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REVIEW

Classification for β-lactamases: historical perspectives

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ABSTRACT

Introduction: β-Lactamases are some of the most prevalent and well-studied families of enzymes, especially in the area of antibiotic resistance. Early attempts to categorize them used either functional names, such as penicillinase or cephalosporinase or structural designations into classes A and B. Increasing diversity of the properties of these enzymes has required a more expansive approach to nomenclature.

Areas covered: Historical designations for early β-lactamases relied heavily on functional names based on the biochemical properties of purified enzymes. As amino acid sequences began to be reported for a select group of these enzymes, classes of β-lactamases were defined, with a major lumping of enzymes into those that had active site serine residues (class A, C, and D) and those that were metallo-β-lactamases (MBLs or class B). More recent classification schemes, as determined through a Medline search, have attempted to incorporate both functional and structural features, using functional groups and subgroups to name β-lactamases within the same structural class. Nomenclature of these enzymes is now under the purview of the NCBI (National Center for Biotechnology Information).

Expert opinion: β-Lactamase nomenclature will continue to evolve with the identification of new enzymes and new functionalities.

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1. Introduction

β-Lactamases are some of the most ubiquitous and ancient enzymes, with their origins estimated to be at least two billion years ago [1]. Their presence has been identified from pre-antibiotic era samples isolated from a variety of pristine environmental sites [2,3]. However, their significance was not appreciated until 1940, when Abraham and Chain [4] first noted that a strain of '*Bacillus coli*,' as well as a culture of *Staphylococcus aureus*, was capable of deactivating the novel antibacterial material known as penicillin [4]. The hydrolytic enzyme produced by this organism, named a 'penicillinase,' was the first representative β-lactamase (E.C. 3.5.2.6) described in the scientific literature, leading to the study of thousands of similar enzymes now known to be responsible for most resistance to the β-lactam antibiotics, especially in Gram-negative bacteria [5]. Because of the diverse nature of these enzymes with homologous catalytic activities, the nomenclature associated with these enzymes has sometimes been ambiguous. Attempts to categorize the enzymes into families have historically been based on functional properties [6] or on structural attributes [7–10], or, sometimes on both [11,12]. In this review, nomenclature based both on functional and molecular characteristics will be discussed from the historical perspective using literature searches based on Medline. Possible future directions in this area will also be considered.

2. Lactamase nomenclature

2.1. Evolution of β-lactamase nomenclature

2.1.1. Historical functional names

Naming of β-lactamases has been a challenging and sometimes confusing task, with researchers utilizing either functional characteristics or molecular properties to categorize these enzymes (Table 1). The historical expansion of functional names is demonstrated in Figure 1, where the initial appearance of currently accepted nomenclature is correlated with the development of the β-lactam antibiotics. The first β-lactamases were named penicillinases by Abraham and Chain [4], due to their ability to destroy the antibacterial properties of the potentially useful therapeutic agent known as penicillin. At that time, the structure of penicillin was unknown, and no other β-lactam structure had been identified. Thus, the name penicillinase was used for more than two decades to designate any enzyme that inactivated penicillin. Chemical studies established that the enzymes that hydrolyzed the β-lactam ring were readily differentiated from acylases (or amidases) that hydrolyzed the N-acyl side chain substituted on the β-lactam [33]. Following the identification of the naturally-occurring cephalosporins whose structures were shown to be related to penicillins, various 'penicillinases' identified from multiple sources were shown to destroy these new cephalosporins preferentially to penicillins. Thus, the name 'cephalosporinase' arose, based initially on the observed degradation of

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Article highlights

- β-Lactamases have been named according to their functional activity or according to their amino acid sequence similarities.
- Molecular classifications of class A, B, C and D have been assigned to all β-lactamases that have amino acid sequences available.
- Functional classifications of groups 1, 2, 3, and 4, with appropriate subgroups, have been assigned primarily based on substrate and inhibitor profiles and experimentally-validated β-lactamase activity.
- Attempts to correlate both molecular and functional characteristics in a single set of nomenclature have been published.

cephalosporin C by a strain of *Enterobacter cloacae* in 1963 [13]. Separation of penicillinases from cephalosporinases was not a trivial task, as the convention had been to name both the penicillin-hydrolyzing and cephalosporin-hydrolyzing enzymes as penicillinases. The first genetic map to identify the chromosomal *ampC* gene in *E. coli* K-12 in 1973 was associated with the '*ampC* penicillinase' [34], which is now viewed as the prototypical AmpC cephalosporinase [35].

Because most cephalosporinases retain the ability to inactivate penicillins, bacteria that produced both a legitimate penicillinase together with a cephalosporinase as chromosomal gene products were not readily recognized to produce two separate β-lactam-hydrolyzing activities. Co-production of the two enzymes was first observed in 1965 in the nonpathogenic *B. cereus*, when the loss of cephalosporinase activity was observed but penicillinase activity was preserved after dialysis with EDTA [36]. Zinc was identified as a cofactor for the *B. cereus* cephalosporinase shortly thereafter [37]. By the mid-1960s, major investigators in the antibiotic resistance field began to use the term 'β-lactamase' to differentiate those enzymes that inactivated penicillins or cephalosporins by hydrolyzing the β-lactam bond from the amidases that hydrolyzed the side chain linkage [33,38–40]

Although Sabath and Abraham [37] demonstrated that the *B. cereus* cephalosporinase activity was reliant on the presence of zinc in the 1960s, the use of the terms 'metalloenzymes' or 'metallo–lactamase' (MBL) did not appear in β-lactamase literature until the mid-1980s [41,42]. In 1985 Bicknell and Waley [43] referred to the enzyme as 'zinc β-lactamase II' from *B. cereus*. Initially these enzymes were distinguished because they required zinc as a cofactor for optimal activity and not because of any particularly unique substrate profile. However, after the introduction of imipenem as a therapeutic agent in 1985 [44], the enzymes were recognized as 'imipenem-hydrolyzing' or 'carbapenem-hydrolyzing' β-lactamase . The term 'carbapenemase' was soon adopted to indicate the ability of these enzymes to hydrolyze the broad category of carbapenems [45,46]. As carbapenemases began to proliferate with interspecies dissemination as the result of gene mobilization [47,48], two different groups of these enzymes were differentiated: the zinc-requiring MBLs [49] and those carbapenemases with serine in their active site that structurally more closely resembled the older penicillinases and cephalosporinases, i.e. the serine β-lactamases, or SBLs [50].

Extended-spectrum β-lactamases (ESBLs) began to emerge in the late 1980s, but the abbreviation ESBL did not appear in the literature until the year 2000 [51–53]. Initially described as 'extended broad-spectrum β-lactamases,' or ESB-Bla [54], the abbreviation ESBL has become the preferred designation. ESBLs have been defined as β-lactamases capable of hydrolyzing aminothiazoleoxime β-lactam antibiotics such as the expanded-spectrum cephalosporins (also known as '3rd generation cephalosporins') and monobactams at rates at least 10% that of benzylpenicillin, and that are strongly inhibited by clavulanic acid [20]. The original ESBLs were variants of the common TEM and SHV penicillinases that differed from their parent enzymes in one to three amino acids [21]. ESBLs populate one of the largest sub-families of β-lactamases, both in terms of sheer numbers of discrete enzymes, but also in terms of causing increasingly high proportions of antibiotic-resistant Gram-negative infections globally [55,56].

A recent trend in nomenclature has been to name species-specific chromosomal cephalosporinases related to the class C AmpC-type β-lactamases according to the species name. The first example of this is the ADC subfamily of Acinetobacter-derived cephalosporinases [57] followed by the PDC subfamily of *Pseudomonas*-derived AmpC-type cephalosporinases [58]. In February 2023, 290 ADC and 537 PDC distinctive cephalosporinases had been identified on the NCBI website [26,59].

Although Gram-positive bacteria were the first pathogens to be compromised clinically by production of penicillinases, the naming of β-lactam hydrolyzing enzymes has focused on β-lactamases produced by Gram-negative bacteria. No commonality of individual β-lactamases has been observed between the two. The best-studied penicillinases from Gram-positive pathogens are from *S. aureus*, with the PC1 penicillinase the prototypical example [60,61]. Attempts were made in the early 1990s to differentiate penicillinase variants based on preferential hydrolysis of various penicillins and cephalosporins [62], but these studies were not followed up with genomic sequencing. Because the major resistance mechanism for β-lactam antibiotics in the staphylococci is due to the presence of the *mec*A gene, many investigators now ignore the subtleties associated with penicillinase alleles and lump the staphylococcal β-lactamases together with the name of BlaZ [63]. Notably, only ten *bla*Z sequences have been deposited in the NCBI Reference Gene Catalog to date [59].

2.1.2. Classification schemes

β-Lactamase researchers, serving as enzymological taxonomists, have been described as either 'lumpers' or 'splitters' (G. Jacoby, personal communication). Those who classify β-lactamases into four separate molecular classes according to amino acid similarities fall into the lumper group, whereby those who separate the enzymes according to microbiological or enzymic properties are regarded as splitters. As seen in the previous section, early β-lactamase investigators lumped all β-lactam-hydrolyzing enzymes into the penicillinase bucket, whereas the tendency today has been to split β-lactamases into multiple functional groups with differentiating enzymatic and molecular characteristics. Recent characterization approaches include both sets of properties compiled into the most comprehensive databases available [26–30].

Table 1. Naming conventions for β-lactamases and the dates with which they have reported.

		Basis for Nomenclature			
Date	Nomenclature	Function	Physical Properties	Sequence[a]	Reference
1940	Penicillinase	X			Abraham and Chain [4]
1963	Cephalosporinase	X			Fleming, Goldner and Glass [13]
1968	Groups	X			Sawai, Mitsuhashi and Yamagishi [14]
1970	Types	X	X		Jack and Richmond [15]
1973	Classes (Functional)	X	X		Richmond and Sykes [6]
1976	Classes (Functional)	X	X		Sykes and Matthew [16]
1980	Classes (Molecular: A and B)			AA	Ambler [7]
1981	Class C			AA	Jaurin and Grundstrom [9]
1988	Class D			AA	Huovinen, Huovinen and Jacoby [10]
1988	Groups 1, 2, 3	X	X	(AA)[b]	Bush [17]
1989	Groups 1 2 (6 subgroups) 3 4	X	X	(AA)[b]	Bush [18–20]
1991	Class A (updated)			AA	Ambler et al. [8]
1995	Groups 1 2 (8 subgroups) 3 4	X	X	AA	Bush, Jacoby and Medeiros [11]
1996–2015	Sequential naming convention	(X)[c]		AA	Jacoby et al.[d] [21,22]
2004	Class B (updated)			AA	Garau et al. [23]
2010	Groups 1 (2 subgroups) 2 (12 subgroups) 3 (2 subgroups)	X	X	AA	Bush and Jacoby [12]
2012	*Klebsiella* spp. *bla* genes; OKP, LEN, OXY enzymes			NS	Institut Pasteur/Brisse[e] [24]
2013	Multiple resistance genes (CARD)[f]	(X)		NS, AA	Jia et al.[f] [25]
2015	NCBI standardized	(X)[b]		NS, AA	NCBI[g] [26,27]
2016	Class A			AA	Philippon et al. [28,29]
2017	Structure-function (BLDB)[h]	X	X	AA	Naas et al.[h] [30]
2020	Class C (updated)			AA	Mack et al. [31]
2022	Consensus (all)	(X)[b]		AA	Bradford et al. [32]

[a]Amino acid sequence (AA) or nucleotide sequence (NS)
[b]Secondary characterization criterion
[c]Secondary characterization criterion for TEM, SHV and OXA families
[d]Lahey database: https://externalwebapps.lahey.org/studies/
[e]Institut Pasteur: http://bigsdb.pasteur.fr/klebsiella/klebsiella.html
[f]CARD (The Comprehensive Antibiotic Resistance Database): https://card.mcmaster.ca/
[g]NCBI: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047 and https://www.ncbi.nlm.nih.gov/pathogens/submit-betaβ-lactamase/
[h]BLDB (Beta-Lactamase DataBase): http://bldb.eu

Approaches to β-lactamase nomenclature have focused primarily on those enzymes found in Gram-negative bacteria. In 1968, Sawai, Mitsuhashi and Yamagishi characterized a set of β-lactamases produced by Japanese enteric bacteria, with their separation into three distinctive groups based on substrate profiles and their reaction to antiserum [14]. This was the first attempt to find systematic similarities among the various β-lactam-hydrolyzing enzymes from Gram-negative organisms, based on the totality of their biochemical properties. Two years later Jack and Richmond examined another set of β-lactamases from 46 strains primarily of British origin and expanded the criteria used to define eight different types of enzymes [15]. Not only were substrate profiles and reaction to antisera reported, but electrophoretic profiles and the effect of potential β-lactamase inhibitors (*p*CMB, or *p*-chloromercuribenzoate and cloxacillin) were studied.

Further analyses by Richmond and Sykes in 1973 [6] and Sykes and Matthew in 1976 [16] broadened the properties that were considered in classifying the increasing number of β-lactamases. Molecular size of the purified enzyme, source of the *bla* gene as either chromosomal or plasmid and isoelectric focusing attributes were included in these classifications, with each of the reviews resulting in five distinctive classes of β-lactamases. The Richmond and Sykes classes [6] were frequently used to characterize new β-lactamases into the late 1980s. Although molecular size was an important factor that

was used to differentiate β-lactamase classes in the Sykes and Matthew scheme [16], this was determined by gel filtration column chromatography that could give only an approximation of the actual molecular weight of the enzyme. In 1980, an accurate molecular size determined by amino acid sequencing was known for only four β-lactamases, all SBLs, demonstrating high sequence homology among themselves. Incomplete sequencing of one MBL from *Bacillus cereus*, an enzyme requiring a metal cofactor for enzymatic activity, demonstrated great molecular and biochemical divergence from the class A β-lactamases, and served as the basis for the Ambler molecular classification of class A and class B enzymes [7]. Molecular class C cephalosporinases were differentiated by their larger molecular mass and low sequence homology with the known class A 'penicillinases' in the Amber scheme [9]. Molecular class D 'oxacillinases' were broken away from their original Class A designation due to low sequence homology, other than the conserved active site Ser-X-X-Lys tetrad [10]. Subsequent studies of the MBLs have further subdivided the class B enzymes into subclasses B1, B2 and B3 [64]. Through the late 1980s, the few complete amino acid sequences of β-lactamases that were available were obtained through tedious manual sequencing of cloned genes or amino acid sequencing of purified enzymes [9,10,65,66].

As genetic sequencing of bacterial genes became more accessible, and affordable, the number of fully sequenced β-lactamases increased rapidly, together with an explosion in the number of novel β-lactamases. Following the clinical introduction of novel β-lactam antibiotics in the oxyimino-cephalosporin and monobactam families in the 1980s, new sets of β-lactamases with unique functionalities began to emerge. These new agents exerted pressure on Gram-negative bacteria to produce variants of previously common β-lactamases, but with significantly different hydrolytic properties. Because of the increasing ease with which sequencing could be conducted, dozens of novel alleles were identified that did not fit into the previous functional categories.

In 1988, Bush published an updated functional scheme that took into account some of the novel functionality, with substrate profiles that included oxyimino cephalosporins and aztreonam, and inhibitor profiles using clavulanic acid and EDTA [17]. Any available molecular data were considered for identifying three functional groups [17]. The following year an expanded scheme introduced the concept of functional groups based on contemporary substrates and inhibitors, and included the minimal amount of new sequence data that were available. This scheme was introduced in three sequential invited review papers in *Antimicrobial Agents & Chemotherapy* [18–21]. Although the three papers were intended to be one Minireview, the journal at that time limited reviews to no more than six printed pages, thus requiring the splitting of the work. Fortunately, when an update was requested in 1995, journal policies had changed, and a single functional classification scheme was published by Bush, Jacoby and Medeiros [11], with additional enzymes and sequence data included [11]. A further expansion was published in 2010 by Bush and Jacoby [12]. In the latter expansion, the initial four major functional groups were reduced to groups 1, 2 and 3, due to the lack of updated information about the poorly characterized enzymes originally classified in group 4. The functional groups proposed by Bush *et al*. were defined by physicochemical attributes, basic biochemical properties in the form of substrate and inhibitor profiles, and amino acid sequences that could be aligned with the well-established molecular classes [7,9,10].

As new β-lactams entered the clinical space and the numbers of novel enzymes with unique sequences concomitantly increased, functional groups were expanded into subgroups. A simplified version of these functional groupings, showing only those subgroups with the most clinically relevant β-lactamases, appears in Figure 2. SBLs in classes A, C, and D, as well as the class B MBLS, are grouped according to their substrate profiles that include penicillins and early generation cephalosporins for all groups and subgroups, except the group 2d penicillinases. Hydrolysis of expanded-spectrum cephalosporins is notable for the group 2be and group 2de ESBLs, whereas carbapenem hydrolysis is a distinguishing factor for groups 2f, (no space), 2df, and the MBLs (group 3). SBLs are all inhibitable to some extent by avibactam, but not necessarily by clavulanic acid. EDTA inhibition distinguishes MBLS from SBLs. Further details for the groupings can be accessed in references 11, 12, and 18. As shown in Table 1, the major contemporary nomenclature databases include NCBI (59), the Institut Pasteur website on *K. pneumoniae* with coverage of *Klebsiella*-related β-lactamases [24], the CARD (Comprehensive Antibiotic Resistance Database) website that includes multiple resistance genes [25], and the BLDB (β-Lactamase Database) that uses crystallographic data to correlate β-lactamase structure with function [30].

2.2.Conventions in naming β-lactamases

β-lactamase names were initially assigned rather randomly, based on various attributes, such as geographical location of original isolates (e.g. MGH, OHIO, Toho), individual names of people (e.g. PER, ROB, TEM), preferential substrates (FOX, OXA, IMI), genetic location of the gene (e.g. CepA, IBC, VIM), genus of the producing bacteria (e.g. SME, KPC, PDC), or specific biochemical properties (e.g. L1 and SHV) [67]. At one point, efforts were made within the β-lactamase community to name plasmid-encoded enzymes with all capital letters, *e.g*. TEM or SHV, while chromosomally-encoded β-lactamases had capital letters only at beginning and end, *e.g*. CcrA or CphA [68]. This convention was abandoned in the early 1990s when plasmidic genes began to be incorporated into the chromosome [67]. With the increase in the number of reported ESBLs in the mid-1990s, it became necessary to establish a clearing house for the naming of new β-lactamases, particularly in the TEM and SHV families, to avoid duplication of names or incorrect assignment of enzymes to a well-defined molecular family [32]. The website curated by George Jacoby at the Lahey Clinic [21] was designated as the location for this resource and was expanded from its initial focus on ESBL assignments to include assignment of names for most plasmid-encoded β-lactamase families. After almost 20 years of curation, the site was closed in 2015, although the database is still maintained as an historical

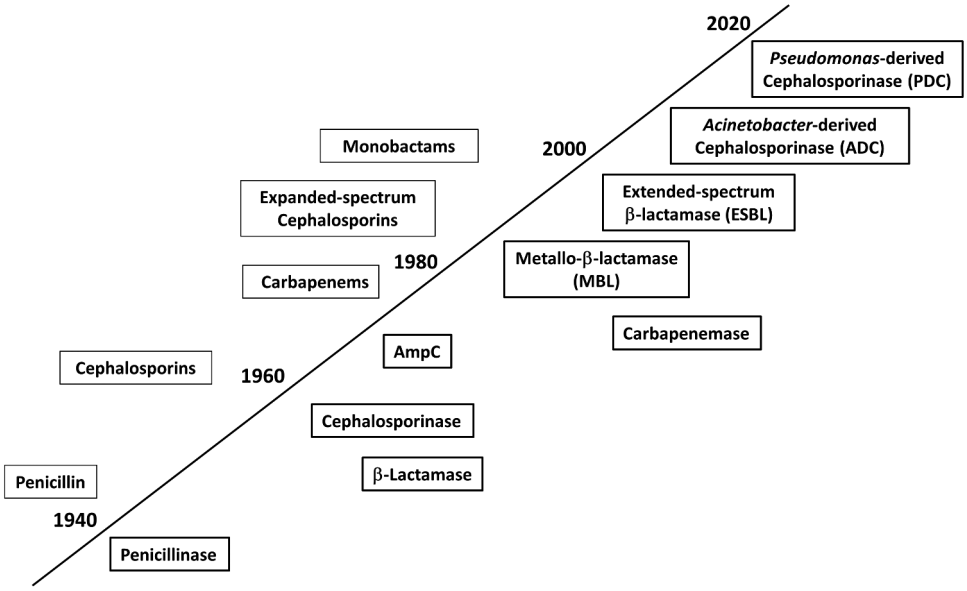

Figure 1. Basic β-lactamase functional descriptions as defined in the literature (below the timeline), associated with the identification of novel β-lactams (above the timeline).

archive [22]. The sequence information and references from the Lahey website were transferred to NCBI that now serves as the centralized group responsible for β-lactamase numerology [26,27,59].

Because thousands of individual, unique, naturally occurring β-lactamase sequences have now been reported [59], naming conventions are even more critical than in the 1990s when only several hundred novel β-lactamases had been characterized [11]. NCBI, with access to large sets of whole-genome sequencing data, is in a unique position to parse the growing amount of sequence information using a systematic and logical set of naming principles [26]. Various groups have proposed consensus sequencing recommendations for class A β-lactamases [8], class B metallo-β-lactamases [23], and class C cephalosporinases [31]. A group of β-lactamase experts, including representatives from NCBI, recently proposed a set of naming conventions that include as their major points [32]:

a. Only naturally-occurring β-lactamases can receive a name that will be based on predicted amino acid sequences, not on nucleotide sequences. The enzyme must have at least one amino acid substitution that is different from a previously assigned enzyme.
b. Assignment and tracking of new allele numbers will be curated by NCBI.
c. Chromosomal class C cephalosporinases (i.e. AmpC) in general will not receive allele numbers.
d. Names associated with geographical locations will not be assigned.
e. Functional assignments of β-lactamases will be made only on the basis of biochemical or microbiological data, not on sequence similarities.

Class D β-lactamase nomenclature remains a serious issue that has not been adequately addressed by the β-lactamase community [69,70]. Class D enzymes, almost exclusively designated with 'OXA' prefixes, were originally named because of their ability to hydrolyze methicillin, oxacillin and other isoxazolyl penicillins more efficiently than other substrates [16]. An alignment and numbering scheme for class D β-lactamases was proposed in 1991 by analyzing three diverse OXA alleles [71]. However, over 1100 OXA alleles have been identified to date [59], with phenotypic designations ranging from pure penicillinases to ESBLs to carbapenemases. The wide functional diversity within this class is accompanied by structural differences, such as low numbers of conserved amino acids, low sequence similarity (as much as 80% difference) and protein lengths ranging from 250 to 275 amino acids for class D β-lactamases from clinically relevant isolates [67,69,72]. When environmental sources are considered, class D alleles are even more varied and have been subdivided into 64 clusters [73]. Because of the wide diversity observed among these enzymes, both on a functional and on a structural level, as has been noted by a number of investigators [32,74,75], there is a need to reconsider the nomenclature for this class.

2.3.Controversies in nomenclature conventions

Naming of β-lactamases and further classification of these enzymes into related sets has presented a challenge to the community of researchers who study antibiotic resistance mechanisms. Attempts to standardize nomenclature using similar naming conventions for different antibiotic resistance mechanisms has been proposed by several groups [76,77], but have been largely unsuccessful. Hall and Schwarz [76] have proposed that there be at least a 2% difference in amino acid sequence from a known β-lactamase in order to name a new enzyme. In the tetracycline area [78], Roberts [79], the curator of tetracycline resistance gene names, requires a new tetracycline resistance gene to encode a protein with <79% amino acid identity with any previously identified *tet* gene product for it to be recognized as a novel allele [80]. However, these conventions have not been well received in the β-lactamase or aminoglycoside worlds,

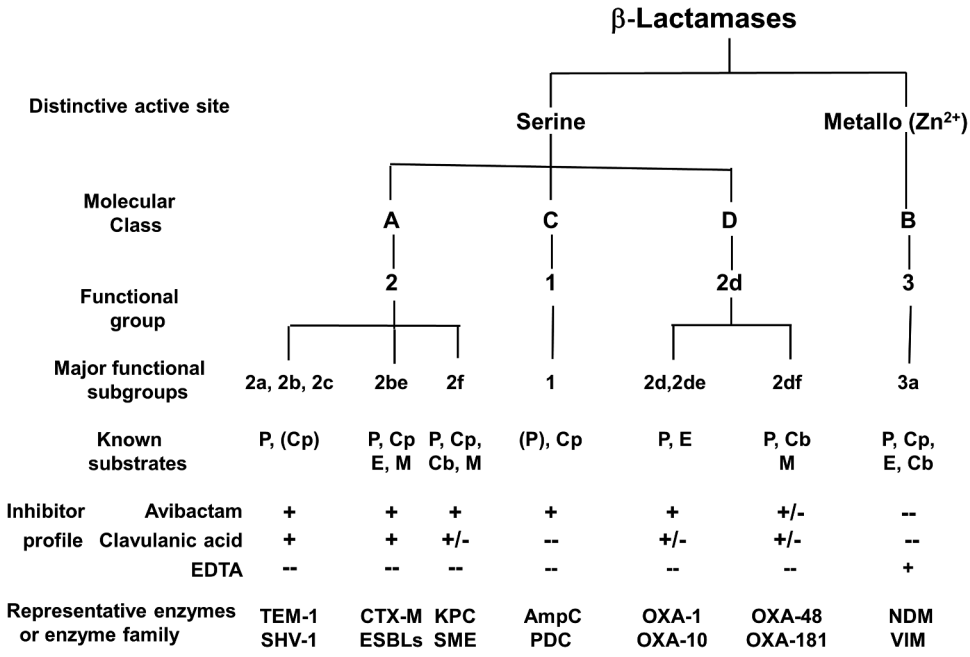

Figure 2. Simplified correlation between molecular and functional nomenclature with a focus on the most clinically relevant β-lactamases. Substrates listed in parentheses represent β-lactams hydrolyzed weakly by that subgroup of enzymes. Inhibitor profiles: (+) effective inhibition; (+/-) inhibition dependent upon the specific enzyme: (–) no useful inhibition. Abbreviations: Cb, carbapenem; Cp, cephalosporin; E, expanded-spectrum cephalosporin; M, monobactam, P, penicillin. Adapted from a more comprehensive figure from reference 5 with permission.

where single amino acid changes have resulted in enzymes with different functionality from the parent [74,81]

Additional controversy has ensued regarding the definition of an ESBL. In the Bush classification schemes, ESBLs have been defined within the functional group 2be, as clavulanic acid-inhibitable β-lactamases that hydrolyze oxyimino-cephalosporins and monobactams. However, there has been an inconsistent application of this definition [82–84]. Some have limited the definition to include only plasmid-encoded β-lactamases that can be mobilized for facile transmission between Gram-negative species [83], whereas others have expanded the definition to define an ESBL-producing Gram-negative organism based on phenotypic resistance to ceftriaxone, ceftazidime or aztreonam [85]. Giske *et al*. [84] have proposed a similar definition: all β-lactamases with activity against expanded-spectrum cephalosporins should be named ESBLs to make it easier for physicians to understand resistance profiles. This proposal has not been well accepted, with detractors noting that there needed to be additional differentiation between class A, C, and D β-lactamases that might all have the ability to hydrolyze ceftazidime, but that might differ in their response to β-lactamase inhibitors or to carbapenems [86]. The expanded definition was also criticized because it would include serine carbapenemases as ESBLs, whose producing organisms would not respond therapeutically to carbapenem therapy often used to treat infections caused by ESBL-producers [86].

3. Conclusions

β-Lactamase nomenclature has become a hybrid model, with both functionality and amino acid sequence similarities used to divide, and subdivide, the thousands of unique β-lactamases known to exist. Although not all antibiotic resistance researchers fully agree on the most appropriate name for a new β-lactamase, they have reached consensus that there needs to be a set of conventions that are followed to bring consistency into the research field. The current consensus of many leaders in the area is that the NCBI should serve as the curator of all genes and their corresponding gene products that are related to antibiotic resistance. Their activities in the β-lactamase arena demonstrate the hybrid approach of assigning new β-lactamase alleles to families of closely related enzymes, with a further designation as to the topline functional behavior of the enzyme. As new antibacterial agents or combinations are introduced clinically, there will inevitably be opportunities to refine the current systems that are in place.

4. Expert opinion

Nomenclature for antibiotic resistance factors has plagued this area of research for many years. Not only β-lactamase names, but the names associated with tetracycline [78] and aminoglycoside resistance genes [81,87] have been confusing over the course of their history. The topic has become even more challenging with the wide availability of whole genome sequencing at a reasonable cost, resulting in thousands of bacterial genomes that can be accessed by the scientific community. The data now being generated from metagenomic analyses of environmental bacterial populations are only adding to the confusion, as names are generated for β-lactamase-like enzymes that may or may not have functional significance [73]. One can envision a time when every natural mutation in a resistance gene will result in a new genetic designation, ostensibly to monitor the evolution of each bacterial species or mobile resistance factor. This approach, which is regarded with some misgiving by the author, is fraught with

complications. Many mutations do not confer any identifiable functional change that warrants a novel enzyme designation. Errors in sequencing are not accounted for. Duplications are difficult to monitor, despite the best attempts to establish centralized databases. As a result, ignorance of the established conventions for naming has resulted in duplications of names, or inappropriate names for novel proteins [67].

Although various platforms are currently in place to define both enzymatic and microbiological properties of β-lactamases, it will take careful curation to ferret out what changes are important functionally and what are simply the result of random, natural mutations conferring little or no effect on physiological activity. As noted by the recent statement from the β-lactamase community, any novel amino acid sequence should be assigned a unique number [32], but it will be important to determine how these changes, either singly or in combination, affect the function of new alleles. The resistance community is now being provided with thousands of bacterial genomic sequences with little or no annotation regarding functional mutations, not only in known antibiotic resistance genes, but also in any essential or ancillary genes. Even when mutations are identified, their effect on function is most often not defined. This is in contrast to early studies on β-lactamases, where distinctive characteristics in antibiotic susceptibilities or enzymatic profiles would serve as a signal to pursue eventual molecular studies. Functional information was usually known before molecular characteristics were identified, whereas sequencing now most frequently precedes functional characterization. Today at least two different groups are compiling information correlating both structure and function for the β-lactamases. The BLDB website has as its ultimate goal the compilation of ' … sequence information as well as biochemical and structural information on all the currently known β-lactamases' [30]. NCBI, in their informational website, states that one of their goals is to enable ' … the retrieval of beta-lactamase, Qnr, and MCR nucleotide and protein accessions along with the antibiotic susceptibility profile of a transconjugant/transformant bearing the beta-lactamase gene(s)' [88]. The ability to compile functionality data for three major resistance factors together with sequencing information adds value to the database and provides greater context. This approach will be critical as the β-lactamase community tackles the issues with the class D/OXA enzymes that require a major sorting out in the future.

As a result of the work of both the BLDB and NCBI groups, it may be possible in the future to categorize new enzymes in a more systematic way, with a stronger correlation between structure and function on an enzymatic level. However, caution needs to be taken to extrapolate enzymatic activity to antibacterial susceptibility. Likewise, enzymatic activity should not be extrapolated from antibacterial susceptibility data which are typically more readily available. Numerous factors work together to influence susceptibility profiles, including the amount of β-lactamase produced, concentration of the antibiotic in the cell with respect to the K_M value for the enzyme with that substrate, antibiotic entry into and efflux out of the bacterial cell, mutations in the essential penicillin-binding proteins, and the number or variety of β-lactamases produced by the cell [89,90]. For example, low hydrolytic activity for a novel β-lactamase with a carbapenem does not necessarily mean that the producing bacterium will be susceptible, if the organism produces a carbapenemase together with the new enzyme. Thus, functional characteristics of a new β-lactamase must be considered as only one predictive tool in the clinical toolbox.

New β-lactam-containing agents or new combinations of agents will continue to exert selective pressure on existing bacteria. It is inevitable that new β-lactamases with distinctive properties will continue to be identified, warranting further classification into either existing or future schemes. With additional refinements in our understanding of structure-function correlations, future classification efforts for β-lactamases should become more informed as they continue to be updated.

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