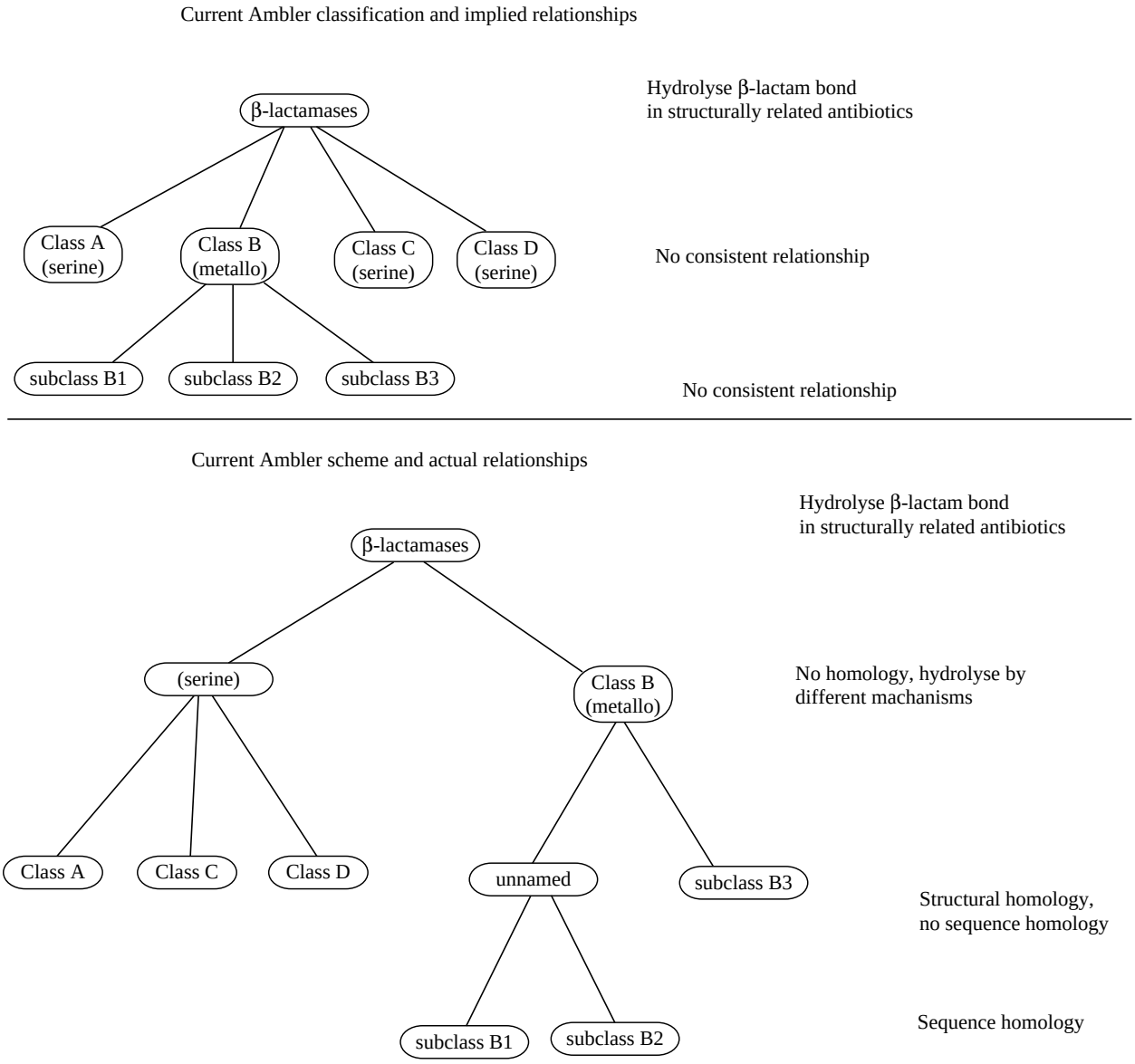

Figure 1. Schematic diagram of Ambler classification system.

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Is it necessary to change the classification of β-lactamases?

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Sir,

β-Lactamase classification schemes are inherently treacherous, particularly if one tries to take into account both structure and function. The decision to classify enzymes based on structure is the obvious way to describe these enzymes now that sequence data are so readily available. Most β-lactamase investigators freely acknowledge that the first structural division of these enzymes involves the molecular separation of serine enzymes from metallo-β-lactamases. Thus, it seems unnecessary to create a new classification nomenclature to acknowledge that fact.

If one decides that the classification of β-lactamases should only rest on the comparison of the sequences, it is clear that Hall and Barlow have a point. The sequences of the B2 β-lactamases are closer to those of the B1 enzymes and the members of the subclass B3 can be considered as 'outliers'.[1] However, is this sufficient to modify a classification that is now widely accepted and which reflects other properties of the enzymes? It may be unfortunate that for historical reasons all the metallo-β-lactamases are included in the sole class B, but there are other data that indicate that the better sequence similarity between B1 and B2 and the differences compared with B3 should not receive too much emphasis.

Enzymes that hydrolyse β-lactams belong to two distinct superfamilies on the basis of both structural and functional factors. The first superfamily contains the active-site serine β-lactamases and DD-transpeptidases/carboxypeptidases [also called penicillin-binding proteins (PBPs)]. Their common ancestor is probably a specifically bacterial enzyme, an essential PBP whose active site and function were modified by evolutionary pressure to yield the three presently described classes of serine β-lactamases. Despite clear structural similarities, the catalytic mechanisms of these three classes are fundamentally different since the general base is Glu-166 in class A,[2] a carboxylated Lys-73 in class D[3] and probably the base form of Tyr-150 in class C.[4] Both the sequences and the mechanisms justify the existence of the three classes, despite the somewhat better structural similarity between class A and class D, which also exhibit significantly smaller masses (25–30 000 Da) versus 39 000 Da for class C. All the members of this superfamily are bacterial proteins.

The second superfamily is more heterogeneous. It contains proteins or protein domains exhibiting the metallo hydrolase/oxidoreductase fold that was first identified in a metallo-β-lactamase.[5] Presently, this second superfamily contains more than 5600 proteins, often composed of several domains.[6,7] Among these, the largest group (>2700 sequences) is ubiquitous and the representative proteins are involved in a function essential for all organisms, t-RNA maturation. The structures of the metallo-hydrolase domains of two of these enzymes have recently been described.[8,9] It is possible that this type of protein is the ancestor of this superfamily which, in the course of evolution, generated a large number of enzymes with very different functions, among which were the metallo-β-lactamases. It seems that the original scaffold was well adapted to evolve into a wide variety of functions.

In metallo-β-lactamases, functional and mechanistic factors clearly distinguish the B1, B2 and B3 enzymes from each other and, on this basis, B2 is not more related to B1 than B3. Firstly, in contrast to B1 and B3 enzymes, which exhibit broad substrate profiles, the activity of B2 enzymes is restricted to carbapenems.[10,11] Secondly, while the B1 and B3 enzymes exhibit maximum activity as di-Zn species, the B2 β-lactamases are inhibited when binding a second Zn^{2+} ion.[11] Thirdly, major differences occur in the residues that act as Zn ligands. As shown in Table 1, if the B1 subclass is taken as a reference, B3 is not more different from B1 than B2. On the contrary, the 3-H site of B1 is nearly entirely conserved in B3, with the sole exception of GOB-1 where H116 is replaced by Q, while in B2, N116 does not seem involved in binding the second Zn^{2+} ions.[12] It is interesting to note here that, even with a high Zn^{2+} concentration in the crystallization medium, the crystal structure of the *Aeromonas hydrophila* (CphA) B2 enzyme only highlights a single Zn^{2+} ion in the DCH site.[13] The replacement of C221 (in B1 and B2) by H121 in B3 might appear as a major change. However, since H121 is next to D120, the ligand exchange occurs without important rearrangements of the tertiary structure. Indeed, in the 3D structure of the N220G mutant of CphA, the Zn ion is slightly displaced in 20% of the molecules and has four ligands rather than three. The fourth ligand is, unexpectedly, R121,[13] a result that confirms that the involvement of residue 121 in Zn ligation in the 'DHH' site does not result in major structural constraints.

The fact that the DCH site binds the sole catalytic Zn ion in subclass B2 clearly sets these enzymes apart from their B1 and B3 counterparts where dinuclear active centres appear to be the rule for optimizing the activity. The comparison of the available 3D structures also shows excellent conservation of the secondary structure elements in all metallo-β-lactamases.[13] The major differences occur in the loops and even there the differences between the subclasses are not entirely clear-cut: if a long L4 loop is a specific attribute of B3, long L2 loops are found in B2 and B3 and not in B1.[1] In consequence, and in contrast to the sequence alignments, functional factors clearly separate subclass B2 from the two other subclasses, and the structural data remain ambiguous.

One might wonder if it is necessary to involve the functional aspects in β-lactamase classification. However, the biological

Table 1. Zn^{2+} ligands in the metallo-β-lactamases

	3-H site			DCH/DHH site			
Subclass	116	118	196	120	121	221	263
B1	H	H	H	D	(R/C)	C	H
B2	[(N)	H	H]	D	(R)	C	H
B3	H/Q	H	H	D	H	(S)	H

Residues between round brackets are **not** Zn^{2+} ligands. The square brackets around the residues in the 3-H site of B2 indicate that there is up to now no structural indications that the inhibitory Zn^{2+} ion binds there. The Q residue in position 116 of GOB-1 raises the question of the possible involvement of another residue in the '3-H' site of this enzyme although residues 116, 118 and 196 are clearly the Zn^{2+} ligands in L1 and FEZ-1, the two B3 enzymes of known structures. An alternative possibility would be that GOB-1 is mainly active as the mono-Zn species with Zn^{2+} in the DHH site, just as the B2 enzymes!

significance of these enzymes lies in their function and not on their structure. Thus, reliance only on the sequences can sometimes be quite misleading, as shown by several examples of erroneous functional annotations of genes in genomic analysis.[14] In the first superfamily mentioned above, the sequence of the class D OXA-2 β-lactamase is more similar to that of the BlaR C-terminal domain than to that of the OXA-1 β-lactamase. On this basis, BlaR would be classified as a class D β-lactamase although its k_{cat} values for penicillins and cephalosporins are in the 10^{-6}–$10^{-5}\,s^{-1}$ range,[15] several orders of magnitude lower than the k_{cat} values exceeding $10^{2}\,s^{-1}$ reported for typical class D β-lactamases.[16] Similarly, in the second superfamily, the sequence of human glyoxalase II is closer to those of B1 and B2 enzymes than those of B3.[1] But glyoxalase should, nevertheless, not be classified as a metallo-β-lactamase.

In conclusion, there has been good acceptance of the structural classification schemes that recognize three families (or classes) of active-site serine enzymes and three (sub)-families of metalloenzymes. It seems unnecessary to make additional modifications to a well-established scheme without consideration of factors such as enzymatic mechanisms and functional behaviour.

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New *aac*(6′)-*I* genes in *Enterococcus hirae* and *Enterococcus durans*: effect on β-lactam/aminoglycoside synergy

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Sir,

Enterococcus faecium species harbour an intrinsic *aac*(6′)-*Ii* gene,[1] which encodes aminoglycoside acetyltransferase AAC(6′) that confers resistance to the synergy of the association of penicillin with tobramycin. Nevertheless, similar genes have not been previously detected in other enterococcal species. *Enterococcus hirae* and *Enterococcus durans* are frequently found in the intestine of animals and less frequently in humans,[2] and are occasionally involved in severe human infections.[3]

The characterization of two novel *aac*(6′)-*Ii*-like genes, species-specific for *E. hirae* and *E. durans*, is reported in this study. In agreement with the nomenclature suggestion of Vanhoof *et al.*[4] for the *E. faecium* acetyltransferase gene [*aac*(6′)-*Ii*], we have named the new genes as *aac*(6′)-*Iih* for *E. hirae*, and *aac*(6′)-*Iid* for *E. durans*.

Eight *E. hirae* strains with seven different PFGE-*Sma*I patterns and three unrelated *E. durans* strains were identified by biochemical and genetic criteria.[5] MICs of penicillin, streptomycin, gentamicin, tobramycin and kanamycin were determined by the recommended agar dilution method, and none of the isolates had either high-level resistance to the aminoglycosides tested or penicillin resistance. Time–kill studies were carried out as previously described.[6] Synergy was observed with