



Review

Mechanistic and Clinical Aspects of β -Lactam Antibiotics and β -Lactamases

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Abstract. Bacterial infections have been a major cause of concern in the recent years due to the emergence of drug resistance strains and inability of the current therapeutic regimens to treat these infections in certain cases. β -Lactam antibiotics have been drugs of choice since the introduction of penicillin. These drugs inhibit bacterial cell-wall-synthesizing enzymes, the so-called penicillin-binding proteins (PBPs) selectively, thus providing an effective strategy for treatment of the bacterial infections. Significantly, bacteria have developed resistance mechanisms to neutralize the antibiotic action of β -lactam drugs. β -Lactamases are enzymes that hydrolyze the β -lactam moiety of these drugs, rendering them inactive. This is the primary mechanism of resistance to this class of antibiotics. There are 255 known β -lactamases to date and the continued use of β -lactams may select for newer variants yet. A discussion of the roles of these enzymes in the manifestation of the drug-resistant phenotype and their implications for pathogenicity of clinical strains of bacteria is presented.

Key words: β -lactams; β -lactamases; antibiotic; drug resistance; bacterial infection.

Bacterial infections have been the focus of concern in the recent years due to the rising incidence of multiple drug-resistant pathogens²². Bacteria most commonly cause respiratory tract infections, urinary tract infections, skin infections, gastrointestinal tract infections, and surgical wound infections. It is estimated that more than 400 species of bacteria exist symbiotically in the gastrointestinal tract of humans⁴². While many of these organisms are nonpathogenic and live in symbiosis with their human host, in certain opportunistic conditions they may turn against the host causing severe infections. The discovery of penicillin – the first β -lactam to be introduced into the clinic – was a boon to the clinicians in the middle of the twentieth century for treatment of bacterial infections, especially those caused by Gram-positive bacteria. In the late 1960s it was generally thought that bacterial infections were not

a concern anymore. However, this perception has changed most dramatically in the past few years in the light of the emergence of resistance virtually to every antibacterial agents in the clinic. The history of developments in the β -lactam antibiotics over the past 50 years and how the advent of clinical resistance to these antimicrobials has driven the field forward, have been reviewed recently by BUSH and MOBASHERY⁸. Furthermore, several recent articles on the problem of antibiotic resistance, and the urgent need to address this issue, have appeared in the literature^{1, 12, 22, 31, 44}.

β -Lactam antibiotics are currently the most commonly used antibiotics because of their safety and selectivity in treatment of infections caused by both the Gram-negative and Gram-positive pathogens. Because of the versatility and common use of β -lactam antibiotics over the past 50 years, bacteria have learned to

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counter the effect of these drugs. Resistance to β -lactam drugs is mainly due to the action of a family of hydrolytic enzymes called β -lactamases, which have been a topic of several recent reviews^{7, 8, 21, 24, 28}. In this review, we would like to approach the subject by briefly reviewing the properties of β -lactam drugs, followed by a description of what is known of β -lactamases.

β -Lactam Antibiotics

β -Lactam drugs continue to play a key role in treatment of bacterial infections and their position at the top of antibacterial armamentarium appears to be secure for the next 10–20 years. This statement is made in the face of the belief in the early 1990s that the market on these antibacterials had been saturated. Nonetheless, new β -lactams are being introduced to the market, each of which shows interesting breadth of biological activity with reduced affinities to the resistance enzymes. There are a considerable number of new β -lactam compounds in development by various pharmaceutical companies today, which testifies to the high level of our dependence on these antibacterial for now and the foreseeable future.

Introduction of the first penicillin in 1940s for treatment of bacterial infections, especially those caused by Gram-positive bacteria, owed much to the serendipitous discovery by ALEXANDER FLEMING¹³ in the late 1920s, and the pioneering efforts of HOWARD FLOREY, ERNST CHAIN and their colleagues¹¹. Penicillin was used extensively during the period of 1940–1960 in treatment of various bacterial infections, and often it was employed prophylactically. Bacterial strains, especially staphylococci, that resisted the action of penicillin were identified soon after its introduction^{19, 47}. These resistant strains have spread widely since.

Researchers focused on the development of semi-synthetic penicillins and cephalosporins during 1950–1960. In the following two decades, various broad-spectrum penicillins and cephalosporins (Fig. 1; the R groups are the sites of structural diversity), such as methicillin, ampicillin, oxacillin, and cephalothin were introduced. These drugs, in particular cephalosporins, were resistant to β -lactamases in general and were useful in treatment of Gram-negative bacterial infections as well. Cephalosporins have undergone development in what is acknowledged as at least three “generations”⁸. During the 1970s, second-generation cephalosporins such as cefazolin, third-generation cephalosporins such as cefoxitin and broad-spectrum penicillins such as ticarcillin were introduced. These compounds were stable to the hydrolytic action of most of the then-

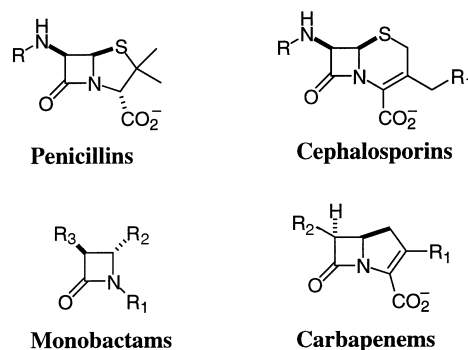


Fig. 1. The general structures for penicillins, cephalosporins, monobactams and carbapenems

-known β -lactamases and exhibited a broad-spectrum of activity.

In the 1980s, the classes of monobactams and carbapenems (Fig. 1) were introduced for the first time, which received much attention due to their broad-spectrum activity, especially against Gram-negative bacteria harboring β -lactamases. Aztreonam (a monobactam), imipenem (a carbapenem), the so-called expanded-spectrum cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone, and the broad-spectrum penicillin, piperacillin were approved for use in the 1980s. Imipenem has been considered the drug of last resort for treatment of serious bacterial infections that show broad resistance to many antibiotics. It shows excellent broad-spectrum of activity⁶. Despite the relative paucity of use of imipenem in the clinic, recently bacteria resistant to imipenem are being isolated, threatening the effectiveness of this versatile antibiotic^{32, 37, 38, 48}.

Another important milestone in countering the effect of β -lactamases was the discovery of clavulanic acid by Beecham scientists in the middle 1970s. This compound is a powerful inhibitor of the class A β -lactamases – the most common in pathogenic bacteria⁸ – which paved the way to the therapeutic strategy of combination formulation⁴³. Thus, two combinations of a penicillin and clavulanate, amoxicillin/clavulanate and ticarcillin/clavulanate were introduced. The success of this strategy paved the way for the introduction of combinations of penicillins with other β -lactamase inhibitors such as sulbactam and tazobactam (e. g., ampicillin/sulbactam and piperacillin/tazobactam) in 1980s and 1990s^{5, 10, 36, 40, 43}.

Mechanistic Aspects of β -Lactam Antibiotics

β -Lactam antibiotics inhibit the bacterial cell wall synthesis by covalently modifying a group of outer membrane enzymes collectively known as “penicillin-binding proteins” (PBPs). PBPs and related enzymes

were recently reviewed by GHUYSEN et al.¹⁴ PBPs catalyze a series of reactions in the assembly of the bacterial cell wall and its regulation. One of these reactions is a critical cross-linking of two peptidoglycan chains, which imparts rigidity to the cell-wall⁸. TIPPER and STROMINGER⁴⁶ suggested that the penicillin nucleus mimics the terminal acyl-D-Ala-D-Ala portion of the structure of the peptidoglycan. This portion of the peptidoglycan binds the PBP during the cross-linking step²⁰. In the first step, an acyl-enzyme complex is formed by the reaction of the active-site serine hydroxyl with the terminal acyl-D-Ala-D-Ala moiety, followed by a second reaction involving another peptidoglycan to form the cross-link. However, penicillin (and other similar β -lactam antibiotics) binds to PBPs, and it forms an acyl-enzyme complex with the PBP by acylating the active-site serine, but the second reaction does not take place, resulting in irreversible inhibition of the catalytic process of PBPs. This causes an incomplete biosynthesis of the bacterial cell wall, an impairment of the structure that leads to bacterial death. Novel variants of PBPs are being identified in bacteria, which resist certain β -lactam antibiotics³¹. Methicillin-resistant *Staphylococcus aureus* (MRSA) is such an organism, and the cause of resistance is the alteration of the PBP structure to reduce its affinity for the penicillin^{16, 41}. This mode of resistance is not common since alteration of the PBP structure in the active-site, where the β -lactams bind, would potentially affect also the normal function of the biosynthetic enzyme.

Because of the inherent problem to the bacterium with selection of altered structures for the PBPs, the vast majority of resistant bacteria have resorted to a different strategy in acquiring resistance to these drugs, namely by producing β -lactamases. MASSOVA and MOBASHERY²⁵ recently discussed the evolutionary and functional relationships of PBPs and β -lactamases. There is compelling evidence that β -lactamases may have evolved from PBPs²⁵. Indeed, both β -lactam antibiotics and β -lactamases are of microbial origins. In the process of competition for resources one strain of bacterium would produce a β -lactam antibiotic to control another organisms within the same environmental niche. A β -lactamase-producing organism would have a distinct advantage in this competition for resources by its ability to destroy the β -lactam antibiotics produced by the other organisms²¹. The extensive use of β -lactam antibiotics in the clinic has allowed for the selection of a number of variants of β -lactamases that show various breadth of activity. Presently, there exist β -lactamases that collectively take all known β -lactam antibiotics as substrates, but fortunately not all are common in clinical strains of bacteria.

β -Lactamases and Their Inhibitors

The first β -lactamase was identified in 1940s even before the introduction of the first penicillin into the clinic, which later was isolated from a strain of *Staphylococcus aureus*^{19, 47}. The vast majority of β -lactamases are plasmid-borne, hence the genes for these enzymes transfer from organism to organism readily¹⁵. Despite the advances in development of new β -lactam antibiotics over the years, the evolution of these enzymes to expand the breadth of substrate profile has kept pace. There are currently 255 known β -lactamases⁸, a list that is growing, threatening the therapeutic options clinically. Several classifications schemes have been proposed for β -lactamases^{2, 3, 9, 17, 33, 39}. These classification schemes of Ambler (classes A, B, C and D) and Bush (groups I, II, III and IV) are widely used in the literature^{2, 9, 17, 33}. The most common group of clinical β -lactamases are those of the TEM and SHV family (class A β -lactamases)²⁶. For example, there are 67 clinical variants of the TEM β -lactamases and 12 variants of the SHV enzymes to date. A list of all the clinically identified β -lactamases is maintained on the World-Wide Web (<http://www.lahey.org/studies/webt.htm>).

The three mechanism-based inhibitors for β -lactamases, clavulanic acid, sulbactam, and tazobactam, acylate the active-site serine of class A β -lactamases, but subsequent to the formation of this complex other chemical events take place that prevent the deacylation step^{25, 27, 29}. It is of interest that certain effective β -lactam antibacterials such as imipenem also inhibit these enzymes by acylating the active site serine and resisting the deacylation step^{27, 45}. Imipenem forms the acyl-enzyme intermediate in the active-site of the enzyme, but resists hydrolysis by interfering with the approach of hydrolytic water, as well as changes its conformation such that the acyl-enzyme species enjoys an extended longevity²⁷.

β -Lactamases in the Clinic

A recent study at the University of Iowa revealed that the number of infections caused by Gram-positive bacteria is on the rise, especially those due to coagulase-negative staphylococci and *Staphylococcus aureus*¹⁸. A considerable number of these strains are methicillin-resistant variants and are difficult to treat. Other common Gram-positive bacteria are streptococci, and enterococci. *Enterococcus faecium* is the most common "drug-resistant" bacterium and among these species resistance with multiple mechanisms is common. The incidence of penicillin-resistant strains of *Streptococcus*

pneumoniae is rising as well, up to 30% in a 1994 survey and other β -lactam antibiotics are inactive towards the strains carrying altered PBPs¹⁸.

Among the Gram-negative bacterial pathogens, *E. coli*, *Pseudomonas*, *Klebsiella*, and *Enterobacter sp.* are the important organisms that manifest resistance by expression of extended-spectrum β -lactamases and chromosomally induced cephalosporinases¹⁸. *Enterobacter sp.* are important nosocomial pathogens, and resistance to expanded-spectrum cephalosporins (such as the third-generation cephalosporins) develops normally in these organisms due to selective mutations in the chromosomal gene. However, in a recent study on 31 clinical isolates of *E. aerogenes* from a hospital in Richmond, Virginia, USA, plasmid-mediated extended-spectrum β -lactamases were identified in addition to inducible chromosomal cephalosporinase, that confer resistance to broad-spectrum penicillins and aztreonam³⁴. Recently, a clinical strain of *Pseudomonas aeruginosa* was isolated that produced three different types of β -lactamases: a class C β -lactamases, and two class D enzymes (OXA-18 and OXA-20); the later two are novel enzymes with broad substrate profiles³⁰. It is a cause for concern that several *P. aeruginosa* and *S. marcescens* strains have been shown to harbor the plasmid-borne metallo- β -lactamase IMP-1, isolated from patients in more than 20 hospitals in Japan¹⁵. Metallo- β -lactamases confer resistance to a variety of β -lactams including broad-spectrum penicillins, cephalosporins and carbapenems¹⁵. While there is no evidence that these particular plasmids are transmitted to other strains, it is likely that such transfer would occur. For example, *Bacteroides* strains normally live in human intestines symbiotically, and under normal conditions they are unharmed. However, they can become virulent opportunistic pathogens under certain conditions, and they often express important resistance factors such as metallo- β -lactamases that they have picked up by transient contact with other pathogenic species such as *P. aeruginosa*⁴².

Among the *Klebsiella sp.*, *K. pneumoniae* causes mainly nosocomial infections, and is placed among the eight most important pathogens in the hospitals in the United States, United Kingdom and Germany³⁵. It is disturbing to note that more than 20% of the 966 clinical isolates of *Klebsiella sp.* collected from 35 centers in Western and Southern Europe produced extended-spectrum β -lactamases and 88% of these strains were resistant to ceftazidime, and the monobactam, aztreonam²³. Members of the *Enterobacteriaceae sp.* transmit plasmid-mediated extended-spectrum β -lactamases, resulting in multiple drug resistance and limited therapeutic choices. Most of these strains carry either the

TEM- or SHV-class of β -lactamases (class A enzymes), and these strains retain the plasmids of the resistance factors for a long time even after the discontinuation of the antibiotic³⁵.

Class B metallo- β -lactamases are expressed mostly along with another β -lactamase. Although until recently this class of enzymes were shown to be only of chromosomal origin, in certain species such as *B. fragilis*, *K. pneumoniae*, *S. marcescens*, metallo- β -lactamase genes have been identified on plasmids, which can easily be transferred to another species³⁷. Carbapenemases, which include metallo- β -lactamases and other carbapenem-hydrolyzing β -lactamases, are increasingly identified in various bacterial species.

Concluding Remarks

β -Lactam antibiotics have been the mainstays in clinical use in antibacterial therapy and they will continue to hold their central importance for the foreseeable future. Our knowledge of the mechanism of action of these antibiotics and also the mechanisms of resistance to them has increased dramatically within the past few years. This advance owes much to the recent success in protein crystallographic efforts that have furnished many useful structures to not only explain the structure-function relationships for these systems, but also to generate hypotheses for testing in mechanistic studies. Whereas some foresee the end for the usefulness of these drugs in the future, we are optimistic that the aforementioned structural and mechanistic information would pave the way for design of the next generations of β -lactam antibiotics. These will be molecules that will be designed in rational drug design programs with both the structures for the targets of antibiotic binding (e. g., PBPs) and those of the active-sites of the resistance enzymes in mind. One thing is clear, and that is that multidisciplinary efforts should be brought to bear on the various mechanisms underlying the processes that antibiotics undergo, in order to keep one step ahead of microorganism in their acquisition of drug resistance mechanisms.

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