Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Antimicrobial Therapeutics Reviews*

Proliferation and significance of clinically relevant β-lactamases

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Inactivation of β-lactam antibiotics by β-lactamases in bacterial infections is associated with some of the most serious infectious disease issues that are currently encountered. The evolution of unique β-lactamases has resulted in more than 1,300 distinct enzymes that have been identified in natural clinical isolates. Of these enzymes, the most deleterious β-lactamases are the extended-spectrum β-lactamases, or ESBLs, that hydrolyze most penicillins and cephalosporins, and the carbapenemases that may inactivate all β-lactam classes of drugs. The most prominent ESBLs worldwide are the CTX-M-14 and CTX-M-15 enzymes. Among enzyme families, the TEM and OXA β-lactamases exhibit the greatest number of variants. The broad groups of carbapenemases are particularly treacherous, especially the KPC serine carbapenemases and the NDM family of metallo-β-lactamases, both of which appear in multidrug-resistant Gram-negative pathogens that are often resistant to most classes of antibiotics. Although new β-lactamase inhibitor combinations are being investigated as a means of controlling infections caused by these organisms, additional approaches are sorely needed.

Keywords: β-lactamase; ESBL; carbapenemase; β-lactam antibiotic; resistance

Introduction

Any treatise on cell wall–active antibiotics must contain a section on β-lactam antibiotics, and any discussion of β-lactam antibiotics must include β-lactamases, the enzymes that have driven the discovery and development of perhaps the most widely used class of antibiotics. The first reported laboratory observation describing a β-lactamase was from Abraham and Chain in 1940,[1] a year before penicillin was used to treat an infected patient.[2] However, enzymatic inactivation of β-lactams existed long before the antibiotic era began. In 2005, Fevre *et al.* calculated that *bla* genes in *Klebsiella oxytoca* originated as long ago as 100 million years.[3] Recently, direct confirmation of the age of β-lactam–inactivating activity in ancient samples has been reported. In a survey of microorganisms collected from a 4 million-year-old isolated cave, 62% of the Gram-positive strains exhibited β-lactam–destroying activity, as evidenced by the loss of a zone of inhibition following incubation with both penicillins and cephalosporins.[4] Fragments of genetic material encoding or derived from β-lactamases have been recovered from permafrost samples dating back 30,000 years.[5] Thus, these early enzymes, or closely related precursors, were present in natural sources where β-lactam–containing molecules were most likely produced, thereby providing a protective environment for the β-lactamase–producing organism.[6–7]

However, it is undisputed that the prevalence and variety of β-lactamases have multiplied dramatically with the therapeutic use of β-lactam antibiotics.[8] Several comprehensive reviews have recently been published describing β-lactamases in general,[9–11] as well as specialized reviews of AmpC cephalosporinases,[12] extended-spectrum β-lactamases (ESBLs),[13] inhibitor-resistant enzymes,[14] and carbapenemases[15–18] (see also Ref. 19), with the last group of enzymes attracting perhaps the largest amount of current interest. The scope of this review is to provide an abbreviated description of β-lactamases, with specific reference to the

doi: 10.1111/nyas.12023

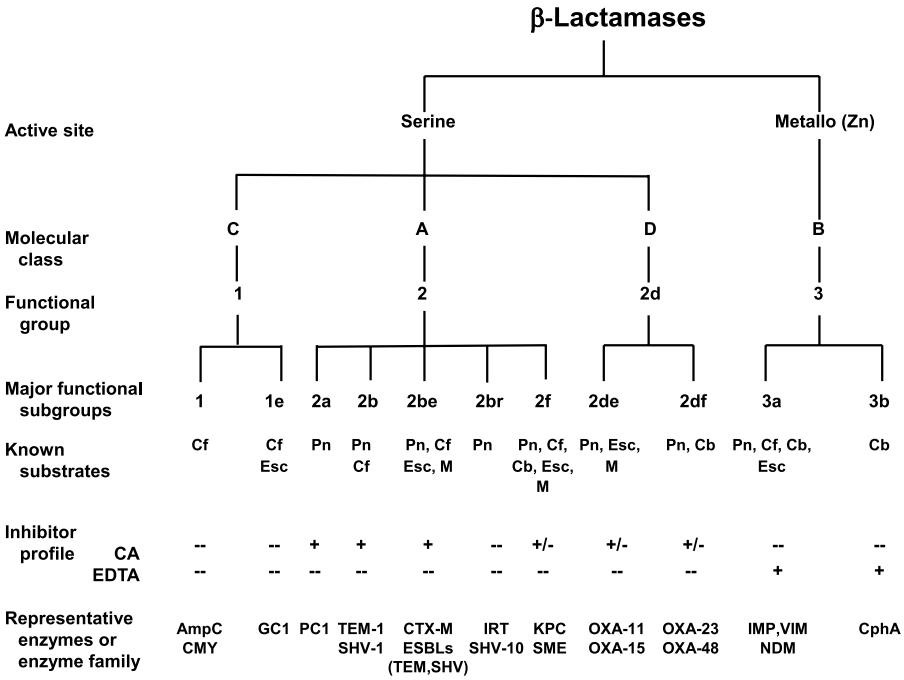

Figure 1. Molecular and functional features of the major groups of β-lactamases. Molecular classes are based on the nomenclature initiated by Ambler.[21] Functional groupings with substrate and inhibitor profiles are based on the nomenclature initially proposed by Bush[22] and expanded in 1995 (Ref. 23) and 2010 (Ref. 11). Subgroups that include the most prevalent enzymes or enzyme families are featured, based on recent epidemiological studies.[11] Substrate and inhibitor profiles were based on Refs. 11, 14, and 20. Abbreviations: Cb, carbapenems; Cf, early marketed cephalosporins; CA, clavulanic acid; EDTA, ethylenediaminetetraacetic acid; Esc, expanded spectrum cephalosporins; M, monobactam; Pn, penicillins. Figure modified from Ref. 24.

explosion and relevance of major new β-lactamases. It is hoped that this review will provide the foundation for other articles in the volume that deal in depth with specific β-lactamases and novel antimicrobial therapeutics designed to restrain their hydrolytic activities.

Nomenclature and classification

Numerous articles have been written describing β-lactamase classifications and nomenclature.[9–11] These enzymes have been lumped and split by various authors, using either molecular or functional characteristics of the enzymes to try to make sense of more than 1,300 uniquely occurring β-lactamase variants.[20] Functional and molecular classification schemes have been aligned as shown in Figure 1, with a clear separation of two molecular classes: the metallo-β-lactamases (MBLs), those that require zinc for β-lactam catalysis, and the serine β-lactamases, those β-lactamases that catalyze β-lactam hydrolysis via an acyl enzyme formed between substrate and an active site serine. Each molecular class has its own characteristics based on identifying features of the primary sequence of each β-lactamase.[21,25–26] Thus, one may categorize a new β-lactamase into a distinctive molecular class as soon as the sequence has been determined, an exercise that has generally become trivial for the β-lactamase laboratory. However, the function of the enzyme is the more critical designation for the clinician who wants to know what substrates (antibiotics) may be spared from hydrolysis so that appropriate therapy can be prescribed.

As seen in Figure 1, the four major β-lactamase classes, A–D, can be separated into functional groups that have distinguishing substrate and inhibitor profiles. Molecular class C is associated with two functional subgroups, 1 and 1e, both of which hydrolyze early cephalosporins efficiently with little effect of β-lactamase inhibitors; however, enzymes in subgroup 1e, sometimes named the ESAC (extended spectrum AmpC) β-lactamases, exhibit enhanced hydrolysis of cephalosporins with aminothiazoleoxime side chains.[27]

Molecular classes A and D, or group 2 β-lactamases, include those serine β-lactamases with the broadest, and largest, functional groups of enzymes, with multiple subgroups that overall demonstrate hydrolysis of all β-lactam antibiotics across the class. Two of the most important among the class A enzyme subgroups are the notable ESBLs (subgroup 2be) and serine carbapenemases (subgroup 2f) that are creating havoc within the global infectious disease community by hydrolyzing all classes of β-lactams.[28] Most purified class A β-lactamases are considered to be inhibited by clavulanic acid, although their response to the common β-lactamase inhibitor combinations such as amoxicillin-clavulanic acid may be variable in whole cell testing. Class D β-lactamases are often underappreciated, but cause serious resistance in organisms like *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.[29]

Another set of β-lactamases of considerable clinical concern is the molecular class B family, or functional group 3 β-lactamases, that can hydrolyze all β-lactams except for the monobactams. These enzymes are especially deleterious, as they are not inhibited by any of the current β-lactamase inhibitor combinations,[30] including agents in late stages of clinical development (see Palzkill[19] and Shlaes,[31] this volume). Although monobactams do not undergo hydrolysis by MBLs, it should be noted that they are hydrolyzed by the group 2f serine carbapenemases.

β-Lactamase nomenclature is a bit daunting for non-β-lactamase specialists. Not only are there formal molecular and functional classification schemes as described above, but also the names ESBLs and carbapenemases are used to span different sets of enzymes in different classification schemes. Initially ESBLs were variants of the common group 2b penicillinases (TEM and SHV) that acquired the ability to hydrolyze cefotaxime or ceftazidime due to a limited number of point mutations.[32] Today, ESBLs include not only the TEM and SHV families of group 2be ESBLs, but also the group 1e cephalosporinases with expanded substrate hydrolysis profiles, the ubiquitous CTX-M family of ESBLs, and the cephalosporin-hydrolyzing group 2de OXA enzymes.[33]

Similarly, carbapenemases, as noted above, include both serine carbapenemases in functional groups 2f and 2df and the group 3 MBLs. Although carbapenemases, for the most part, also hydrolyze many expanded-spectrum cephalosporins, it is important that they retain the carbapenemase name. Infections caused by traditional ESBL-producing pathogens can often be treated successfully with a carbapenem,[34–35] whereas carbapenem monotherapy to treat infections caused by carbapenemase-producing organisms is not generally recommended.[36–37] Carbapenemases and ESBLs should, therefore, be viewed distinctly from a clinical perspective and should preserve their distinguishing names.

β-Lactamase evolution

Penicillinases from the Gram-positive cocci were the first β-lactamases to be studied in the laboratory. These enzymes arose in staphylococcal clinical isolates rapidly after penicillin began to be used in the 1940s.[38] As new β-lactam–containing molecules were introduced into clinical practice, new β-lactamases were identified that could hydrolyze each of these novel antibiotics;[39] it was as if the enzymes were simply sitting in the environment waiting for their favorite substrate to appear. Shortly following the introduction of expanded spectrum cephalosporins, such as cefotaxime and ceftazidime, extended-spectrum β-lactamases (ESBLs) containing point mutations began to be identified in clinical isolates that were resistant to these new agents.[40] As β-lactamase inhibitor combinations such as amoxicillin-clavulanic acid and ampicillin-sulbactam were used more frequently, inhibitor resistant TEM β-lactamases (IRTs) with point mutations began to appear, conferring resistance to all the marketed inhibitor combinations.[41] The emergence of these variants provides a clear example of adaptive evolution occurring in real time. Carbapenems, the most potent and broadest spectrum β-lactam antibiotics, did not remain unscathed. β-Lactamases that included broad-spectrum carbapenems in their substrate profiles could also hydrolyze almost all other β-lactam classes; exceptions were enzymes in the subgroup of zinc-containing β-lactamases that had a high selectivity for carbapenem hydrolysis and little hydrolysis of other β-lactams.[16]

Identification of new β-lactamases has been increasing proportionally to the increased ease, and decreased cost, of genetic sequencing. The number of unique β-lactamases described from clinical isolates is estimated to be at least 1,300, based on earlier

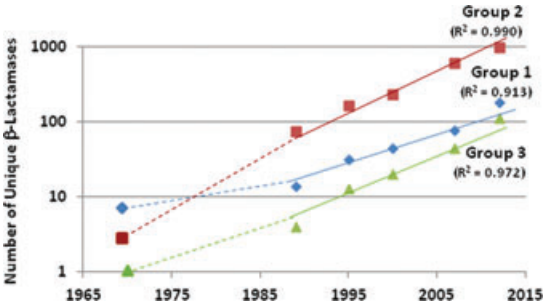

Figure 2. Exponential increase in identification of naturally occurring β-lactamases from 1989 to 2012. R^2 values were determined based on an exponential analysis of enzyme numbers from 1989 (Ref. 22) to 2012 sources.[9,20,42] The dotted lines were extrapolated from 1970 to 1989.

Table 1. Number of variants in major enzyme families (10 or more members) with transferable β-lactamases identified from 1961 to 2012[3,9,20,42]

Enzyme type	Molecular class	1961	2000	2012
All TEMs	A	0	86	197
All SHVs	A	0	26	168
CTX-M	A	0	9	134
OPK	A	0	0	36
K-OXY	A	0	6	28
LEN	A	0	1	26
GES	A	0	1	22
CARB	A	0	6	14
KPC	A	0	0	12
VIM	B	0	2	37
IMP	B	0	3	42
CMY	C	0	6	95
ACT	C	0	2	19
All OXAs	D	0	28	255

compilations of enzymes,[23] literature reviews, and a continuous updating of the two major websites that enumerate the numbers of variants in β-lactamase families.[20,42] When the numbers of enzymes are enumerated according to the functional group, there is an exponential increase in enzymes identified in each of the three major β-lactamase groups from 1989 through 2012, as seen in Figure 2. The largest numerical increase is due to the proliferation of group 2 β-lactamases, especially the group 2be ESBLs and group 2d OXA enzymes. Increases in the number of β-lactamases per family (including only those families with at least ten members in November 2012) are shown in Table 1, focusing on enzymes that may be transferable among species. Of the large enzyme families from molecular class A, all contain β-lactamases with penicillin and cephalosporin hydrolyzing capabilities, with ESBLs represented in all the first seven families. Some members of the GES family, as well as all the KPC enzymes, possess carbapenemase activity beyond the hydrolysis of the penicillins and cephalosporins.[43–44]

The β-lactamase families in Table 1 comprise closely related enzymes that have been reported to be encoded on mobile elements, thus allowing for the rapid spread of the parental gene. Under antibiotic pressure, mutations in the parental enzyme have been selected that allow the producing organisms to survive multiple onslaughts of antimicrobial agents. In Figure 3, the prevalence of each major enzyme family, or related families, is shown as the percentage of the total number of β-lactamases in the literature at that date. In 1989, over half of the β-lactamases known in the literature did not belong to a major molecular family. Today, the major β-lactamase families or groups of related families comprise more than 80% of the known β-lactamases. During the past 40 years, the percentage of TEM β-lactamases peaked in 2000 when 25% of all known β-lactamases were derived from TEM-1, many of them with ESBL activity. SHV β-lactamases and CTX-M ESBLs continue to increase in numbers, as do the MBL families. Of all the β-lactamases, the CTX-M and OXA families of enzymes have had the most rapid expansion over the past dozen years. The CTX-M β-lactamases, first identified and sequenced in the early 1990s,[45–46] increased from 3% to 10% of the known β-lactamases in the past 12 years. The OXA β-lactamases, a diverse group of enzymes with substrate profiles that may include primarily penicillins, or penicillins and expanded-spectrum cephalosporins, or even carbapenems, are now represented with 20% of the total number of reported β-lactamase sequences.[20]

Discussion

Several reasons can be proposed for the skyrocketing increase in β-lactamases. In clinical isolates from the 1970s, usually only one or two β-lactamases could be detected.[47] Today, the standard is for hospital-acquired pathogens to produce multiple β-lactamases, with reports of as many as

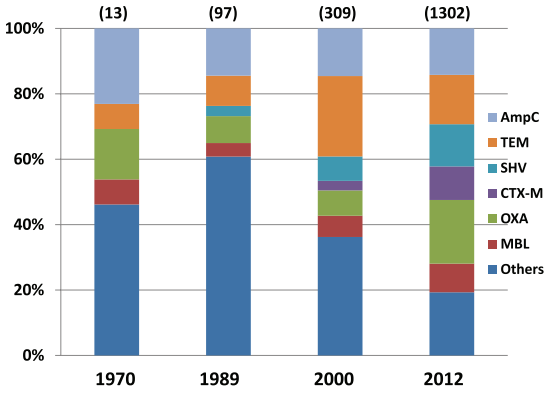

Figure 3. The frequency with which major β-lactamase families have occurred with respect to the total number of enzymes identified from natural sources.[19,42] The total number of identified β-lactamases for each of the designated years is shown on the top of each column.

eight different enzymes in a single strain,[48] indicating that new β-lactamase genes continue to be added to plasmids or gene cassettes in an organism that already contained *bla* genes. With a larger population of diverse nucleotide sequences in the environment, the probability of a functional mutation is increased, especially under the pressure of antibiotics that are used both appropriately and inappropriately.

Although gene sequencing is much more facile and inexpensive than ever before, thereby leading to more sequences that are available for analysis, it is also likely that more pathogens are being identified as resistant and are requiring additional follow-up. This is partly due to the lowering of carbapenem and cephalosporin breakpoints by the Clinical Laboratory Standards Institute (CLSI),[49] resulting in antibiograms with higher percentages of resistance.[50] Thus, more isolates are undergoing secondary testing that potentially can unveil a new β-lactamase.[51] Detection of these resistant strains is important, not only to the clinical or molecular microbiologist, but also to the clinicians who are able to initiate infection control policies as rapidly as possible,[10,52] and, perhaps, contain a potential outbreak situation at an early stage.[53–54]

Infectious disease physicians and clinical microbiologists have identified ESBLs and carbapenemases in Gram-negative bacteria as perhaps the most critical β-lactamase issues.[55–56] ESBLs began to be a problem 25 years ago, and continue to appear in multidrug-resistant enteric bacteria. The CTX-M-14 and CTX-M-15 enzymes have become the dominant ESBLs worldwide,[9,57] especially in cephalosporin-resistant *E. coli* and *K. pneumoniae* isolates that may be susceptible only to carbapenems, colistin or tigecycline, or possibly temocillin.[31,58] Carbapenemases, both serine and metallo-enzymes, are major problems worldwide.[59] The KPC serine carbapenemases have become entrenched in many hospitals in the Americas, southern Europe, and China[60] and have proven to be very difficult to eliminate from hospitals, even with stringent infection-control policies.[61] The MBL NDM-1 has generated a lot of scientific and political interest[62–63] and has become one of the most widespread β-lactamases in highly drug-resistant Gram-negative pathogens throughout northern Europe and the Asia Pacific region.[63] KPC and NDM carbapenemases generally appear in multidrug resistant organisms that are resplendent with additional antibiotic resistance determinants, thus making their eradication more difficult.

The need for new approaches to contain the proliferation of new multidrug-resistant β-lactamase–producing pathogens is urgent. Various proposals have been made about possible avenues to new drugs, including unexploited β-lactamase inhibitor combinations (see Ref. 31), or other creative approaches such as novel "eco-evo" drugs[64] that will consider the impact of such interventions on the ecology of the environment, leading, perhaps to slower evolution of resistant entities. Because of the ubiquity of β-lactamases, however, their continued expansion will place unforeseen demands on the infectious disease community that must continue to explore novel therapeutic approaches.

Conflict of interest

Retirement income was received from Johnson & Johnson, Bristol-Myers Squibb, and Pfizer. Research funding was received from Cubist Pharmaceuticals.

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