MINIREVIEW

Updated Functional Classification of β-Lactamases

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Two classification schemes for β-lactamases are currently in use. The molecular classification is based on the amino acid sequence and divides β-lactamases into class A, C, and D enzymes which utilize serine for β-lactam hydrolysis and class B metalloenzymes which require divalent zinc ions for substrate hydrolysis. The functional classification scheme updated herein is based on the 1995 proposal by Bush et al. (K. Bush, G. A. Jacoby, and A. A. Medeiros, Antimicrob. Agents Chemother. 39:1211–1233, 1995). It takes into account substrate and inhibitor profiles in an attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates. Major groupings generally correlate with the more broadly based molecular classification. The updated system includes group 1 (class C) cephalosporinases; group 2 (classes A and D) broad-spectrum, inhibitor-resistant, and extended-spectrum β-lactamases and serine carbapenemases; and group 3 metallo-β-lactamases. Several new subgroups of each of the major groups are described, based on specific attributes of individual enzymes. A list of attributes is also suggested for the description of a new β-lactamase, including the requisite microbiological properties, substrate and inhibitor profiles, and molecular sequence data that provide an adequate characterization for a new β-lactam-hydrolyzing enzyme.

Hydrolysis of β-lactam antibiotics by β-lactamases is the most common mechanism of resistance for this class of antibacterial agents in clinically important Gram-negative bacteria. Because penicillins, cephalosporins, and carbapenems are included in the preferred treatment regimens for many infectious diseases, the presence and characteristics of these enzymes play a critical role in the selection of appropriate therapy.

β-Lactamase production is most frequently suspected in a Gram-negative bacterial isolate that demonstrates resistance to a β-lactam antibiotic. Due to more sophisticated molecular approaches than were previously available, it has become increasingly easy to obtain nucleotide sequences, with their deduced amino acid sequences, for the genes encoding these enzymes in β-lactam-resistant clinical isolates. By late 2009, the number of unique protein sequences for β-lactamases exceeded 890 (16; G. Jacoby and K. Bush, http://www.lahey.org/ /Studies/ [a site that contains additional literature and GenBank accession number references for β-lactamases in various functional groups]). Thus, it is important that a systematic process be established for tracking these enzymes.

Classification of β-lactamases has traditionally been based on either the functional characteristics of the enzymes (16, 55) or their primary structure (2). The simplest classification is by protein sequence, whereby the β-lactamases are classified into four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs (2, 3, 29, 46). Classes A, C, and D include enzymes that hydrolyze their substrates by forming an acyl enzyme through an active site serine, whereas class B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis. Although a structural approach is the easiest and least controversial way to classify such a diverse set of enzymes, a functional classification provides the opportunity to relate these varied enzymes to their clinical role, i.e., by providing selective resistance to different classes of β-lactam antibiotics. Functional groupings, admittedly, can be more subjective than structural classes, but they aid the clinician and laboratory microbiologist in correlating the properties of a specific enzyme with the observed microbiological resistance profile for a clinical isolate. Historically, functionality has been the overriding consideration in defining the role of a particular β-lactamase in the medical setting (55). Thus, it seems appropriate to continue to group these diverse enzymes according to their hydrolytic and inhibition properties.

UPDATED FUNCTIONAL CLASSIFICATION

Table 1 depicts an expanded version of the functional classification scheme proposed initially by Bush in 1989 (13) and expanded in 1995 (16). This table aligns structural and functional classifications as closely as possible, based on the available information in the public domain. New functional subgroups have been added to the scheme as a result of identification and expansion of major β-lactamase families in which variants continue to be identified on a regular basis (Table 2). As in the earlier functional classifications, enzymes were aligned based on their ability to hydrolyze specific β-lactam classes and on the inactivation properties of the β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam. A description of each of the functional groups follows.

Group 1 cephalosporinases. Group 1 enzymes are cephalosporinases belonging to molecular class C that are encoded on the chromosomes of many Enterobacteriaceae and a few other

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organisms (27). They are more active on cephalosporins than benzylpenicillin and are usually resistant to inhibition by clavulanic acid and active on cephapirins, such as cefoxitin. They have a high affinity for aztreonam (Kᵢ values as low as 1 to 2 nM), in contrast to the class A cephalosporinases (14, 15). A few have unusual properties, such as a lack of activity on cefoxitin (6), inhibition by clavulanate or tazobactam (5, 69), or production of resistance to cefotaxime but not ceftazidime (73). In many organisms, including Citrobacter freundii, Enterobacter cloacae, Serratia marcescens, and Pseudomonas aeruginosa, AmpC expression is low but inducible on exposure to \(\beta\)-lactams, such as amoxicillin, ampicillin, imipenem, and clavulanic acid (17, 27, 34, 67). In other organisms, including Acinetobacter baumannii and Escherichia coli, one or more components of the induction system are missing. When produced in large amounts, especially in a host with reduced \(\beta\)-lactam accumulation, group 1 enzymes can provide resistance to carbapenems, especially ertapenem (11, 28, 51). Plasmid-mediated group 1 enzymes in the CMY, ACT, DHA, FOX, MIR, and other families have been known since 1989 but are currently less common than plasmid-mediated subgroup 2e extended-spectrum \(\beta\)-lactamases (ESBLs) (27).

The new subgroup 1e enzymes are group 1 variants with greater activity against ceftazidime and other oxyimino-\(\beta\)-lactams as a result of amino acid substitutions, insertions, or deletions (44). They have been termed extended-spectrum AmpC (ESAC) \(\beta\)-lactamases and include GC1 in E. coli (45) and plasmid-mediated CMY-10 (33), CMY-19 (64), CMY-37 (1), and others (21). An AmpC variant from P. aeruginosa with increased activity against imipenem has also been recently described (57). Clinically significant resistance is most often conferred when the producing organism also has a porin mutation (36).

**Group 2 serine \(\beta\)-lactamases.** Functional group 2 \(\beta\)-lactamases, including molecular classes A and D, represent the largest group of \(\beta\)-lactamases, due primarily to the increasing identification of ESBLs during the past 20 years (Fig. 1). Subgroup 2a penicillinases represent a small group of \(\beta\)-lactamases...
TABLE 2. Major families of β-lactamases of clinical importance

<table>
<thead>
<tr>
<th>Enzyme family&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Functional group or subgroup</th>
<th>No. of enzymes&lt;sup&gt;b,c&lt;/sup&gt;</th>
<th>Representative enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMY</td>
<td>1, 1e</td>
<td>50</td>
<td>CMY-1 to CMY-50</td>
</tr>
<tr>
<td>TEM</td>
<td>2b, 2be, 2br</td>
<td>172</td>
<td>TEM-1, TEM-2, TEM-13</td>
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<tr>
<td></td>
<td>2b</td>
<td>12</td>
<td>TEM-3, TEM-10, TEM-26</td>
</tr>
<tr>
<td></td>
<td>2be</td>
<td>79</td>
<td>TEM-30 (IRT-2), TEM-31 (IRT-1), TEM-163</td>
</tr>
<tr>
<td></td>
<td>2br</td>
<td>36</td>
<td>TEM-50 (CMT-1), TEM-158 (CMT-9)</td>
</tr>
<tr>
<td></td>
<td>2ber</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>SHV</td>
<td>2b, 2be, 2br</td>
<td>127</td>
<td>SHV-1, SHV-11, SHV-89</td>
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<td>2b</td>
<td>30</td>
<td>SHV-2, SHV-3, SHV-115</td>
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<tr>
<td></td>
<td>2be</td>
<td>37</td>
<td>SHV-10, SHV-72</td>
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<td></td>
<td>2br</td>
<td>5</td>
<td></td>
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<td>CTX-M</td>
<td>2be</td>
<td>90</td>
<td>CTX-M-1, CTX-M-44 (Toho-1) to CTX-M-92</td>
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<tr>
<td>PER</td>
<td>2be</td>
<td>5</td>
<td>PER-1 to PER-5</td>
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<td>VEB</td>
<td>2be</td>
<td>7</td>
<td>VEB-1 to VEB-7</td>
</tr>
<tr>
<td>GES</td>
<td>2f</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>GES-2 to GES-7 (IBC-1) to GES-15</td>
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<tr>
<td>KPC</td>
<td>2f</td>
<td>9</td>
<td>KPC-2 to KPC-10</td>
</tr>
<tr>
<td>SME</td>
<td>2f</td>
<td>3</td>
<td>SME-1, SME-2, SME-3</td>
</tr>
<tr>
<td>OXA</td>
<td>2d, 2de, 2df</td>
<td>158</td>
<td>OXA-1, OXA-2, OXA-10</td>
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<tr>
<td></td>
<td>2d</td>
<td>5</td>
<td>OXA-11, OXA-14, OXA-15</td>
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<td>2de</td>
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<td>OXA-23 (ARI-1), OXA-51, OXA-58</td>
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<td>2df</td>
<td>48&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>IMP</td>
<td>3a</td>
<td>26</td>
<td>IMP-1 to IMP-26</td>
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<tr>
<td>VIM</td>
<td>3a</td>
<td>23</td>
<td>VIM-1 to VIM-23</td>
</tr>
<tr>
<td>INd</td>
<td>3a</td>
<td>8</td>
<td>IND-1, IND-2, IND-2a, IND-3 to IND-7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Enzyme families include those for which numbers have been assigned based on primary amino acid structures (G. Jacoby and K. Bush, http://www.lahey.org/Studies/).
<sup>b</sup> Compiled through December 2009.
<sup>c</sup> The sum of the subgroups in each family does not always equal the total number of enzymes in each family, because some enzyme numbers have been withdrawn, and some enzymes have not been assigned a functional designation by the investigators who provided the amino acid sequence.
<sup>d</sup> GES-1, unlike other members of the GES family, has little detectable interaction with imipenem (49).
<sup>e</sup> Nine clusters of OXA carbapenemases with their individual members have been designated in Table 6 in reference 52.

with a relatively limited spectrum of hydrolytic activity and are the predominant β-lactamases in Gram-positive cocci, including the staphylococci (30) and occasionally enterococci (74). These enzymes preferentially hydrolyze benzylpenicillin and many penicillin derivatives, with poor hydrolysis of cephalosporins, carbapenems, or monobactams at rates usually <10% those for benzylpenicillin or ampicillin. An exception is the facile hydrolysis of nitrocefin by the subgroup 2a enzymes.

![FIG. 1. Increase in numbers of group 1, 2, and 3 β-lactamases from 1970 to 2009. Shown are group 1/class C cephalosporinases (black), group 2/class A and class D β-lactamases (blue), and group 3/class B metallo-β-lactamases (red).](image-url)
Subgroup 2a β-lactamases are inhibited by clavulanic acid and tazobactam with 50% inhibitory concentrations (IC\textsubscript{50}) of usually <1 µM, assuming at least 5 min of preincubation of enzyme and inhibitor. The majority of these enzymes are chromosomal, although some staphylococcal penicillinases are plasmid encoded. This subgroup, which numbered 20 in 1995, has increased to only 25 in 2009. This may be because a true penicillinase does not cause significant clinical resistance for those β-lactams in predominant current use.

Subgroup 2b β-lactamases readily hydrolyze penicillins and early cephalosporins, such as cephaloridine and cephalothin, and are strongly inhibited by clavulanic acid and tazobactam. They include the TEM-1, TEM-2, and SHV-1 enzymes, the most common plasmid-mediated β-lactamases identified in the 1970s and early 1980s (38, 58). Since the 1995 β-lactamase compilation (16), at least 9 TEM and 29 SHV 2b enzymes have been described (G. Jacoby and K. Bush, http://www.lahey.org /Studies/) often in the course of characterizing other β-lactamases in unusually resistant clinical isolates.

Subgroup 2be comprises ESBLs. These broad-spectrum enzymes retain the activity against penicillins and cephalosporins of subgroup 2b β-lactamases and in addition hydrolyze one or more oximino-β-lactams, such as cefotaxime, ceftazidime, and aztreonam, at a rate generally >10% that of benzylpenicillin. The first and largest subset of subgroup 2be was derived by amino acid substitutions in TEM-1, TEM-2, and SHV-1 that broadened their substrate spectrum at a cost of lower hydrolyzing activity for benzylpenicillin and cephaloridine (53). TEM and SHV ESBLs have been joined by the functionally similar but more rapidly proliferating CTX-M enzymes that are related to chromosomally determined β-lactamases in species of \textit{Klebsiella} (8). As the name implies, most (but not all) CTX-M enzymes hydrolyze cefotaxime more readily than ceftazidime. Many hydrolyze cefepime as well. Unlike TEM or SHV ESBLs, CTX-M enzymes are inhibited by tazobactam at least an order of magnitude better than by clavulanic acid (8, 65). Finally, there are less common ESBLs unrelated to TEM, SHV, or CTX-M, including BEL-1, BES-1, SFO-1, TLA-1, TLA-2, and members of the PER and VEB enzyme families. Characteristically, subgroup 2be β-lactamases remain sensitive to inhibition by clavulanic acid, a feature used in their detection by clinical laboratories (19).

Subgroup 2br enzymes are broad-spectrum β-lactamases that have acquired resistance to clavulanic acid (IC\textsubscript{50} ≥ 1 µM) and related inhibitors while retaining a subgroup 2b spectrum of activity. Currently 36 of the 135 functionally characterized TEM enzymes have this property and include enzymes such as TEM-30 and TEM-31 (IRT-2 and IRT-1, respectively), as well as 5 of the corresponding functionally characterized 72 SHV enzymes (e.g., SHV-10). No CTX-M β-lactamase demonstrates this characteristic to date (G. Jacoby and K. Bush, http://www.lahey.org/Studies/).

Subgroup 2ber includes TEM enzymes that combine an extended spectrum with relative resistance to clavulanic acid inhibition. Although all have clavulanic acid IC\textsubscript{50} greater than that of TEM-1 (0.08 µM), for some 2ber enzymes the increase in clavulanic acid resistance is modest. They have also been termed CMT (complex mutant TEM) β-lactamases and include TEM-50 (CMT-1) (56, 61).

Subgroup 2c penicillinases are characterized functionally by their ability to hydrolyze carbencillin or ticarcillin at least 60% as rapidly as benzylpenicillin, with cloxacinil or oxacillin hydrolyzed at rates less than half those for benzylpenicillin (16). These penicillinases are generally easily inhibited by clavulanic acid or tazobactam, most often with IC\textsubscript{50} of <1 µM. Because carbencillin is an antibiotic that is currently used infrequently and is not tested for stability by most investigators, only a few new 2c β-lactamases have been described over the past decade (18, 40, 47).

Subgroup 2ce contains the recently described extended-spectrum carbencillinase RTG-4 (CARB-10) with expanded activity against cefepime and cefpirome (50).

Subgroup 2d includes β-lactamases distinguished by their ability to hydrolyze cloxacillin or oxacillin at a rate of >50% that for benzylpenicillin and hence are known as OXA enzymes. Carbencillin may also be readily hydrolyzed. Most members of the OXA family, however, are currently identified according to their conserved amino acid motifs rather than according to function. Many β-lactamases in this subgroup are inhibited by NaCl; they typically have clavulanic acid IC\textsubscript{50} of ≥1 µM. OXA-related enzymes now comprise the second largest family of β-lactamases (Table 2).

In the new subgroup 2de are cloxacillin- or oxacillin-hydrolyzing enzymes with an extended spectrum that includes oximino-β-lactams but not carbapenems. The majority of 2de enzymes are derived from OXA-10 by between 1 and 9 amino acid substitutions and include enzymes such as OXA-11 and OXA-15. They have most often been found in Turkey and France in isolates of \textit{P. aeruginosa}, where the level of resistance they produce is higher than that in \textit{E. coli} (9). Resistance to ceftazidime is usually more pronounced than resistance to cefotaxime or aztreonam. However, organisms producing a few oxacillinases, such as OXA-1 or OXA-31, may be susceptible to ceftazidime but resistant to cefepime (4).

New subgroup 2df β-lactamases are OXA enzymes with carbapenem-hydrolyzing activities. They appear most frequently in \textit{Acinetobacter baumannii} and are usually produced by genes that are located on the chromosome (66), although plasmidborne OXA-23 and OXA-48 enzymes have been identified in the \textit{Enterobacteriaceae} (16, 48). The 2df enzymes have been divided into nine clusters according to amino acid homologies (52, 59, 62, 66). Although subgroup 2df enzymes are defined functionally according to their ability to hydrolyze cloxacillin or oxacillin, only a few subgroup 2df enzymes have been tested using these substrates (66). Of those tested, only OXA-50 had no detectable oxacillin hydrolysis. The characterized OXA carbapenemases have weak hydrolytic activity for carbapenems, demonstrated by \textit{k}_{\text{cat}} values for imipenem and meropenem that are generally ≥1 s\textsuperscript{-1}, with imipenem hydrolyzed faster and more efficiently than meropenem. These rates compare to much higher \textit{k}_{\text{cat}} values for benzylpenicillin or oxacillin, substrates that were usually hydrolyzed at least 40- to 50-fold faster than the carbapenems (66). Although the producing organisms are generally highly resistant to carbapenems, \textit{E. coli} transformants or transconjugants that produce these enzymes are usually susceptible to the carbapenems (66). The enzymes, and their producing organisms, are typically unresponsive to inhibition by clavulanic acid.

Characteristics of the subgroup 2e cephalosporinases include the ability to hydrolyze extended-spectrum cephalospor-
rins and to be inhibited by clavulanic acid or tazobactam. The inducible, chromosomal cephalosporinases in the Proteae often belong to this subgroup. They can be confused with the group 1 AmpC enzymes or with ESBLs because they may appear in similar organisms and with comparable resistance profiles. Subgroup 2e enzymes can be differentiated from AmpC enzymes by their poor affinity for aztreonam, in contrast to the enzymes by their ability to hydrolyze carbapenems, but some serine

**DISCUSSION**

Ideally, a strong structure-function relationship should be observed among the various β-lactamase groupings. The optimal outcome would result in a categorization scheme that placed all β-lactamases into a single classification grid aligning both structure and function. This kind of relationship is beginning to be accomplished with the MBLs. However, at the current time, many β-lactamases are described only on the basis of a protein sequence, with little functional description. Therefore, a set of criteria has been proposed for the description of a new β-lactamase, including both structural and functional information. Table 3 outlines the information needed for a full characterization.

The reasons for β-lactamase diversity are many. At least the serine-based varieties are ancient enzymes, estimated to have been evolving for more than 2 billion years starting from a time before the divergence of bacteria into Gram-negative and Gram-positive varieties (25). They are found in bacteria living in a wide variety of environments and hence are subject to different selective pressures. They are well-studied enzymes
that have attracted the attention of many investigators in the 70 years since they were first described. They are adaptable enzymes that have evolved to avoid being crippled by compounds intended as inhibitors and to attack β-lactam antibiotics designed to resist their action (39). Finally, bla genes have profited from the many mechanisms for horizontal gene transfer between bacteria to spread to new hosts and to become part of multiresistance plasmids now common in clinical isolates with resulting promiscuous dissemination. Given these many factors, it is a safe prediction that β-lactamases will continue to evolve, as will classification schemes needed for their description.

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REFERENCES


Karen Bush received her B.A. from Monmouth College (IL) and her Ph.D. in Biochemistry from Indiana University. She was involved in antibiotic Discovery and Development programs at E. R. Squibb & Sons, Bristol-Myers Squibb, American Cyanamid/Lederle/Wyeth, Astra, and, most recently, Johnson & Johnson Pharmaceutical Research & Development, where she was a Distinguished Research Fellow and the Microbiology Team Leader in Preclinical Anti-Infective Research. She is semiretired and is currently an Adjunct Professor of Biology at Indiana University Bloomington. Her primary interests have been related to antiinfective drug discovery based on attempts to counteract antibiotic resistance, particularly with respect to β-lactams. She began studying β-lactamases in 1977 and still retains an active interest in tracking the new enzymes that continue to be described.

George A. Jacoby trained at Harvard Medical School, the National Institutes of Health, the National Institute for Medical Research at Mill Hill, and Massachusetts General Hospital, where he was a consultant in the Infectious Disease Unit for 25 years before moving in 1993 to head the Infectious Disease Department at the Lahey Clinic. He retired from clinical work in 2002 and now heads a research lab at Lahey, where he works on bacterial resistance to antimicrobial agents, especially quinolones and β-lactams. He is an Associate Professor of Medicine at Harvard Medical School and began working with β-lactamases in 1963.