Chapter 12
The Importance of β-Lactamases to the Development of New β-Lactams

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1 Introduction

β-Lactams are considered to be among the safest, most efficacious, and most widely prescribed antibiotics for the treatment of bacterial infections. Their therapeutic use began with the introduction of benzylpenicillin (penicillin G) during World War II (1, 2), and continues with the development of newer cephalosporins and carbapenems for antibiotic-resistant infections. These agents act by inhibiting bacterial cell wall synthesis, as a result of their strong covalent binding to essential penicillin binding proteins (PBPs) that catalyze the last steps of cell wall formation in both Gram-positive and Gram-negative bacteria (3, 4). However, resistance to these agents has been a major concern to all who use, or have used, β-lactams therapeutically.

Resistance mechanisms associated with β-lactams include modification or acquisition of a low-affinity bacterial target (i.e., a PBP); inactivation of the antibiotic by β-lactamases; and decreased concentration of the β-lactam at the site of the target, due to increased efflux or decreased entry of the drug (5–7). In Gram-positive bacteria, especially staphylococci, low-affinity PBPs now represent the most important β-lactam resistance mechanisms (8), in contrast to the selection of penicillin-resistant staphylococci due to increasing numbers of strains that began producing penicillinases soon after the therapeutic introduction of penicillin G (9, 10). In Gram-negative bacteria, the appearance of β-lactamases with increased catalytic efficiency for β-lactams of multiple classes has remained the major resistance mechanism (11). However, the combination of increased β-lactamase production with decreased β-lactam concentrations within the periplasm results in perhaps the most effective β-lactam resistance mechanism (12).

Because the most common β-lactam resistance mechanism overall is β-lactamase production, it is no coincidence that the emergence of new β-lactamases can be correlated with the introduction of new β-lactam molecules into clinical practice. In this chapter, the origin and hydrolytic action of β-lactamases will be described, together with the most common classification schemes. In addition, the identification of new enzymes will be shown to have a close relationship with recently developed antibacterial drugs and their increased use as therapeutic agents.

2 Hydrolytic Activity

All PBPs and β-lactamases interact with β-lactam antibiotics in reactions that result in the hydrolysis of the antibiotic to form an inactive chemical substance no longer possessing antibacterial activity. The reaction can proceed by at least two separate mechanisms, dependent upon the characteristics of the active site of individual enzymes. All known PBPs react with β-lactams via a conserved active-site serine (13). However, β-lactamases belong to families of enzymes that can utilize either an active-site serine or a metallo (zinc) ion to mediate hydrolysis (14).

PBPs and serine β-lactamases hydrolyze β-lactams by forming an acyl enzyme complex via the active-site serine residue (see Fig. 1). In this scheme, acylation and deacylaation occur at different rates for the two sets of enzymes, with their classification as a PBP or β-lactamase based on the rates at which each step occurs. Thus, for PBPs, acylation may be rapid, but deacylation must be quite slow, to allow the enzyme to remain inactive during at least one cell division cycle (15, 16). For β-lactamases, both acylation and deacylation are generally rapid, with $k_{cat}$ values approaching the limit for a diffusion-controlled reaction (17, 18).
3 β-lactamase Origins

Much speculation abounds concerning the origin of β-lactamases. They have been reported to be a part of the bacterial armamentarium for centuries before the introduction of β-lactams into clinical practice (19), with claims of β-lactamase identification in bacterial samples analyzed from soil clinging to plants from the seventeenth century (20). Although most of the newer β-lactamases are plasmid-encoded, many bacteria have β-lactamase genes incorporated into their chromosomes, thus endowing them with a form of permanence as they are passed from one generation to the next. The appearance of β-lactamase genes on plasmids, in fact, appears to be a fairly recent occurrence; studies of culture collections from 1917 to 1954 showed that the same conjugative plasmids existed in the older strains, but β-lactam-inactivating activities were not associated with these plasmids (21, 22). Datta and Hughes concluded that plasmid-encoded resistance determinants were introduced by transposons that accumulated in previously existing plasmids.

If β-lactamases have been a part of the physiology of bacteria for thousands of years, the question remains as to their origin and their reason for existence. Serine β-lactamases most likely evolved from PBPs, as there are many notable similarities between the two sets of enzymes. Not only do they catalyze the same enzymatic reactions using conserved amino acids, but they have also been found to exhibit very similar three-dimensional structures (14). Even the metallo-β-lactamases appear to be folded in a spatial pattern that resembles PBPs and serine β-lactamases.

In the few organisms that do not produce traditional β-lactamases, notably Streptococcus pneumoniae (23) and Helicobacter pylori (24), resistant PBPs may play that role through a more rapid deacylation reaction than for other PBPs. This has been reported for S. pneumoniae, where resistant PBP2x variants demonstrate from 70- to 110-fold increases in deacylation rates, compared to the corresponding PBP from a susceptible strain (25, 26). In amoxicillin-resistant H. pylori, several surrogate β-lactam-hydrolyzing enzymes have been identified: a) a mutant form of PBP 1A (24) and b) HpcB, an unusual cysteine-rich protein that may play a role as a PBP from a new structural class (27).

Because β-lactams are prevalent in soil samples that contain β-lactam-producing actinomycetes and bacteria (28, 29), it is an obvious suggestion that β-lactamases exist in bacteria to provide an ecological advantage to the β-lactamase-producing cells (30). A soil bacterium that can out-compete its bacterial neighbors by destroying potent β-lactams secreted into the soil would have a distinct evolutionary advantage (31). Notably, many of the first “penicillinases” that were described in the literature in the 1940s were from soil organisms, e.g., Nocardia spp., Streptomyces spp., and Bacillus spp. (32).

However, others argue that β-lactams in the soil would not diffuse far enough to be a threat to surrounding bacteria (33). To ensure survival, bacteria generally conserve resources for only the most critical functions. Thus, when bacteria produce large amounts of β-lactamase in preference to other proteins, there must be a reason other than protection against natural predators. Investigators such as A. Medeiros believe that β-lactamases instead have a major, but poorly understood, role in bacterial physiology (34), possibly by serving to regulate cell growth. Although this latter argument cannot be dismissed lightly, the proliferation of β-lactams in soil isolates suggests that a protection mechanism may have been an important selecting factor in bacterial physiology.

4 Classification Schemes

Classification schemes for β-lactamases have been described since 1970, when eight β-lactamases were separated into categories (35). For the most part, these schemes have focused on differences in enzymes that appear in Gram-negative bacteria, where increased numbers of both chromosomal- and plasmid-encoded enzymes contribute to resistance. There has been less interest in the β-lactamases in Gram-positive bacteria, primarily because the enzymes in Gram-positives that contribute to clinical resistance have been mainly the staphylococcal penicillinases, a rather homogenous set of enzymes that have also appeared.