM-1 mutagenesis studies have im-

plicated the Lys234Arg substitution in carboxypenicillin hydrolysis (120).

The LSBL4 cluster includes two single  $\beta$ -lactamases, AER-1 and plasmid-encoded PSE-3, which has already been classified as a functional group 2c enzyme, but it also includes chromosome-encoded enzymes from enterobacterial species, such as CKO-1, MAL-1, and HER-1 (Table 1). The amino acid sequence of CKO-1 was very similar to that of MAL-1 (98% identity), a chromosome-encoded  $\beta$ -lactamase detected in a strain of *Levinea malonatica* (currently known as *C. koseri*) in previous studies (4, 31). These two enzymes displayed moderate levels of sequence identity to the AER-1 (52%), PSE-3 (53%), HER-type (48 to 52%), ORN-type (>50%), PLA-type (>50%), and TEM- and SHV-type (46 to 50%) enzymes. In terms of molecular structure, the triads 234KTG236 and 234KSG236 (PSE-3) clearly separate this cluster from true carbenicillinases (cluster LSBL2).

A putative  $\beta$ -lactamase was compared with these clusters. This enzyme is produced by "*Candidatus* Hamiltonella defensa," which probably belongs to the *Enterobacteriaceae* (Fig. 1) (121, 122). It appears to be a typical penicillinase (cluster LSBL1), based on its amino acid sequence, which includes specific residues and is very

TABLE 2 Conserved residues in class A  $\beta$ -lactamases

Location of residue <sup>g</sup>	% conserved residues					
	Reported by:			Total <sup>d</sup>	Subclass A1 <sup>e</sup>	Subclass A2 <sup>f</sup>
	Ambler et al. <sup>a</sup>	Matagne et al. <sup>b</sup>	Risso et al. <sup>c</sup>			
37E	100	89	88	77	97	28
45G	100	100	97	97	99	96 G/A
66F	100	96	91	88	99	80 Y/F
<b>70S</b>	100	100	100	100	100	100
<b>73K</b>	100	100	100	100	100	100
81L	100	96	90	95	99	80 L
107P	100	100	93	97	97	94
<b>130S</b>	100	100	100	99	99	100
131D	100	100	100	100	100	100
<b>132N</b>	95	96	97	95	96	90
134A	100	100	96	90	98	91 A/G
136N	100	93	91	79	99	94 D
144G	100	96	96	95	97	90
156G	100	93	97	93	95	85
157D	100	91	83	78	99	58I
164R	100	89	79	73	92	34 A
<b>166E</b>	100	100	93	100	100	100
169L	100	91	77	76	93	81 M
179D	100	91	78	77	97	99 N
180T	100	91	79	76	95	80 W/Y
199L	100	93	92	90	98	80
207L	100	93	82	86	88	100 L/I
233D	100	91	85	75	93	99 H/R
<b>234K/R</b>	100	100	100	100	100	100
<b>235T/S</b>	100	100	97	99	100	99 T
236G	100	100	100	100	100	100

<sup>a</sup> Twenty representative enzymes were examined (96).

<sup>b</sup> Forty-six representative enzymes were examined (12).

<sup>c</sup> Seventy-five putative enzymes were examined (97).

<sup>d</sup> There were a total of 268 representative and putative enzymes.

<sup>e</sup> There were a total of 213 representative and putative enzymes for subclass A1.

<sup>f</sup> There were a total of 55 representative and putative enzymes for subclass A2.

<sup>g</sup> Major residues involved in the catalytic mechanism and/or in substrate binding are shown in boldface type (12).

similar to the  $\beta$ -lactamases TEM-1 (74% identity) and GIL-1 (73% identity). Most LSBL1-type enzymes are chromosome encoded in various species of enterobacteria. It therefore appears highly probable that TEM-1 originates from an enterobacterium.

Finally, this group of enzymes (LSBLs), which are not naturally able to inactivate oxyimino- $\beta$ -lactams and are not resistant to inhibitors (clavulanate, tazobactam, and sulbactam), displayed no well-defined consensus substitutions, with known exceptions reported for various TEM and SHV mutants (12, 114, 115). The Lahey Clinic website lists at least 220 TEM variants and 190 SHV variants with amino acid substitutions such as 104Lys, 164Ser/His, 182Thr, 237Thr/Gly, 238Ser, and 240Lys/Arg, which expand their inactivation spectra, and 69Leu/Val/Ile, 130Gly, 165Arg, 244Cys/Ser/Thr, 265Met, 275Leu/Gln, and 276Asp, which increase their resistance to inhibitors (see <http://www.lahey.org/Studies/>). In addition, the Ser-to-Thr substitution at Ambler position 69 enables RTG-4 to hydrolyze cefepime and ceftiprome (123).

The class *Gammaproteobacteria* includes an interesting cluster (ESBL1) of widespread CTX-M-type enzymes (cefotaximase), represented mostly by chromosome-encoded  $\beta$ -lactamases produced by several species of *Enterobacteriaceae*: *Citrobacter amalonaticus* (formerly *Levinea amalonatica*) ("Cdi"), *Citrobacter sedlakii*

(SED-1), *Citrobacter rodentium*, *Klebsiella oxytoca* (K1; OXY-1), *Kluyvera ascorbata* (KLUA-1), *Kluyvera cryocrescens* (KLUC-1), *Kluyvera georgiana* (KLUG-1), *Leminorella grimontii* (GRI-1), *Leminorella richardii* (RIC-1), *Proteus vulgaris* (CumA; BlaP), *Proteus penneri* (HUG-1), *Rahnella aquatilis* (RAHN-1), *Serratia fonticola* (FONA-1), and the more recently identified *Ewingella* sp. (SMO-1) (Table 1) (124, 125). The first example of probable gene transfer in this group is that of the gene encoding the ESBL SFO-1 in *S. fonticola* (71, 126), followed by those in *K. ascorbata* and other *Kluyvera* species, the source of most CTX-M enzymes (48, 49, 124). The *bla*<sub>OXY</sub> gene was furthermore recently shown to be present on a plasmid in *K. pneumoniae* and *K. oxytoca* isolates (127).

At least three susceptibility patterns were easily identified for species in which the  $\beta$ -lactamase was chromosomally encoded: the "penicillinase" resistance phenotype, with low levels of expression (*K. oxytoca* and *C. amalonaticus*); an unusual pattern originally identified in *K. ascorbata* strains displaying low levels of resistance to ticarcillin and cephalothin, with synergy being observed between these older  $\beta$ -lactams and clavulanate, which was subsequently confirmed with a combination of cefuroxime and clavulanate; and overproduction of chromosomally encoded  $\beta$ -lactamases, resulting in resistance to cefuroxime, cefotaxime, and ceftriaxone but not to ceftazidime (*C. amalonaticus*, *K. oxy-*



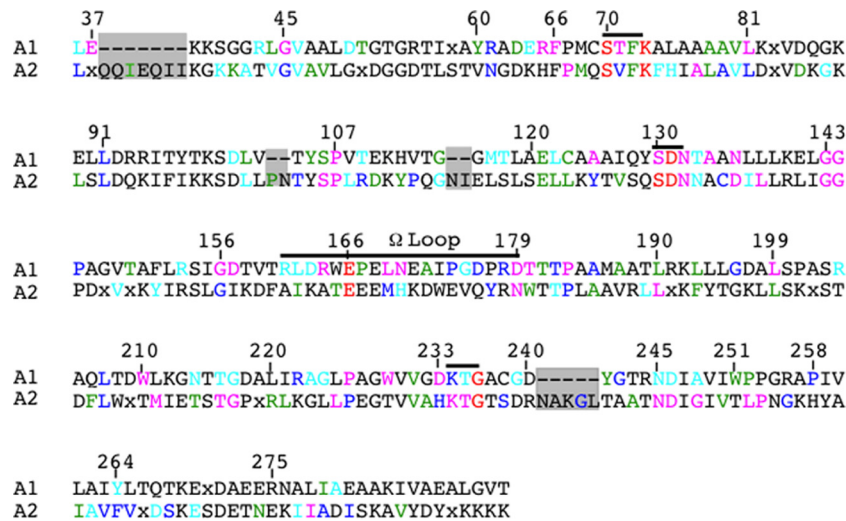


FIG 2 ABL consensus sequences after multiple alignments of the amino acid sequences from the 120 subclass A1 and 55 subclass A2  $\beta$ -lactamases (196). Signal peptides as well as N-terminal ends have been omitted because they show little sequence similarity. Numbering follows the numbering scheme described by Ambler et al. (96). The black lines indicate residues involved in the catalytic mechanism and/or substrate binding. Additional residues that are typical of subclass A2 are shaded in gray. Dashes indicate gaps within the alignment, and x indicates variable residues. A colorimetric scale was used to express the percentage of residues in each cluster deduced from Jalview (197), where red indicates a 100% conserved residue, purple indicates a 90 to 99% conserved residue, dark blue indicates an 80 to 89% conserved residue, light blue indicates a 70 to 79% conserved residue, and green indicates a 60 to 69% conserved residue.

*toca*, *P. vulgaris*, *R. aquatilis*, and *S. fonticola*) (116, 128–131). This diversity of profiles may justify a functional classification into three groups due to poor substrate choice for *C. amalonaticus*: groups 2a, 2b (Cdi), and 2be (4). In addition, more accurate analyses clearly identified that enzymes such as ESBLs are particularly reactive against cefuroxime, cefotaxime, ceftriaxone, cefepime, cefpirome, and aztreonam but react poorly with ceftazidime. Finally, like most class A ESBLs, these enzymes were highly susceptible to  $\beta$ -lactamase inhibitors.

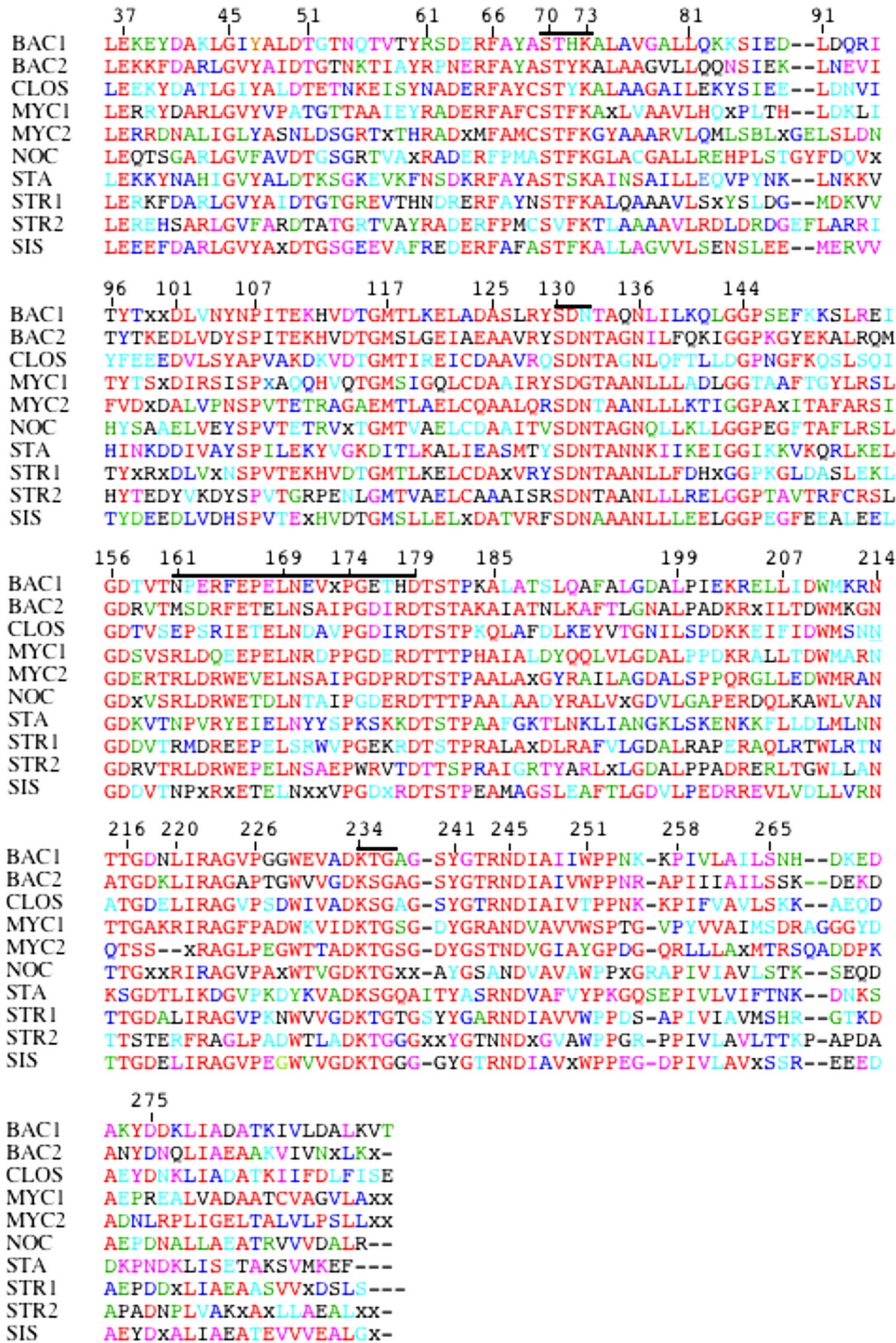
Highly conserved residues and motifs were identified within this homogeneous cluster: 42GRLGxALIxT51, 161RLDRxEPxLNT/SAXxGDxRD TT181, and 231VGDKTGxGDYGT TN DIAVxWP252 (Fig. 4 and 5). Some key residues of ESBL1 enzymes can account for differences in susceptibility patterns (124, 132–134). For example, OXY-type and *C. amalonaticus* enzymes have an Ala residue at position 237, whereas other types of enzymes have a Ser residue, which has been identified as playing a role in the extension of the substrate specificities of TEM and SHV ESBLs to cefotaxime (12, 124). In the CTX-M-4 enzyme, the Ser237Ala substitution decreases both relative levels of cefotaxime hydrolysis and susceptibility to clavulanate inhibition. In contrast, the Ala237Ser substitution in the OXY-1 enzyme increases resistance to cefotaxime and cefepime (135). The ESBL1 Arg276 residue was predicted to fulfill the role of the Arg244 residue detected in LSBL1 types. Relative rates of oxyimino- $\beta$ -lactam hydrolysis are decreased by substitution of Arg276, suggesting that this residue contributes to extending the activity spectrum of the enzyme.

As for TEM- and SHV-type enzymes, ESBL1 enzymes can evolve such that they inactivate ceftazidime more efficiently or confer higher levels of acquired resistance, but few data are available concerning such changes. Ceftazidime-resistant mutants with point mutations (Pro167Ser and Asp240Gly substitutions) were isolated in clinical practice for commonplace CTX-M enzymes with higher catalytic efficiencies against ceftazidime (124, 136, 137). Such evolution through single mutations has also been

reported for the chromosome-encoded  $\beta$ -lactamase (OXY-2) of some *K. oxytoca* isolates, which have acquired resistance to ceftriaxone, cefotaxime, and aztreonam through an Ala237Ser substitution. Some isolates that are resistant to both ceftazidime and cefotaxime, due to the Ala237Ser and Pro167Ser substitutions, have also been recovered (135, 138, 139). Finally, for this highly prevalent group of ESBL enzymes, acquired resistance to inhibitors remains rare in clinical practice, in contrast with the situation for TEM-type enzymes (12, 136, 140). Only one *K. oxytoca* isolate that was resistant to amoxicillin-clavulanate has been recovered from a blood culture. This isolate had a serine-to-glycine substitution at Ambler position 130, as reported for SHV-10 (141).

The chromosomal enzymes produced by several *Yersinia* species appear to belong to a single cluster. *Yersinia enterocolitica* (mostly biotypes 1B, 2, 3, 4, and 5) is naturally resistant to some  $\beta$ -lactams, such as penicillins (ampicillin, amoxicillin, and ticarcillin) and first-generation cephalosporins (cephalothin and cephalixin), but it remains susceptible to cefuroxime and oxyimino- $\beta$ -lactams (142–144). Synergy is usually detected with combinations of ticarcillin or carbenicillin and clavulanate, rather than with amoxicillin plus clavulanate, which is inactive against the second chromosomal  $\beta$ -lactamase (cephalosporinase/BlaB). The reversal of carboxypenicillin by clavulanate is related to the constitutive production of a class A-type enzyme (BlaA) (Table 1) (4). This enzyme has been classified as a member of functional group 2e.

High levels of amino acid sequence identity (>80%) have been demonstrated for  $\beta$ -lactamases of several species of *Yersinia*, including *Y. enterocolitica*, *Y. enterocolitica* subsp. *palaearctica*, *Y. intermedia*, *Y. frederiksenii*, and *Y. rohdei*. The ESBL1 cluster is most similar to these enzymes (56 to 58% identity with RAHN-1, FON-1, and CTX-M types). In addition to the strictly and highly conserved class A residues, these two clusters have multiple residues and motifs in common (Fig. 4). It seems highly probable that BlaA is an ESBL.



**FIG 3** ABL consensus sequences from the 88 subclass A1  $\beta$ -lactamases produced by two phyla (*Firmicutes* and *Actinobacteria*) and 10 clusters: BAC1 (*Bacillus* 1), BAC2 (*Bacillus* 2), CLO (*Clostridium*), MYC1 (*Mycobacterium* 1), MYC2 (*Mycobacterium* 2), NOC (*Nocardia*), STA (*Staphylococcus*), STR1 (*Streptomyces* 1), STR2 (*Streptomyces* 2), and SIS (*Nocardioiopsis*). Signal peptides as well as N-terminal ends have been omitted because they show little sequence similarity. Numbering follows the numbering scheme described by Ambler et al. (96). The black lines indicate residues that are involved in the catalytic mechanism and/or in substrate binding. Dashes indicate gaps within the alignment, and x indicates variable residues. A colorimetric scale was used to express percentages of residues in each cluster deduced from Jalview (197), where red indicates a 100% conserved residue, purple indicates a 90 to 99% conserved residue, dark blue indicates an 80 to 89% conserved residue, light blue indicates a 70 to 79% conserved residue, and green indicates a 60 to 69% conserved residue. Representative  $\beta$ -lactamases and bacterial species were examined by cluster as follows (those indicated by GI numbers were not present in the phylogenetic study). The BAC1 cluster contains BCL-1 (*B. clausii*), BlaIII (*B. cereus*), BlaP (*B. licheniformis*), *B. cereus*, *B. hemi-cellulosilyticus* (GI:569807732), *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. pseudomycoides* (GI:493024478), *B. sonorensis* (GI:493686415), *B. thuringiensis*, and *B. weihenstephanensis*. The BAC2 cluster contains BlaI (*B. cereus*), *B. amyloliquefaciens*, *B. anthracis*, *B. atrophaeus* (GI:498487062), *B. cereus*, *B. siamensis* (GI:515503247), *B. subtilis*, *B. thuringiensis*, *B. vallismortis*, *P. brevis*, and *P. dendritiformis*. The CLO cluster contains *C. boltea*, *C. botulinum*, *C. butyricum*, *C. clostridioforme*, *C. kluveri*, and *C. senegalense* (GI:497978020).

TABLE 3 Conserved residues among 72 subclass A1  $\beta$ -lactamases from Gram-positive bacteria

Position <sup>a</sup>	CS <sup>b</sup>	% conservation	Other residue(s)
36	Leu	94	Ile ( <i>C. botulinum</i> , <i>C. butyricum</i> , <i>S. lentus</i> , <i>S. saprophyticus</i> )
37	Glu	100	
42	Ala	90	Val (BlaU, BlaF, <i>B. brevis</i> , <i>C. botulinum</i> , <i>C. butyricum</i> , <i>M. fortuitum</i> ), Gly (MAB-1, <i>M. abscessus</i> , <i>M. massiliense</i> ), Thr ( <i>S. saprophyticus</i> )
45	Gly	100	
48	Ala	88	Gly (CAD-1, <i>S. clavuligerus</i> , <i>S. saprophyticus</i> , <i>L. weihenstephanensis</i> ), Met ( <i>M. kansasii</i> ), Val (BlaC, <i>M. canettii</i> , <i>M. marinum</i> , <i>M. ulcerans</i> )
66	Phe	99	Met ( <i>N. brasiliensis</i> )
70	Ser	100	
73	Lys	100	
81	Leu	100	
107	Pro	94	Asp ( <i>S. prunicolor</i> , <i>S. sviceps</i> ), Thr (BlaF, <i>S. lavendulae</i> )
117	Met	92	Ile (PC1, BlaZ, <i>S. capitis</i> , <i>S. lentus</i> ), Leu (BlaL, <i>S. lavendulae</i> )
125	Ala	94	Val (BlaL, <i>S. lavendulae</i> , <i>S. prunicolor</i> , <i>S. sviceps</i> )
130	Ser	100	
131	Asp	100	
132	Asn	92	Gly (BlaC, <i>M. canettii</i> , <i>M. kansasii</i> , <i>M. marinum</i> , <i>M. ulcerans</i> , <i>K. versatilis</i> , <i>S. usitatus</i> ), Ser (BlaIII)
134	Ala	96	Gly (MAB-1, <i>M. massiliense</i> , <i>M. rhodesiae</i> )
136	Asn	100	
143	Gly	92	Ala ( <i>S. badius</i> ), Asp ( <i>C. bolteae</i> , <i>C. clostridioforme</i> , <i>P. alvei</i> , <i>P. dendritiformis</i> , <i>S. aureofaciens</i> )
144	Gly	100	
156	Gly	100	
157	Asp	100	
164	Arg	92	Ala (BlaC, <i>M. canettii</i> ), Gln ( <i>M. kansasii</i> , <i>M. marinum</i> , <i>M. ulcerans</i> ), His ( <i>C. kluyveri</i> )
166	Glu	100	
169	Leu	100	
170	Asn	89	Met (PC1), Gly (BlaL), Ser (BlaU, <i>S. badius</i> , <i>S. globisporus</i> , <i>S. rapamycinicus</i> , <i>S. violaceusniger</i> ), Thr ( <i>L. rocourtiae</i> )
174	Pro	99	Ala ( <i>M. smegmatis</i> )
179	Asp	100	
180	Thr	99	Val ( <i>S. lavendulae</i> )
181	Ser/Thr	100	
182	Thr/Ser	100	
183	Pro	87	Ala (BlaF, <i>B. anthracis</i> , <i>B. amyloliquefaciens</i> , <i>B. atrophaeus</i> , <i>B. cereus</i> I, <i>B. licheniformis</i> , <i>B. megaterium</i> , <i>B. siamensis</i> , <i>B. subtilis</i> , <i>B. thuringiensis</i> , <i>B. vallismortis</i> , <i>P. dendritiformis</i> )
185	Ala	88	Gln (ACI-1, <i>A. intestini</i> , <i>C. bolteae</i> , <i>C. botulinum</i> , <i>C. butyricum</i> , MAB-1 <i>M. massiliense</i> ), Glu (CAD-1)
199	Leu	97	Ile ( <i>L. rocourtiae</i> ), Met ( <i>S. saprophyticus</i> )
207	Leu	88	Phe ( <i>C. bolteae</i> , <i>C. butyricum</i> , <i>C. clostridioforme</i> , <i>S. albus</i> , <i>L. weihenstephanensis</i> ), Tyr (CAD-1, <i>C. maltaromaticum</i> , <i>L. rocourtiae</i> )
214	Asn	96	Ser (BlaL, <i>M. marinum</i> , <i>M. ulcerans</i> )
216	Thr/Ser	97	Ile (ACI-1, <i>A. intestini</i> )
222	Arg/Lys	99	His ( <i>S. sviceps</i> )
224	Gly	93	Ala (MAB-1, <i>M. massiliense</i> , <i>P. vortex</i> , <i>S. afghaniensis</i> ), Glu (ACI-1, <i>A. intestini</i> )
226	Pro	97	Arg (BlaU), Ser (BlaZ, <i>S. capitis</i> )
233	Asp	100	
234	Lys	100	
235	Thr/Ser	100	
236	Gly	100	
241	Tyr	89	His (FAR-1, R39, <i>S. clavuligerus</i> , <i>N. dassonvillei</i> , <i>N. synnemataformans</i> ), Lys (MAB-1, <i>M. massiliense</i> ), Phe ( <i>L. rocourtiae</i> )
242	Gly	92	Ala (PC1, BlaZ, <i>B. megaterium</i> , <i>S. albus</i> , <i>S. capitis</i> , <i>S. saprophyticus</i> )
245	Asn	97	Leu (AST-1, <i>N. cyriacigeorgica</i> )
246	Asp	99	Glu ( <i>S. cellulosa</i> )
258	Pro	93	Arg (BlaF, <i>M. smegmatis</i> , <i>M. rhodesiae</i> , <i>M. tusciae</i> ), Ser ( <i>M. kansasii</i> )
265	Ser/Thr	97	Val (BlaU, <i>M. smegmatis</i> )

<sup>a</sup> Amino acid position.<sup>b</sup> CS, ABL consensus (96). Residues in boldface type are considered to be involved in the catalytic mechanism and/or in substrate binding. Shaded residues correspond to conserved residues proposed by Matagne et al. (12).

The tiny ESBL2 cluster comprises three representative enzymes (ERP-1, DES-1, and BES-1) and a single putative enzyme (*Desulfovibrio desulfuricans*). ERP-1 (from *Erwinia persicina*) was the first enterobacterial extended-spectrum  $\beta$ -lactamase to be shown to be pathogenic to plants (37). The kinetic parameters of the

ERP-1 enzyme place it in group 2be (Table 1). However, its  $\beta$ -lactam resistance phenotype suggested that it might be a narrow-spectrum penicillinase, similar to those of *C. koseri* and *K. oxytoca*. The plasmid-encoded ESBL BES-1 was identified in *Serratia marcescens* isolates with strong resistance to penicillins and aztreonam

The MYC1 cluster contains BlaC (*M. tuberculosis*), *M. bovis* (GI:654314144), *M. canettii*, *M. kansasii*, *M. marinum*, and *M. ulcerans*. The MYC2 cluster contains BlaF/MFO-1 (*M. fortuitum*), *M. smegmatis*, *M. vulneris* (GI:602525319), *M. farcinogenes* (GI:633837907), and *M. mageritense* (GI:602541679). The NOC cluster contains AST-1 (*N. asteroides*), FAR-1 (*N. farcinica*), *N. brasiliensis*, and *N. cyriacigeorgica*. The STA cluster contains BlaZ (*S. aureus*), PC1 (*S. aureus*), *S. capitis*, *Staphylococcus epidermidis*, *Staphylococcus equorum*, *Staphylococcus haemolyticus*, *S. saprophyticus*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *Staphylococcus stepanovicii*, *Staphylococcus xylosum*, and *Enterococcus faecalis*. The STR1 cluster contains BlaL (*Streptomyces cacaoi*), BlaU (*S. cacaoi*), *S. albus*, *S. badius*, *Streptomyces filamentosus* (GI:493088671), *S. globisporus*, *S. rapamycinicus*, and *S. violaceusniger*. The STR2 cluster contains BlaF (*Streptomyces fradiae*), BlaL (*Streptomyces albus*), *S. aureofaciens*, *S. afghaniensis*, *S. avermitilis*, *Streptomyces bottropensis*, *S. clavuligerus*, *S. lavendulae*, *S. rimosus*, and *S. scabiei*. The SIS cluster contains R30 (*Actinomodura* sp.), *Nocardopsis alba* (GI:504722104), *N. dassonvillei*, *N. halotolerans*, and *N. synnemataformans*.



FIG 4 ABL consensus sequences after multiple alignments of amino acid sequences from 12 clusters among 171 subclass A1 β-lactamases produced by Gram-negative bacteria: the LSBL, ESBL, YERS (for *Yersinia*), CARBA (for carbapenemases), BURK (for *Burkholderia*), XANT (for *Xanthomonas*), and FRAN (for *Francisella*) clusters. Signal peptides as well as N-terminal ends have been omitted because they show little sequence similarity. Numbering follows the numbering scheme described by Ambler et al. (96). The black lines indicate residues that are involved in the catalytic mechanism and/or in substrate binding. Dashes indicate gaps within the alignment, and x indicates variable residues. A colorimetric scale was used to express the percentage of residues in each cluster deduced from Jalview (197), where red indicates a 100% conserved residue, purple indicates a 90 to 99% conserved residue, dark blue indicates an 80 to 89% conserved residue, light blue indicates a 70 to 79% conserved residue, and green indicates a 60 to 69% conserved residue. Representative β-lactamases and bacterial species examined by cluster are as follows. The LSBL1 cluster contains GIL-1 (*C. gillenii*), LAP-1 (*E. cloacae*), LEN-1 (*K. pneumoniae*), OHIO-1 (*K. pneumoniae*), OKP-A (*K. pneumoniae*), ORN-1 (*Raoultella ornithinolytica*), PLA-1 (*Raoultella planticola*), SHV-1 (*K. pneumoniae*), TEM-1 (*Shigella flexneri*), TER-1 (*Raoultella terrigena*), “*Ca. Hamiltonella defensa*,” and *K. variicola*. The LSBL2 cluster contains AmpC (*Vibrio fischeri*), CARB-3 (*P. aeruginosa*), MP-1 (*Moritella marina*), PLES-1 (*P. shigelloides*), PSE-1 (*P. aeruginosa*), VHH-1 (*V. harveyi*), VHW-1 (*V. harveyi*), VAK-3 (*Vibrio alginolyticus*), *E. lysicola*,

and distinctly higher levels of resistance to cefotaxime than to ceftazidime from Brazilian hospitals (Table 1) (20). The third  $\beta$ -lactamase, DES-1, was identified in some strains of *D. desulfuricans*, a Gram-negative anaerobe phylogenetically related to the *Deltaproteobacteria* (36). The susceptibility pattern of strains carrying this enzyme was poorly defined as resistance to penicillins and cefotaxime, whereas DES-1 is actually an ESBL (Table 1). These ESBLs have amino acid identities of 48 to 57% with respect to the most closely related ESBL1 enzymes from *Yersinia* and *Burkholderia*. A comparison of the BES-1, ERP-1, and DES-1 sequences with that of ESBL1 identified several amino acid residues likely to underlie the extended hydrolysis spectrum (e.g., Ser/Thr237) (Fig. 4). The CARBA cluster (cluster 4) (Fig. 1) corresponds essentially to the carbapenemases of functional group 2f, which have been detected sporadically in clinical isolates on a number of occasions over the 30 years or so since their initial discovery (13, 92, 145–147). Most of these enzymes have been found in *Enterobacteriaceae*, including *Enterobacter cloacae* (FRI-1 and NMC-A), *Serratia marcescens* (IMI-1 and SME-1), *Klebsiella pneumoniae* (KPC-2), *Serratia fonticola* (SFC-1), and even *Pseudomonas fluorescens* (BIC-1) (Table 1) (4, 21, 47, 51, 57, 70, 74). Bacteria producing these enzymes have reduced susceptibility to imipenem, but the obtained MICs are highly variable, such that the bacteria concerned may display anything from a slight decrease in susceptibility to full resistance. This cluster includes chromosome-encoded enzymes (BIC, IMI, NMC, SFC, and SME types) and more troublesome plasmid-encoded types (KPC and FRI). In terms of hydrolysis, carbapenems such as imipenem are the distinctive substrates for enzymes from this cluster. Moreover, these enzymes are inhibited more strongly by tazobactam than by clavulanate. They are able to hydrolyze a broad range of other  $\beta$ -lactams, including cephalosporins and penicillins, but they hydrolyze extended-spectrum cephalosporins such as ceftazidime only inefficiently. In contrast, aztreonam is efficiently degraded. Chromosome-encoded enzymes mediate carbapenem resistance that is not coupled to resistance to extended-spectrum cephalosporins, whereas KPC-type enzymes mediate resistance to both carbapenems and extended-spectrum cephalosporins. The hydrolysis substrate spectrum of KPCs includes aminothiazole-oxime cephalosporins such as cefotaxime and inhibitors such as clavulanate (148). Boronic acid was also recently identified as an inhibitor of KPCs (149).

NMC-A- and IMI-type enzymes, which have amino acid sequences displaying  $\sim$ 97% identity, are related to SME-type enzymes, to which they are  $\sim$ 70% identical at the amino acid level. However, IMI-type enzymes also display 55 to 59% amino acid sequence identity to KPCs. Several motifs were identified in com-

parisons of their amino acid sequences (Fig. 4 and 5): 64ERFPLC SSFKGFLAAAVL81, 160FRLDRWELE/DLNT/SAIPGDxRDTST/SP183, and 231VGDKTGT/SCGxYGTANDYAVxWP252. These enzymes have conserved cysteine residues at positions 69 and 238, with the formation of a disulfide bridge that modifies the shape of the active site through changes in the distances between several active-site residues (21). The disulfide bridge is essential for the hydrolysis of carbapenems, penicillins, and cephalosporins. The serine-to-alanine substitution at position 237 decreased the  $k_{cat}$  values for imipenem; cephalothin hydrolysis rates were reduced by a factor of 5, whereas benzylpenicillin  $k_{cat}$  values remained unchanged (150). However, no single residue has yet been identified as being responsible for carbapenem resistance (147, 151). With the exception of KPC types, all types (BIC, FRI, IMI, NMC, SFC, and SME) have an additional residue at position 139a (Glu) (Fig. 4 and 5).

For most of the enzymes of this cluster, the low levels of activity observed for inhibitors such as clavulanate may reflect significant hydrolysis of these substrates. An Arg residue at position 220 has been identified as being critical, with a major effect on  $\beta$ -lactamase inhibitor kinetics (152). Moreover, the amino acid present at position 276 (Asp or Glu for this cluster) plays a structural rather than a kinetic role (147, 152). The amino acid substitutions observed in inhibitor-resistant  $\beta$ -lactamases derived from TEM-1 and TEM-2 include the Asp276 substitution, which has been identified in several variants (<http://www.lahey.org/studies/temtable.asp>).

The ESBL3 cluster (cluster 25) (Fig. 1) includes a predominant family of enzymes, GES or IBC enzymes, with at least 26 members, only a few of which display measurable enzymatic activity against carbapenems (<http://www.lahey.org/Studies>) (18, 19, 42, 46, 95) (Table 1). GES variants have now been identified in a broad range of countries. In contrast, only three variants of BEL family enzymes have been described. GES and BEL enzymes are mostly plasmid or integron encoded and are thus found mostly in *Enterobacteriaceae*. However, a third type of ESBL  $\beta$ -lactamase was recently described as being chromosome encoded (SGM-type enzymes) for several species of *Sphingobium*, which are widespread in nature, particularly in aquatic environments (72). The susceptibility pattern conferred by these enzymes was characterized mostly as an ESBL phenotype. The hydrolysis profile of these enzymes includes activity against penicillins, most expanded-spectrum cephalosporins, and aztreonam but not cephamycins and carbapenems. These enzymes are inhibited by clavulanate, and some are also inhibited by tazobactam and imipenem (Table 1). Several variants of GES enzymes (e.g., GES-2, GES-4, GES-5, GES-16, and GES-20) have activity spectra that have expanded to in-

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*L. damsela*, *O. antarctica*, *Providencia alcalifaciens*, *P. leiognathi*, *V. caribbeanicus*, *V. parahaemolyticus*, and *V. splendidus*. The LSBL3 cluster contains CARB-5 (*P. aeruginosa*), SCO-1 (*A. baumannii*), *A. faecalis*, and *T. xiamenensis*. The LSBL4 cluster contains AER-1 (*A. hydrophila*), CKO-1 (*C. koseri*), PSE-3 (*P. aeruginosa*), HER-1 (*E. hermannii*), MAL-1 (*L. malonatica*), *C. pulveris*, *K. radicincitans*, and *P. putida*. The ESBL1 cluster contains BlaP (*Proteus mirabilis*), CdiA (*C. amalonaticus*), CTX-M-1 (*E. coli*), CTX-M-2 (*S. Typhimurium*), CTX-M-8 (*C. amalonaticus*), CumA (*P. vulgaris*), FEC-1 (*E. coli*), FON-1 (*S. fonticola*), GRI-1 (*L. grimontii*), HUG-1 (*P. penneri*), KI (*K. oxytoca*), KLUA-1 (*K. ascorbata*), KLUC-1 (*K. cryocrescens*), KLUG-1 (*K. georgiana*), MEN-1 (*E. coli*), OXY-1 (*K. oxytoca*), RAHN-1 (*R. aquatilis*), RIC-1 (*L. richardii*), SED-1 (*C. sedlakii*), SFO-1 (*E. cloacae*), SMO-1 (*Ewingella* sp.), TOHO-1/CTX-M-44 (*E. coli*), and *C. rodentium*. The YER cluster contains BlaA (*Y. enterocolitica*), *Y. frederiksenii*, *Y. intermedia*, and *Y. rohdei*. The ESBL2 cluster includes BES-1 (*S. marcescens*), DES-1 (*D. desulfuricans*), and ERP-1 (*E. persicina*). The CARBA cluster contains BIC-1 (*P. fluorescens*), FRI-1 (*E. cloacae*), IMI-1 (*E. cloacae*), KPC-2 (*K. pneumoniae*), NMC-A (*E. cloacae*), SFC-1 (*S. fonticola*), and SME-1 (*S. marcescens*). The ESBL3 cluster contains BEL-1 (*P. aeruginosa*), GES-1 (*K. pneumoniae*), IBC-1 (*E. cloacae*), SGM-1 (*Sphingobium* sp.), and *S. paucimobilis*. The BURK cluster contains BPS-1 (*B. pseudomallei*), BURTH (*B. thailandensis*), PenA (*B. multivorans*), PEN-1 (*B. cenocepacia*), PENI (*B. pseudomallei*), and *B. mallei*. The XANT cluster contains L2 (*S. maltophilia*), PME-1 (*P. aeruginosa*), XCC-1 (*X. campestris*), *S. maltophilia*, *X. axonopodis*, *X. campestris*, *X. oryzae*, and *X. vasicola*. The FRAN cluster contains FPI-1 (*F. philomiragia*), FTU-1 (*F. tularensis*), *F. novicida*, *F. philomiragia*, and *F. tularensis*.

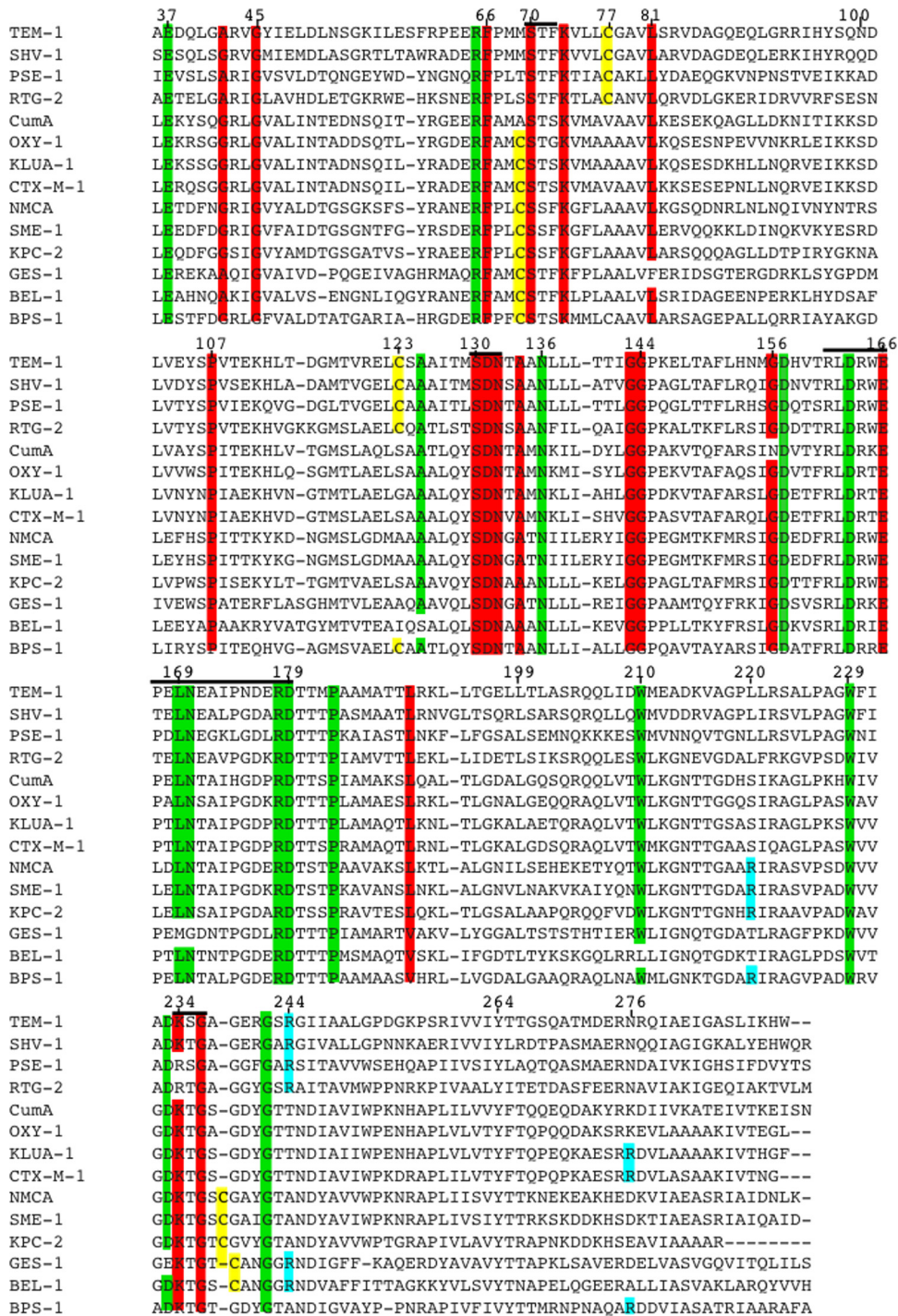
TABLE 4 Conserved residues in 145 subclass A1  $\beta$ -lactamases from Gram-negative bacteria

Position <sup>a</sup>	CS <sup>b</sup>	% conservation	Other residue(s)
37	Glu	95	Gln (XCC-1, <i>A. beijerinckii</i> ), Leu ( <i>X. bovienii</i> ), Lys (VHH-1), Ser ( <i>A. gyllenbergii</i> )
42	Gly/Ala	98	Ser ( <i>V. alginolyticus</i> ), Val (BOR-1, <i>B. parapertussis</i> ), Trp ( <i>A. beijerinckii</i> , <i>A. gyllenbergii</i> , <i>X. bovienii</i> )
43	Arg/Lys	92	Gln (IBC-1, GES-1, <i>A. gyllenbergii</i> , <i>V. caribbeanicus</i> , <i>X. bovienii</i> ), Ser (OHIO-1, KPC-2), Tyr (AmpC), Leu ( <i>Y. rohdei</i> )
44	LIMV	96	Phe ( <i>C. testosteroni</i> )
45	Gly	100	
46	VILM	90	Phe (LAP-1), Thr (XCC-1), Tyr (OKP-A, GIL-1, TEM-1, "Ca. Hamiltonella defensa," PLA-1, TER-1, ORN-1)
61	Arg/Lys/His	93	Asn (CARB-3, BlaP, FTU-1, FTP-1, MP-1, PSE-1, PAL-1, SGM-1, <i>S. paucimobilis</i> ), Asp ( <i>O. antarctica</i> )
65	Arg/His	94	Ala ( <i>Rhizobium</i> species), Pro ( <i>N. winogradskyi</i> ), Ser ( <i>B. ambifaria</i> ), Tyr (BRO-1, <i>Francisella</i> species)
66	Phe	100	
70	Ser	100	
71	Thr	93	Ser (BIC-1, IBC1, GES-1, KPC-2, NMC-A, SFC-1, SME-1), Val (FPH-1, FTU-1)
73	Lys	100	
78	Ala/Gly	96	Ser (BRO-1, FONA, LUT-1, RAHN-1, SFO-1, SMO-1), Val ( <i>B. ambifaria</i> )
81	Leu	99	Phe (IBC-1, GES-1)
102	LIMV	98	His (ERP-1), Ser (RIC-1)
107	Pro	100	
117	MLIV	97	Phe (PAL-1), Thr (VHH-1, VHW-1, <i>V. parahaemolyticus</i> , <i>V. splendidus</i> )
125	Ala	98	Ile ( <i>A. faecalis</i> , <i>A. gyllenbergii</i> , SCO-1), Ser (BEL-1)
130	Ser	98	Gly ( <i>F. novicida</i> , <i>F. tularensis</i> )
131	Asp	100	
132	Asn	99	Ser (FTU-1)
134	Ala	99	Ser (BRO-1)
136	Asn	99	His (VHW-1)
143	Gly	96	Asn (CKO-1/MAL-1, <i>O. antarctica</i> ), Arg ( <i>H. marinus</i> ), Asp ( <i>C. vibrioides</i> , <i>V. splendidus</i> )
144	Gly	95	Asp (XCC-1, <i>X. campestris</i> , <i>X. ozaenae</i> , <i>X. vasicola</i> ), Glu ( <i>X. axonopodis</i> ), Val (VHW-1)
149	Thr	86	Asn (BES-1, BRO-1, AER-1, <i>C. pulveris</i> , <i>Francisella</i> sp., <i>P. putida</i> , <i>Yersinia</i> sp.), Gln (ROB-1), Gly ( <i>P. stutzeri</i> )
153	Arg/Lys	91	Ala (FPH-1), Leu ( <i>F. novicida</i> , <i>F. tularensis</i> ), Gln (BES-1, "Ca. Hamiltonella defensa," OXY-1, <i>P. temperata</i> , PLES-1), His (TEM-1)
156	Gly	93	Ala ( <i>F. novicida</i> , <i>F. tularensis</i> ), Asn (BlaP, CumA, HUG-1), Asp (GRI-1, VAK-3), Ser ( <i>P. leiognathi</i> )
157	Asp	98	Ala (BRO-1), Asn ( <i>J. lividum</i> )
161	Arg/Lys	95	Cys ( <i>X. vasicola</i> ), Gln (AER-1, ERP-1, <i>J. lividum</i> , <i>L. damsela</i> , MP-1), Ile (FPI-1, FTU-1)
162	LVI	93	Asn (L2), Ser/Thr ( <i>C. segnis</i> , XCC-1, <i>Xanthomonas</i> sp.)
163	Asp	94	Ala ( <i>F. tularensis</i> , <i>F. philomiragia</i> ), Asn (ROB-1), Lys (FPH-1), Thr (FTU-1)
164	Arg/His	97	Ala (FTU-1), Gly ( <i>F. philomiragia</i> ), Asn (FTU-1, <i>F. novicida</i> , <i>F. tularensis</i> )
166	Glu	100	
169	LMIV	100	
170	Asn	97	Gly (IBC-1, GES-1), Ser (GES-variants), Thr (PAL-1, <i>X. bovienii</i> ), Val ( <i>A. beijerinckii</i> , <i>A. gyllenbergii</i> )
175	Gly	90	Asn ( <i>G. lovleyi</i> , PLES-1, ROB-1, TEM-1, <i>V. caribbeanicus</i> , <i>Yersinia</i> sp.), Asp ( <i>C. segnis</i> , <i>Rhizobium</i> sp.), Ser (SFO-1)
176	Asp/Glu	97	Ala ( <i>V. caribbeanicus</i> ), Ser/Thr ( <i>Francisella</i> sp.)
178	Arg	93	Asn ( <i>V. caribbeanicus</i> ), Asp ( <i>F. novicida</i> , <i>F. tularensis</i> ), Ile (FPH-1, FTU-1), Leu ( <i>H. intermedia</i> ), Ser (BRO-1)
179	Asp	96	Asn (FPH-1, FTU-1, <i>Francisella</i> sp.)
180	Thr/Ser	96	Lys (FPH-1, FTU-1, <i>Francisella</i> sp.)
181	Thr/Ser	98	Ala (BRO-1, FTU-1, <i>J. lividum</i> )
182	Thr/Ser	98	Lys (BRO-1), Met (TEM-1), Val ( <i>O. carboxidovorans</i> )
183	Pro	98	Ala (OHIO-1), Thr ( <i>A. beijerinckii</i> )
185	Ala	86	Asn (GIL-1), Gln (ROB-1), His (ERP-1), Ile ( <i>F. novicida</i> , "Ca. Hamiltonella defensa"), Lys (HER-1), Pro (BRO-1), Ser (BEL-1, LEN-1, OKP-A, OHIO-1, SHV-1, <i>G. lovleyi</i> , <i>J. lividum</i> , <i>R. cervicalis</i> ), Thr ( <i>V. splendidus</i> )
186	MIV	99	Ala (PSE-3)
190	LMIV	99	Trp ( <i>C. segnis</i> )
199	Leu	98	Met ( <i>S. paucimobilis</i> , <i>Sphingobium</i> sp.)
207	LI	95	Lys (PSE-1), Phe ( <i>C. segnis</i> , <i>D. fructosivorans</i> , KPC-2, <i>Francisella</i> species), Tyr (IMI-1, NMCA, SME-1)
210	Trp	97	Leu (BEL-1), Tyr (FPH-1, FTU-1, <i>G. lovleyi</i> )
222	Arg/Lys	90	Ala ( <i>Francisella</i> , ERP-1), Cys ( <i>N. winogradskyi</i> ), Gln (BES-1, CTX-M-1, CTX-M-8, FEC-1, MEN-1, KLUC-1, KLUG-1, <i>Y. frederiksenii</i> ), Leu (MIN-1), Pro (DES-1)
226	Pro	95	Ala ( <i>X. axonopodis</i> ), Asn ( <i>G. arctica</i> ), Asp ( <i>M. nanhaiticus</i> ), Gly (L2, <i>S. maltophilia</i> ), Thr ( <i>X. campestris</i> , <i>X. vasicola</i> , <i>X. ozaenae</i> )
229	Trp	99	Val (PLES-1)
232	Gly/Ala	99	Arg (L2), Tyr (ROB-1)
233	Asp/Glu	94	Asn ( <i>F. philomiragia</i> ), Gly ( <i>C. testosteroni</i> , <i>D. acidovorans</i> , <i>D. tsuruhatensis</i> ), His (DES-1), Ser (SGM-1, <i>Sphingobacterium</i> sp., <i>S. paucimobilis</i> )
234	Lys/Arg	100	
235	Thr/Ser	100	
236	Gly	100	
264	Tyr/Phe	97	Leu (FPH-1, FTU-1), Met (ROB-1), Ser ( <i>V. splendidus</i> )

<sup>a</sup> Amino acid position.<sup>b</sup> CS, ABL consensus (96); ILMV, isoleucine (I), leucine (L), methionine (M), and valine (V). Residues in boldface type are considered to be involved in the catalytic mechanism and/or in substrate binding. Shaded residues correspond to originally identified conserved residues (12).

clude carbapenems (95). To date, 26 GES variants with sequence differences of 1 to 3 amino acid residues have been reported (<http://lahey.org/studies/other.asp>). The  $\beta$ -lactamases phylogenetically closest to the GES enzymes are those of the BEL family, with amino acid similarities of 50% to 54%. SGM-1 displays 34

to 41% amino acid sequence identity to the GES-5 and BEL-1 enzymes. Very closely related putative  $\beta$ -lactamases (with an amino acid sequence identity of 77 to 80%) have been identified only on the chromosomes of several bacteria from the *Sphingobium* genus (72).



**FIG 5** ABL amino acid sequences from 15 representative  $\beta$ -lactamases of subclass A1 (196). Signal peptides as well as N-terminal ends have been omitted because they show little sequence similarity. Numbering follows the numbering scheme of Ambler et al. (96). The black lines indicate residues that are involved in the catalytic mechanism and/or in substrate binding. Dashes indicate gaps within the alignment. Red, strictly or highly conserved residue for subclasses 1 and 2; green, strictly or highly conserved residue for subclass 1; light blue, basic amino acid according to location (positions 220, 244, and 276); yellow, cysteine.

A consensus sequence was proposed for this cluster on the basis of various highly conserved positions (Fig. 4). Some of the following residues deserve further attention: Cys69, Cys238, Arg/Lys222, Ser/Thr237, Arg220 (for *Sphingobium*), and Arg244 (for the GES/IBC and BEL families) (Fig. 3). These  $\beta$ -lactamases have conserved Cys residues at positions 69 and 238 that may form a disul-

fide bridge, thus modifying the overall shape of the active site (42). The GES-1, BEL-1, and SMG-1 enzymes cannot confer resistance to carbapenems (18, 72, 92, 95). Another residue must therefore be involved, because one of the most alarming characteristics of GES enzymes such as GES-2 is their apparent ability to evolve into carbapenemases. This process involves a single-amino-acid sub-

stitution, Glu170Asn in GES-2 and GES-13 and Glu170Ser in GES-4, -5, -14, -15, -16, and -20 (92, 95). Surprisingly, SMG-1 and all the  $\beta$ -lactamases from the BEL family (BEL-1 to BEL-3) have an Asn residue at position 170 but do not mediate resistance to carbapenems. This suggests that other residues are involved in carbapenem hydrolysis. The Gly residue at position 243, present in all of the enzymes of this cluster other than SGM-type enzymes, is not conserved in other class A  $\beta$ -lactamases (Fig. 4 and 5). The Gly243Ser substitution observed in GES-9 is associated with the efficient hydrolysis of aztreonam and ceftazidime (153). GES-11, bearing a Gly243Ala substitution, hydrolyzes oxyimino- $\beta$ -lactams such as cefotaxime, ceftazidime, and aztreonam more efficiently than does GES-1. Furthermore, this substitution increases sensitivity to  $\beta$ -lactam inhibitors such as clavulanate and tazobactam (154). The impact of the residue at position 237 is crucial, as in several TEM, SHV variant, or even OXY-type enzymes. A Thr residue is present at position 237 in the GES enzymes, as opposed to the Ser residue present in the BEL and SGM families (Fig. 3). These residues are also conserved in other clusters. A single Thr237Ala substitution differentiates GES-12 and GES-11, and this substitution results in GES-11 hydrolyzing aztreonam and ceftazidime twice as efficiently as GES-12, through major effects on the affinity of the enzyme for the two antibiotics (154). Finally, the highest MICs among GES enzymes, obtained, e.g., with cefotaxime and aztreonam, were conferred by combinations of up to four substitutions (155).

The *Betaproteobacteria* class includes several groups of Gram-negative aerobic or facultative bacteria that are often highly versatile in their degradation capacities. Some species of the *Burkholderia* genus, such as *B. pseudomallei*, *B. mallei*, *B. thailandensis*, *B. cepacia*, *B. cenocepacia*, and *B. multivorans*, are pathogenic to both humans and animals (156–159). At least two patterns of natural  $\beta$ -lactam resistance have been identified in this class of bacteria. High levels of resistance to penicillins and to early cephalosporins (cephalothin and cefuroxime) were observed, and this resistance was almost completely reversed in the presence of clavulanate (160). The MICs of cefotaxime and aztreonam were significantly higher than that of ceftazidime. Several enzymes were identified in *B. pseudomallei*: BPS-1 as a cephalosporinase and PenI as an extended-spectrum  $\beta$ -lactamase (Table 1) (4, 25, 63). The spectrum of hydrolysis of the inducible PenB enzyme of *B. cenocepacia* encompassed mostly penicillins, but it also included, to a lesser extent, expanded-spectrum cephalosporins and aztreonam. Finally, the natural  $\beta$ -lactamase of *B. multivorans* (formerly *B. cepacia* 249) was originally characterized as a penicillinase but was more recently proposed to be a carbapenemase (4, 62, 63).

Phylogenetic comparisons showed that these enzymes were closely related and clustered together. They display 64 to 99% amino acid sequence identity. Two groups were distinguished: group 1 included enzymes from *B. pseudomallei*, *B. mallei*, *B. thailandensis*, and *B. oklahomensis*, and group 2 included enzymes from *B. cenocepacia*, *B. ambifaria*, *B. dolosa*, *B. multivorans*, *B. ubonensis*, and *B. vietnamensis* (61). In all the  $\beta$ -lactamases of this cluster, 166 residues were strictly or highly conserved, including, in particular, Cys69, Cys123, Arg220, Arg222, Ser237, and Asp276 (Asn for *B. oklahomensis*) (Fig. 4). Several conserved sequence motifs were identified, 64ERFPFCSTxKxMLxAAVLA82, 160FRLDRxExELNTALPGDxRDTTTPAAMAAS189, and 233DKTGTGDYGTxNDxGV249; moreover, several molecular modifications were identified for these enzymes. Unlike PenI (*B. pseudomallei*),

PenA (*B. multivorans*) has a Phe residue at position 72 (63). The Ser72Phe substitution promotes clavulanate resistance in *B. pseudomallei* by an increase in the  $K_i$  of the enzyme for this inhibitor (160). Surprisingly, a Phe residue was found in all the group 2 enzymes studied, but synergy between penicillins and clavulanate was reported after the transfer of PenB from *B. cenocepacia* to *Escherichia coli* (61).

The  $\Omega$ -loop contains two critical, strictly conserved active-site residues: Glu166 and Asn170. Substitutions involving other residues can lead to cephalosporin resistance (161–164). For example, Pro167 is a classically variable residue identified in ceftazidime-resistant isolates of *B. pseudomallei* and in *in vitro* mutants. Another mutation identified in *B. pseudomallei* isolates is Cys69Tyr, which leads to high levels of ceftazidime resistance.

The mechanisms responsible for an expansion of the spectrum of the PenA  $\beta$ -lactamase of *B. thailandensis* have recently been investigated *in vitro* for ceftazidime resistance (26). Twelve positions displaying single-amino-acid substitutions are located in the active-site pocket, such as Cys69Phe and Cys69Tyr, or in the  $\Omega$ -loop (positions 162, 164, 166, 169, 170, 171, 172, 174, 176, and 179 but not position 167). Interestingly, a single-amino-acid deletion (Glu168del) may expand the *in vitro* spectrum of inactivation to ceftazidime in *B. thailandensis* (165). Additional single-amino-acid deletions have recently been reported (27). Another mechanism of acquired resistance involves the overexpression of the chromosomal  $\beta$ -lactamase of *B. pseudomallei* or the loss of a penicillin-binding protein or (PBP) and also porin (164, 166–168). Two chromosomally encoded  $\beta$ -lactamases (LUT-1 and MIN-1) have also been identified in this cluster (cluster 5) (Table 1 and Fig. 1) (53, 55). In conclusion, the chromosome-mediated  $\beta$ -lactamases of this cluster may be considered ESBLs with a considerable potential to evolve toward a broader profile of resistance.

Finally, additional clusters of enzymes were found in several Gram-negative bacteria from other genera, such as *Francisella*, *Stenotrophomonas*, *Xanthomonas*, and others. However, given the limited impact of these genera in medicine, we will not consider these enzymes further in this review (Fig. 1 and 4) (4, 40, 41, 86, 169).

## MOLECULAR CHARACTERISTICS OF SUBCLASS A2 $\beta$ -LACTAMASES

A phylogenetic analysis of  $\beta$ -lactamases clearly identified a small group of representative enzymes (CblA, CfxA, CEF-1, CepA, CGA-1, CIA-1, CME-1, CSP-1, PER-1, SPU-1, TLA-1, TLA-2, and VEB-1) (clusters 1 and 2) (Fig. 1). The chromosome-encoded enzyme types (e.g., CblA, CepA, CGA-1, CIA-1, CME-1, and CSP-1/SPU-1) were naturally produced by several bacteria from various genera, including *Bacteroides*, *Capnocytophaga*, *Chryseobacterium*, and *Elizabethkingia* (Table 1). The transferable-element-encoded types PER-1, TLA-1, TLA-2, and VEB-1 were reported for several bacterial species from the major phylum *Proteobacteria* and, more precisely, from the orders *Enterobacteriales* and *Pseudomonadales* (*Acinetobacter* and *Pseudomonas*) (10, 95). A gene coding for a CfxA-type enzyme was identified on a mobilizable transposon and detected in several *Bacteroides*, *Prevotella*, and *Capnocytophaga* species (76, 170–172).

These representative enzymes were functionally classified as hydrolyzing predominantly cephalosporins, such as cephalothin, ceftazidime, and cefotaxime, but also aztreonam and penicillins (4, 5, 29, 30, 32, 33, 81, 84, 173). Significant synergy was generally



reported when such substrates were combined with an inhibitor such as clavulanate. Some of these enzymes (CblA, CfxA, and CepA) were classified as functional group 2e enzymes, as they are inhibited by clavulanic acid, whereas others (CIA-1, CGA-1, CME-1, PER-1, TLA-1, TLA-2, and VEB-1) were classified as group 2be enzymes (Table 1). Insight into the role of these enzymes can best be obtained by transferring the corresponding gene to a recipient bacterium, due to the presence of at least two  $\beta$ -lactamases in the donor strain. For example, for *Chryseobacterium indologenes*, *E. coli* transconjugants or transformants were found to be resistant to amoxicillin, ticarcillin, narrow-spectrum cephalosporins, cefuroxime, and third-generation cephalosporins such as ceftazidime and aztreonam and to have reduced susceptibility to cefotaxime. The MICs of these  $\beta$ -lactams were reduced by clavulanate. The expression of class B  $\beta$ -lactamases such as IND can account for the resistance of *C. indologenes* to cefoxitin and carbapenems (174). The *E. coli* strains producing  $\beta$ -lactamases identified to date have a typical ESBL phenotype, including inhibition by clavulanic acid.

In terms of molecular structure, these enzymes have been described as class A  $\beta$ -lactamases with characteristic active-site motifs (Fig. 2). Multiple-sequence alignment revealed that they belong to subclass A2 on the basis of several strictly or highly conserved residues (Table 2 and Fig. 2). A sequence analysis of 13 representative  $\beta$ -lactamases highlighted the locations of conserved residues displaying low levels of variation, particularly for enzymes of the CfxA type (Fig. 6). Some strictly or highly conserved residues (Leu36, Ala42, Gly/Ala45, Pro67, Ser70, Lys73, Pro107, Ser130, Asp131, Asn132, Gly/Ala134, Gly143, Gly144, Glu166, Leu190, Leu225, Lys234, Thr235, and Gly236) were common to enzymes of both subclasses (subclasses A1 and A2), whereas a number of residues were conserved only among subclass A2 enzymes, including Lys40, Asn61, Val71, Thr104, Cys135, Asp136, Met169, Tyr177, Asn179, Met211, His/Arg233, Gly/Ala243, Leu252, Val263, Phe264, Val265, Ser268, and Asn275.

These  $\beta$ -lactamases have four insertion blocks/segments relative to subclass A1 enzymes (Fig. 2 and 6): 37a to 37g, 103a-103b, 112a-112b, and 240a to 240d. Three of these insertion blocks were examined in PER-1-type enzymes, and the most relevant structural trait was found to be the presence of an expanded active site, as suggested by other studies (98).

Variants, mostly bearing point mutations, were identified in these types of enzymes. However, the sites of these mutations were different from those in TEM/SHV-type enzymes, and no marked changes in enzyme activity were observed (<http://www.lahey.org/Studies/>). The eight variants of PER-type enzymes had amino acid sequences that were 86% to 98% identical to that of PER-1. The lowest levels of sequence identity were recorded for PER-2 (or CFI-1 for cefitibutenase, also known as ARG-1) and PER-6 (95). Seven variants were reported for VEB-type enzymes, with a level of identity of ~99% (95, 175–177). Three variants were identified for CIA-type enzymes (sequence identity of 98%), and two were identified for CME-type enzymes (99%). Two possible TLA-type variants were suggested but with a level of identity as low as 53%. Among microaerophilic and anaerobic bacteria, only three variants were reported for CfxA-type enzymes (sequence identity of 98%) (76, 170–172).

These enzymes had no features in common with the subclass A1  $\beta$ -lactamases isolated from Gram-negative species. For instance, the Cys residues at positions 77 and 123 in penicillinases

(cluster LSBL) were not conserved in the enzymes of this class. The Cys69 and Cys238 residues identified in the CARBA, ESBL3, and FRAN clusters were not conserved (Fig. 3 and 4) (29, 40, 41, 178–180). In subclass A2  $\beta$ -lactamases, cysteine residues were found in a limited number of positions close to the conserved SVFK or SDN motif: those at position 78 (CIA-type enzymes), 81 (Cfx-type enzymes), or 135 (CblA, CepA, CGA-1, CIA-1, CME-1, CSP-1 [SPU-1], PER-1, TLA-1, and VEB-1) (Fig. 6). In CME-2, the Cys135 and Cys276 residues may form a disulfide bridge, as already demonstrated for the Cys69 and Cys238 residues of NMC-A (57, 181). Finally, only CfxA-type enzymes were found to have 4 cysteine residues (positions 40, 81, 214, and 251).

The  $\Omega$ -loop, an important structural element of  $\beta$ -lactamases, is present in subclass A2 enzymes but differs significantly from the  $\Omega$ -loops found in subclass A1 derivatives. For example, in subclass A2 enzymes, the 161RFDRxExxLN170 motif is absent. Moreover, CblA, CepA, CIA-1, CGA-1, CME-1, PER-1, TLA-1, TLA-2, and VEB-1 have a histidine rather than an asparagine residue at position 170 (Fig. 4). This asparagine residue is involved in the positioning of the active-site water molecule, a function that it fulfills together with the highly conserved Glu166 and Ser70 residues (182, 183). The equivalent histidine residue may similarly contribute to the catalytic properties of CME-2, but this remains to be confirmed by site-directed mutagenesis. Other signature residues, such as Met169, Tyr177, and Asn179, may also be considered to be defining features for these enzymes.

As for conserved basic residues, Arg244 is conserved in several class A enzymes (clusters LSBL1, LSBL2, LSBL3, LSBL4, ESBL3, and XANT). In its absence, another basic residue (Arg or Lys) is generally present at position 220 (e.g., in subclass A2, clusters ESBL2, BURK, and FRAN [see below]) or at position 276 (cluster ESBL1) (12).

The function of a few residues in a single  $\beta$ -lactamase (PER-1) was assessed by site-directed mutagenesis, which showed a lack of involvement of any of the residues responsible for the cephalosporinase activity in the TEM and SHV families in the substrate profile of this enzyme (184), as discussed above and below.

## STRUCTURE-FUNCTION RELATIONSHIPS OF CLASS A ENZYMES

A great diversity of amino acid sequences was observed between the different clusters of class A  $\beta$ -lactamases. Numerous tertiary structures have been determined for these proteins, mostly by X-ray crystallography (<http://www.rcsb.org/pdb/home/home.do>), facilitating further explorations of the differences between the two subclasses A1 and A2. These enzymes are produced by Gram-positive bacteria (*Bacillus anthracis*, *Bacillus licheniformis*, *M. bovis*, *M. fortuitum*, *M. tuberculosis*, *Staphylococcus aureus*, and *Streptomyces albus*) or Gram-negative bacteria (TEM types, SHV types, CumA, CTX-M types, KPC-2, L2, NMC-A, OXY-1, PenA, PenI, PER-1, PSE-4, SED-1, SME-1, Toho-1, and *Francisella tularensis*). All of the enzymes for which structures have been determined, with the exception of PER-1 and PER-2, belong to subclass A1 (98, 99). They all have the same overall structure with similarities in the structural features surrounding the active site, such as two subdomains generating a cleft (Fig. 7 and 8) (12, 23, 149, 185–187). One of the subdomains (the alpha subdomain) is largely  $\alpha$ -helical. In contrast, the other subdomain (the alpha/beta subdomain) consists of a five-stranded  $\beta$ -sheet flanked by  $\alpha$ -helices. These two subdomains form a cleft that harbors the active site,

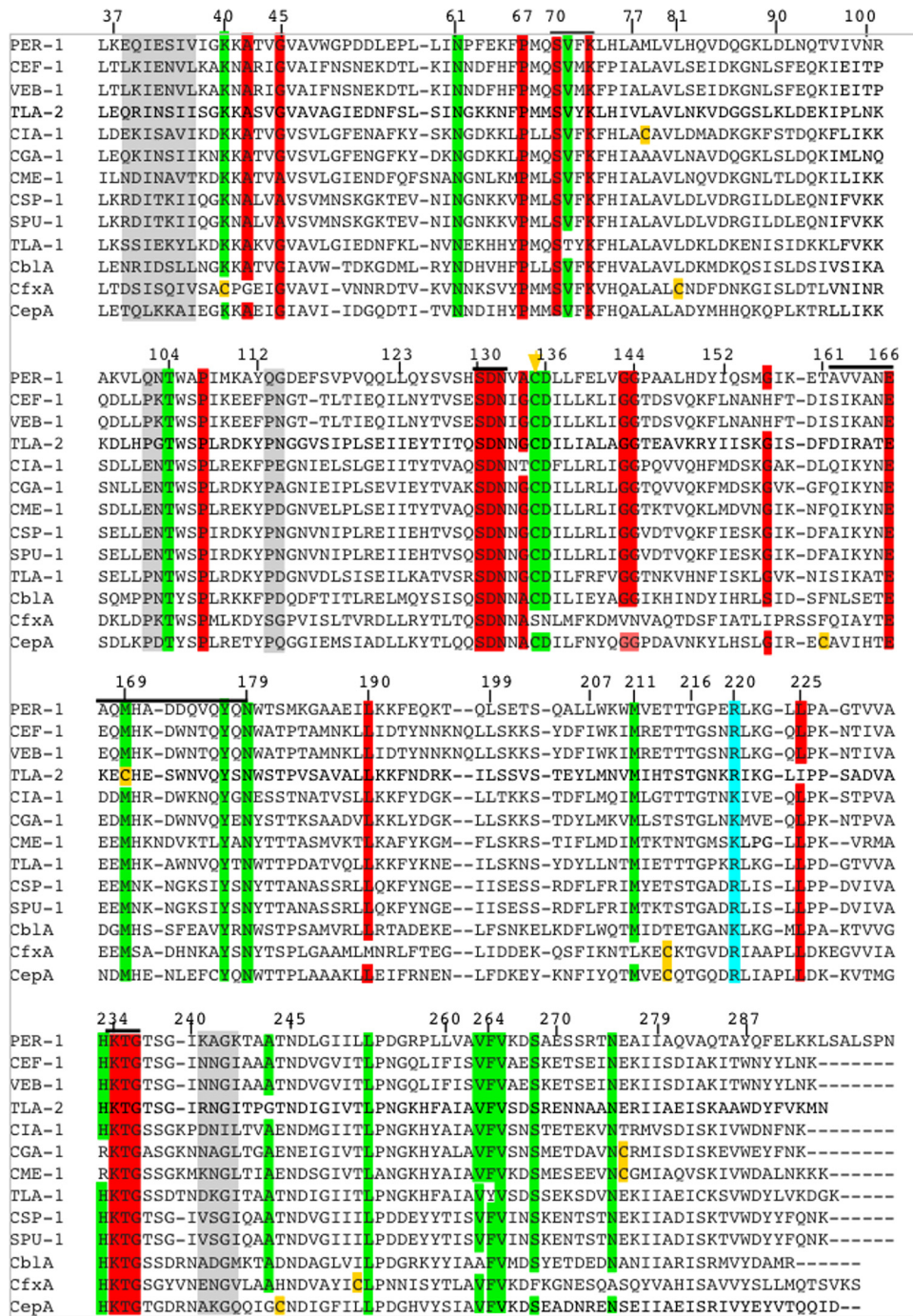


FIG 6 ABL residues of amino acid sequences from 13 representative  $\beta$ -lactamases of subclass A2 (196). Signal peptides as well as N-terminal ends have been omitted because they show little sequence similarity. Numbering follows the numbering scheme of Ambler et al. (96). The black lines indicate residues that are involved in the catalytic mechanism and/or in substrate binding. The additional residues that are typical of subclass A2 are shaded in gray. Dashes indicate gaps within the alignment. Red, strictly or highly conserved residue for subclasses 1 and 2; green, strictly or highly conserved residue for subclass 2; light blue, basic amino acid at position 220; yellow, cysteine.

which contains the catalytic Ser70 residue and the deacylation water primed by interactions with Glu166, Asn170, and Ser70.

Interestingly, two  $\beta$ -lactamases, PER-1 and PER-2, the structure of which was recently determined, differ from the other enzymes in terms of their structure (98, 99) (see below). Both enzymes belong to subclass A2 and display high levels of catalytic activity against most  $\beta$ -lactam substrates.

The strictly conserved residues essential for catalytic activity are described above. Here, we consider additional sequence regions of potential interest in terms of our understanding of the diversity of  $\beta$ -lactamases, the specificity of their functions, and the role of the mutated residues in the newly discovered enzymes.

In the  $\beta$ -lactamases of classes A, C, and D, an active-site serine residue (Ser70) mediates nucleophilic attack on the carbonyl

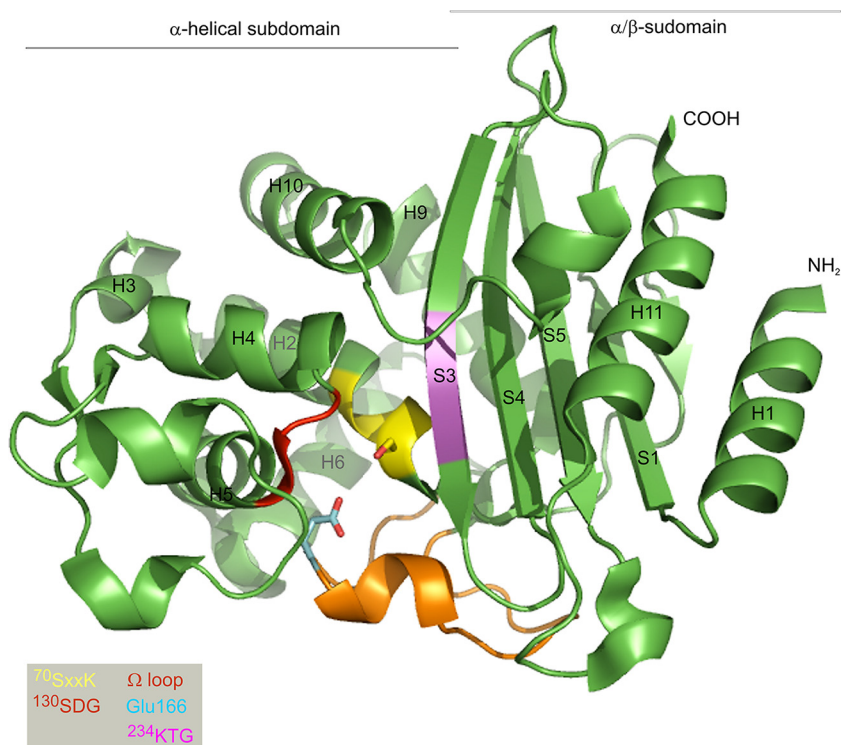


FIG 7 Secondary and tertiary structures of the class A  $\beta$ -lactamase of *Mycobacterium tuberculosis*, with the spatial arrangement of the three catalytic-center-defining amino acid groupings, the  $\alpha$  domain (left), and an  $\alpha/\beta$  domain (right) (23). The helices are represented as H1 to H11, and the strands are represented as S1 to S5. The figure was created with PyMOL (Delano Scientific).

group of the  $\beta$ -lactam ring of the antibiotic molecule. In class B enzymes, catalytic activity is dependent on one or two essential  $Zn^{2+}$  ions. Additional residues crucial for catalysis are Lys at position 73 and the Ser-Asp-Asn triad at positions 130 to 132 (SDN). Here, we address the sequence differences among class A enzymes, making them correspond with structural differences, confirming our subclass definition.

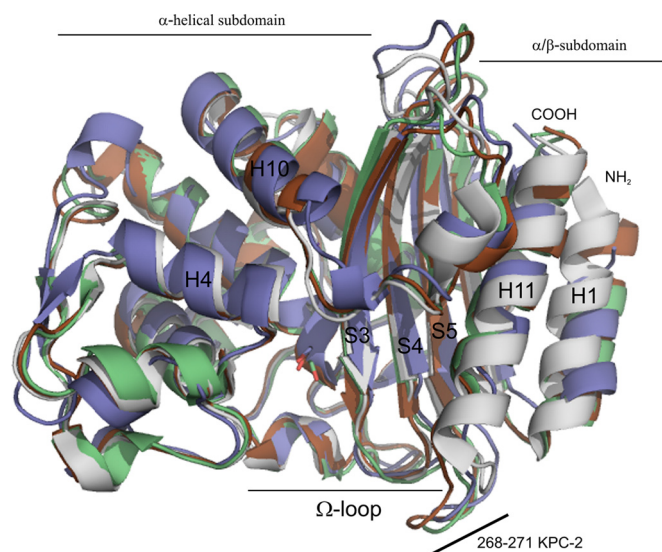
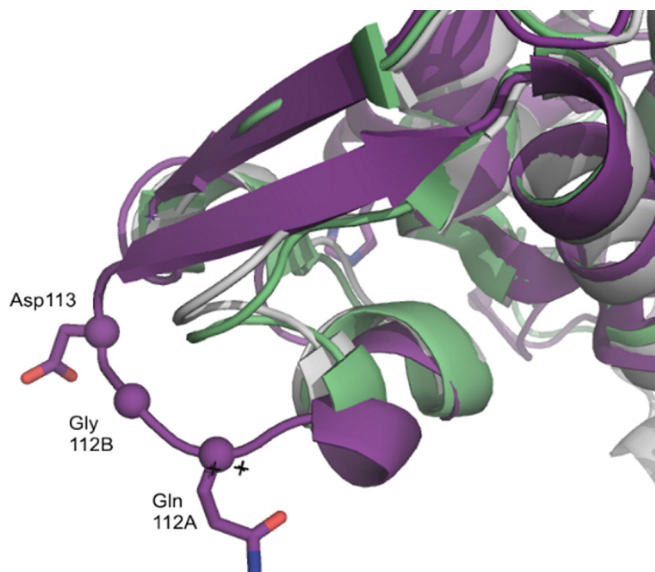


FIG 8 Superposition of four subclass A1  $\beta$ -lactamases: PC1 (blue), TEM-1 (gray), OXY-1 (brown), and KPC-2 (green) (149, 185–187).

We compared enzymes from subclasses A1 and A2 for which structures have been determined: TEM-1, KPC-2, SME-1, CTX-M-9, and PER-1 (subclass A2). Clinical interest in PER-1 has increased recently due to the detection of this enzyme in multiple *Pseudomonas aeruginosa* isolates in hospitals in Asia (188), in isolates of *Acinetobacter baumannii* in Kuwait (189), and in isolates of *Providencia stuartii* in Tunisia (190).

CFB enzymes (subclass A2) have a specific insertion of 4 amino acids after position 240 (nomenclature of 240abcd) (Fig. 2 and 6). These inserted residues are arranged as a loop, as in PER-1, for example (98). This loop is strongly displaced with respect to the position of all other classes of enzymes with, for instance, a maximal additional elongation of 6.28 Å for the protein backbone between the  $C_{\alpha}$  of Arg241 in TEM-1 and the  $C_{\alpha}$  of Ala240b in PER-1. The presence of a longer loop, of 9 rather than 5 amino acids, between the S3 and S4  $\beta$ -sheets, close to the active site, may increase the accessibility of the active site. Note that residue 240 is not resolved in most structures, with the exception of those for PER-1 and SME-1.

Another insertion is observed in subclass A2 enzymes, at positions 112a and 112b (112ab); moreover, the amino acid at position 113 differs from that observed in subclass A1 enzymes. X-ray crystallography has shown that the 112ab insertion results in a longer loop between a short  $\alpha$ -helix (H3) and a  $\beta$ -sheet in this region, again resulting in a  $\beta$ -sheet that is more extended and better defined in the structure of PER-1 than in that of KPC-2 or TEM-1 (Fig. 9). This  $\beta$ -sheet interacts with a neighboring sheet containing residues 94 to 98. In the structure of TEM-1, the region of the extended Asn-Gly-Asp loop is occupied by  $K^{+}$  ions. Note that in



**FIG 9** Detailed view of the conformation of the region spanning positions 100 to 115 in PER-1. PER-1 is shown as a purple ribbon, KPC-2 is shown in green, and TEM-1 is shown in gray.  $C_{\alpha}$  atoms of residues 112A, 112B, and 113 are represented as spheres, and their side chains are displayed as sticks. Black crosses represent  $K^{+}$  ions that cocrystallized with TEM-1.

other subclass A2 enzymes, residues at the 112ab positions are not strictly conserved (Fig. 6); they nevertheless favor the same local secondary structure as that in PER-1.

Position 136 distinguishes between the different clusters of serine-dependent class A  $\beta$ -lactamases: an Asn residue is present at this position in most subclass A1 enzymes, such as TEM-1, SME-1, and PenP, whereas most subclass A2 enzymes, such as PER-1, have a basic Asp residue at this position (Fig. 6). The nature of the residue at position 136 has another impact on the overall fold of the enzyme. Tranier and coworkers highlighted the tendency of the aspartate residue at position 136 in PER-1 to favor a *trans* conformation for the peptide bond connecting residues 166 and 167, through hydrogen bonding with the side chain of Asp136 (98). This has major consequences on the conformation of the  $\Omega$ -loop. For instance, the His170 ring of PER-1 is displaced by 7.4 Å with respect to the side chain of Asn170 in the TEM-1  $\beta$ -lactamase. When comparing the structure of PER-1 with that of SME-1 or TEM-1, it is observed that it is  $N_{\delta_2}$  of the Asp residue and not its carbonyl group, as in the latter enzymes, which points toward the outside of the protein; this suggests a change in the local potential electrostatic interactions with partner proteins or domains with respect to enzymes harboring an Asn at this position.

Conversely, a neutral Asn residue is observed at position 179 in subclass A2 proteins, whereas most subclass A1 proteins harbor an Asp residue at this position (Fig. 2 and 6). In the X-ray structure of TEM-1,  $O_{\delta_2}$  of Asp179 is located 2.75 Å from  $N_{\epsilon}$  and 2.89 Å from  $N_{\delta_2}$  of residue Arg164. In PER-1, the equivalent protein domain is thus more hydrophobic, with the side chain of Asn179 being surrounded by the aliphatic residues Val163 and Ala164 (Fig. 10A). Moreover, the side chain of Asn179 is tilted by  $\sim 100^{\circ}$  with respect to that of Asp179 of TEM-1, confirming that there are differences in the environments of these two residues. Note that position 164 has multiple variants among extended-spectrum TEM  $\beta$ -lactamases, with, e.g., a His in TEM-11 and TEM-16 and a Ser in TEM-5,

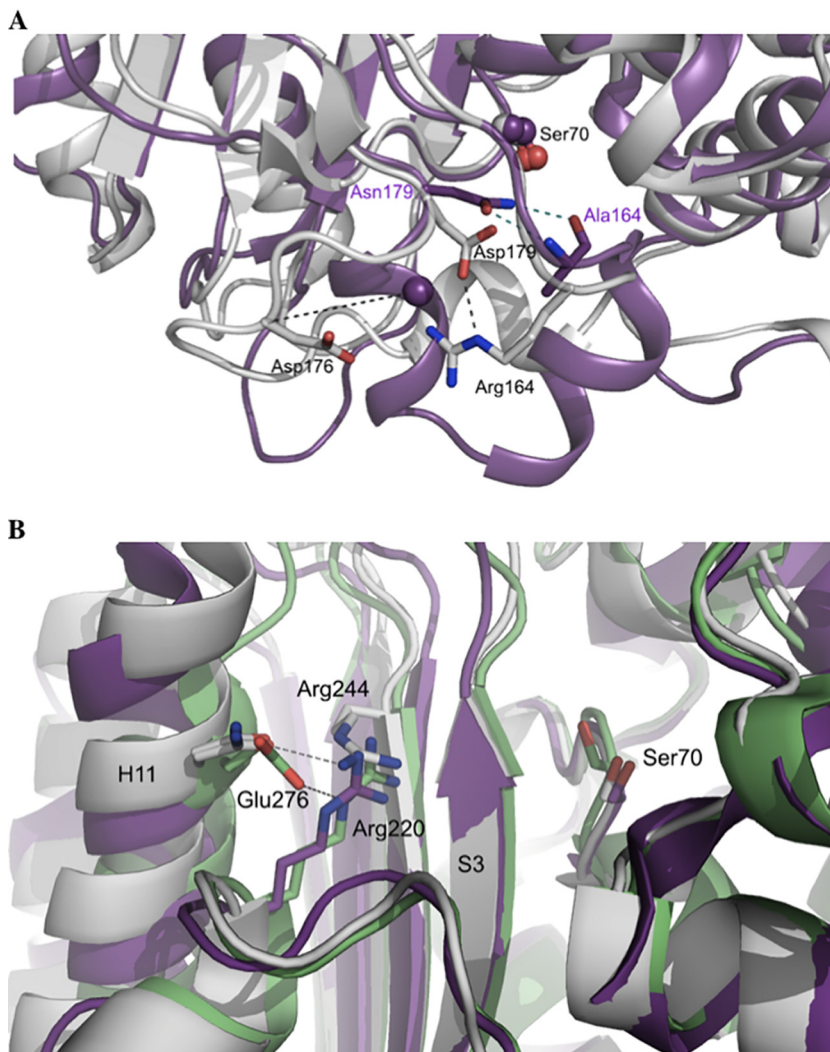
TEM-7, and other variants (12, 114, 115). The Arg161Val substitution in PER-1 may therefore significantly affect the substrate specificity of this enzyme.

The conserved residues within the  $\Omega$ -loop among subclass A2 enzymes are different from those of subclass A1 enzymes, with the exception of residue Glu166 (Fig. 2 and 6). Subclass A2 enzymes have a Met residue at position 169 and a Tyr residue at position 177. Moreover, most subclass A1 enzymes have an Asp residue at position 179, whereas CFB enzymes have an Asn residue at this position. Overall, this creates a less charged environment in the  $\Omega$ -loop of CFB enzymes at the enzyme surface. It also alters the interaction with the disordered/loop region around residue 165 (Asn or Thr for subclass A2), abolishing the ionic interaction between  $O_{\delta_2}$  of Asp179 and  $N_{\epsilon}$  of Arg164, which occurs in other class A enzymes (2.85 Å) (Fig. 10A).

Moreover, in subclass A2 enzymes as well as in *M. tuberculosis* and *M. canettii*  $\beta$ -lactamases, at position 164, an Ala, Tyr, His, or Glu residue can be found, which discards all possibility of ionic or hydrogen bond interactions with the residue at position 179 (22, 23, 191). This interaction occurs instead between the side chain of the residue at position 179 and the backbone atoms of the residue at position 164 (Fig. 10A). As for the residues surrounding position 179, residues 175 to 178 of PER-1 are organized as an  $\alpha$ -helix, whereas the X-ray structures of other  $\beta$ -lactamases suggest that this stretch of amino acids corresponds to a loop region. Gln176 is thus separated from the equivalent Asp residues in TEM-1 and CTX-M-9 by 8.1 Å and 7.9 Å, respectively (98).

All the representative subclass A2 enzymes harbor an Arg or Lys residue at position 220 (Fig. 6 and 10B), as do some subclass A1  $\beta$ -lactamases such as KPC-2 (cluster CARBA) (Fig. 5). Comparisons of the structure of KPC-2 with that of TEM-1  $\beta$ -lactamase (branch LSBL) indicated that the position of the guanidine moiety of Arg220 was similar to that of the Arg244 residue in TEM-1 (1.18 Å between their  $C_{\zeta}$  atoms) (Fig. 10B), with position 244 being conserved as an Arg residue in some subclass A1 (e.g., clusters BAC1, BAC2, PASE, CARB, and RTG) and subclass A2 enzymes. Papp-Wallace and coworkers deduced that Arg220 played a critical role in the interaction of imipenem antibiotics with PenA from *B. cepacia* by mutating this residue to a Gly residue; they also showed that this residue played a key role in antibiotic sensitivity by extensive mutagenesis studies of KPC-2 (63, 152). This suggests that these positions are functionally equivalent in the two subclasses of enzymes, as discussed above for protein sequences, providing a positively charged docking site for a hydroxyl or carboxyl group of the substrate.

The most striking feature of a majority of ESBL enzymes is the occurrence of a hydroxylated residue (Ser or Thr), rather than an Ala or Gly residue, at position 237 (e.g., STA, LSBL1, and LSBL2) (Fig. 4 and 5) (12, 114, 115). Indeed, the importance of the Thr237 residue of KPC-2 was highlighted in recent studies (192). This  $\beta$ -lactamase also has several structural features in common with the subclass A2 enzyme PER-1 that have never before been described. These similarities are not directly apparent when considering the sequences of the proteins. The 268ProAsnLysAsp271 stretch of KPC-2 forms a loop that protrudes toward the outside of the protein, as shown by its X-ray structure (Fig. 11). This loop occupies the same position as the 240a-d loop of PER-1. The Tyr241 residue of KPC-2 overlaps the side chain of the Arg241 residue of TEM-1 and could participate in the displacement of this loop. The side chain of these



**FIG 10** Local structural differences between class A1 and class A2 enzymes. (A) Differences in the structure of the  $\Omega$ -loop between subclass A1 (TEM-1 in gray) and subclass A2 (PER-1 in purple)  $\beta$ -lactamases. (B) Equivalence of Arg220 from subclass A2 (PER-1) and Arg244 from subclass A1 (TEM-1). In panel A, Cyan dots show ionic interactions between the side chains of Asp179 and Arg164 in TEM-1. Red dots evidence a salt bridge between the side chain of Asn179 and the main-chain atoms of Ala164 in PER-1. Black dots highlight the displacement of the  $C_{\alpha}$  atom of residue 176 between the two enzyme structures, and the  $C_{\alpha}$  atom of Gln176 in PER-1 is shown as a sphere. In panel B, TEM-1 is shown in gray, KPC-2 is shown in green, and PER-1 is shown in purple. The side chains of Arg220 from PER-1 and KPC-2 are shown as sticks (left), as is that of Arg244 from TEM-1; also shown are the side chain of Asn276 from TEM-1 (O atom distant from  $N_{\epsilon}$ -Arg244 by 2.78 Å) and that of Glu276 from KPC-2 (O atom distant from  $N_{\epsilon}$ -Arg220 by 2.83 Å). The side chains of Ser70 for each enzyme are visible on the right.

residues is adjacent to that of Pro174, which is conserved in many subclass A1 enzymes (e.g., cluster LSBL1 with TEM-1, cluster CARBA with KPC-2, and cluster YER) but is replaced by a Gln residue in PER-1 (Fig. 2 and 6). The corresponding loop spanning positions 173 to 177 in PER-1 is thus displaced with respect to the configuration of TEM-1 and KPC-2. In TEM-1, the equivalent residues (Ser268 to Ala270) are buried deeper within the fold of the protein (Fig. 11). The loop spanning positions 268 to 271 of KPC-2 therefore probably plays a critical role in selecting  $\beta$ -lactam substrates for this enzyme as a function of their bulkiness.

Given the major structural differences between PER types and subclass A1  $\beta$ -lactamases, it is not surprising that site-directed mutagenesis studies of PER-1 found no direct relationship between the cephalosporinase activity of this enzyme and the type of residue at positions 164, 179, 238, and 240 (184, 193). These res-

idues are responsible for the extended-spectrum profiles of TEM and SHV enzymes (12, 114, 115). Enzyme inactivation by clavulanate was unaffected by amino acid substitutions at positions 69, 165, 244, and 275 of the PER-1 enzyme, as for IRT  $\beta$ -lactamases (194). Finally, protein engineering studies of PER-1 identified two residues whose mutations resulted in significant kinetic effects. First, the replacement of Thr104 with a Glu completely abolished the catalytic activity of the enzyme toward penicillins and reduced the  $k_{cat}$  values for cephalosporins by a factor of 50 to 700 (193). According to the X-ray structure of PER-1, this mutation should disrupt the interaction of the  $\gamma$ -hydroxyl group of Thr104 with Asn132. The replacement of Thr237 with an alanine residue increased the  $k_{cat}/K_m$  ratio of the mutant protein with respect to cephalosporin substrates by 1 to 2 orders of magnitude, and the reverse Ala237Thr mutation improved the hydrolysis of cephalosporin substrates by TEM and OXY enzymes (12, 114, 115, 135, 193).

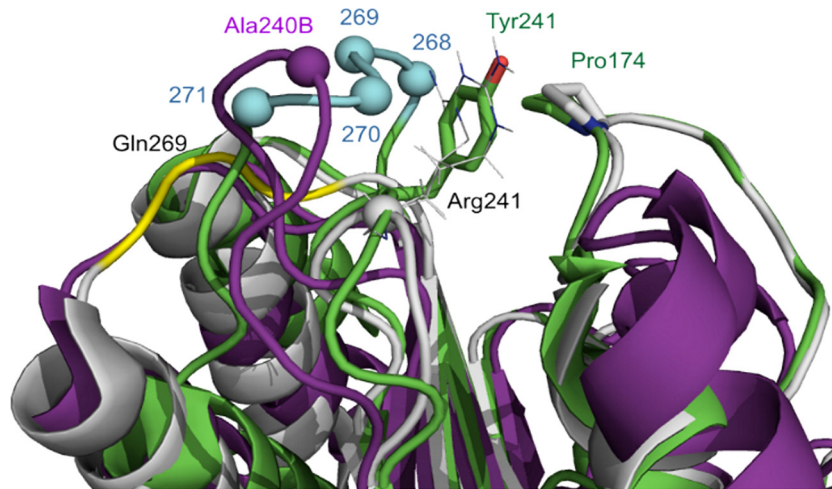


FIG 11 Superposition of PER-1 (purple), KPC-2 (green), and TEM-1 (gray) and significant enlargement of the substrate-binding cavity in PER-1. The loop spanning positions 268 to 271 of KPC-2 occupies a position similar to that of the 240A-to-240D insert of PER-1. The  $C_{\alpha}$  atom for Lys240A from PER-1 is shown as a sphere; the stretch spanning positions 268 to 271 of KPC-2 is highlighted in cyan, with  $C_{\alpha}$  atoms being represented as spheres. Arg241 is shown as thin lines, with its  $C_{\alpha}$  atom shown as a sphere and two minimized positions for its side chain. The side chains of Tyr141 from KPC-2 as well as Pro174 from KPC-2 and TEM-1 are shown as sticks. The yellow segment corresponds to Ser268-Ala270 for TEM-1.

#### ANNOTATION IN DATA BANKS

Knowledge regarding the molecular characteristics of  $\beta$ -lactamases has increased tremendously in recent years. The new findings obtained should make it possible to unambiguously distinguish between class A  $\beta$ -lactamases and, e.g., DD-peptidases, which belong to the same functional superfamily (195). For instance, the reference NCBI protein sequence under GenBank accession number [WP\\_015205777](#), deposited in the database in 2013 as a class A  $\beta$ -lactamase, was identified after a Blast alignment on the usual platforms (e.g., NCBI) as a penicillin-binding protein (20 of 30 runs), a  $\beta$ -lactamase or class A  $\beta$ -lactamase (7 times), or a hypo-

thetical protein (3 times) with an average length of 518 residues. The mean number of amino acids for 123 representative class A  $\beta$ -lactamases was nevertheless 299, for a standard deviation of 11. Karen Bush recently underlined that molecules of this size were no more than 31 kDa (13). The presence of several residues and motifs, 70SerxxLys73, 130SerAspAsn132, Pro107, Gly144, Gly/Ala156, Glu166, and 234LysThrGly236, moreover provides evidence for the existence of a novel class A enzyme. Nevertheless, as described above, several exceptions have to be considered (Table 5) (12, 96). The 234LysThrGly236 triad may differ between clusters in Gram-negative bacteria naturally producing an LSBL: Arg-

TABLE 5 Molecular characteristics of class A  $\beta$ -lactamases according to subclass

Subclass	Cluster	Typical enzyme(s)	Residue(s) at amino acid position(s):										
			70	73	77	130–132	136	166	179	233	234–236	237–238	245–246
A1a	Gram positive	PC1, BlaU, BlaL	S	K		SDN <sup>a</sup>	N	E	D	D	KT <sup>b</sup> G	AG	ND
	MYC1	BlaC	S	K		SDG	N	E	D	D	KTG	T <sup>b</sup> G	ND
A1b	LSBL1/4	TEM-1, SHV-1	S	K	C <sup>c</sup>	SDN	N	E	D	D	KT <sup>b</sup> G	AG	G <sup>d</sup> I <sup>e</sup>
	LSBL2	PSE-1, CARB-3	S	K	C <sup>c</sup>	SDN	N	E	D	D	RSG	AG	G <sup>d</sup> I <sup>e</sup>
	LSBL3	BlaP, RTG-2	S	K	C <sup>c</sup>	SDN	N	E	D	D	RTG	AG	G <sup>d</sup> I <sup>e</sup>
A1c	ESBL1/2	CTX-M, BES-1	S	K		SDN	N	E	D	D	KT <sup>b</sup> G	T <sup>f</sup> G	ND
	CARBA	NMCA, KPC-2	S	K		SDN	N	E	D	D	KTG	TC <sup>g</sup> G	ND
	ESBL3	GES-1, BEL-1	S	K		SDN	N	E	D	E <sup>h</sup>	KTG	T <sup>b</sup> C	ND
	BURK	BPS-1, PenI	S	K		SDN	N	E	D	D	KTG	TG	ND
A2a		PER-1, VEB-1	S	K		SDN	D	E	N	H <sup>i</sup>	KTG	T <sup>b</sup> S <sup>j</sup>	ND

<sup>a</sup> Ser for some *Bacillus* species.

<sup>b</sup> Or an analogue (Ser).

<sup>c</sup> Presence of a disulfide bridge between Cys77 and Cys123.

<sup>d</sup> Or an analogue (Ala) or Ser.

<sup>e</sup> Or an analogue (Val).

<sup>f</sup> Ala or Gly for some *Enterobacteriaceae* (*K. oxytoca* and *C. amalonaticus*).

<sup>g</sup> Presence of a disulfide bond between Cys69 and Cys238.

<sup>h</sup> Or an analogue (Asp).

<sup>i</sup> Or an analogue (Arg).

<sup>j</sup> Or Glu for CblA and CfxA types and some CepA types.

SerGly or ArgThrGly types. Thirty-three residues are strictly conserved, 43 are highly conserved among the enzymes of subclasses A1 and A2, and several of these residues are highlighted in our alignment as potential directions for future research (Table 5).

The diversity, specificity, and stability of amino acid sequences must thus be taken into account to ensure that the nucleotide and peptide sequences of class A  $\beta$ -lactamases be appropriately deposited and named.

## CONCLUSIONS

The treatment of infectious diseases with  $\beta$ -lactams is a highly challenging task due to the emergence and spread of new  $\beta$ -lactamases, which have become a real public health problem. These emerging enzymes are mostly class A enzymes. The new role played by clinical biologists in molecular diagnosis and the development of genomics have also had significant consequences for the discovery of new *bla* genes. There has been significant progress in DNA sequencing, and this has led to a large number of class A enzymes being repeatedly reported in databanks, in some cases being deposited under an inappropriate name or classification. These multiple examples clearly highlight the need to improve class A  $\beta$ -lactamase identification from amino acid sequences, as originally proposed by Ambler et al. (6, 96).

Class A is very large and can be divided into subclasses. One of these subclasses, subclass A2, has received surprisingly little attention from scientists. Site-directed mutagenesis experiments have been performed for only one  $\beta$ -lactamase in this subclass, PER-1. These experiments showed that mutations occurring at positions classically identified as being involved in the expansion of the inactivation spectrum had no impact on its overall hydrolytic behavior (193). These findings indicated that this enzyme has a one-of-a-kind reaction profile. In addition, amino acid substitutions at various positions have been found not to affect the inactivation of IRT enzymes by clavulanate (194). Overall, our analysis highlights the need for more investigations on specific subgroups and the relevance of subclass A2 as a distinct subtype of class A  $\beta$ -lactamases.

## ACKNOWLEDGMENTS

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We have no disclosures to make or conflicts of interest to declare.

## ADDENDUM

A novel plasmid-encoded carbapenemase, named BKC-1, for Brazilian *Klebsiella* carbapenemase 1, was recently detected among *K. pneumoniae* clinical isolates (A. G. Nicoletti et al., Antimicrob Agents Chemother 59: 5159–5164, 2015, <http://dx.doi.org/10.1128/AAC.00158-15>). This class A enzyme displays the highest level of identity (63%) to a  $\beta$ -lactamase of *Sinorhizobium meliloti*. This new class A carbapenemase (subclass A1, cluster RHI, for *Rhizobiales*) showed some molecular particularities, such as the presence of Cys69 but the absence of Cys238 and the insertion of an Arg residue at position 171 and two residues (Arg and His) at position 241.

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