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REVIEW

β-Lactam antibiotics, β-lactamases and bacterial resistance

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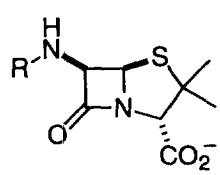
β-Lactams are the most prescribed antibacterials for the treatment of bacterial infections. Bacteria have developed mechanisms to resist the action of β-lactam drugs, of which the catalytic function of β-lactamase is the most important. These enzymes catalyse hydrolysis of β-lactam antibiotics, whereby the drug is destroyed. This review offers a perspective on the function of these enzymes and their clinical implications.

Antibiotics are widely used worldwide, and in the USA alone approximately 160 million prescriptions for antibiotics were written in 1996 and more than 50 million pounds of these drugs were produced for use [**1**]. Among the various classes of antibiotics, β-lactam antibiotics, such as penicillins (1) and cephalosporins (2), are the most frequently used agents in treatment of bacterial infections. β-Lactam antibiotics have been in clinical use for more than 50 years. The susceptibility of various pathogenic bacteria to the β-lactam antibiotics has changed dramatically over the years due to their widespread and liberal usage. The cases of resistance to these antibiotics are common in pathogenic bacteria, prompting the search for new antibiotics with novel mechanisms of action. In essence, drug resistance is the bacterial fight for survival in the face of the challenge by a chemotherapeutic agent. The most common mechanism of resistance to β-lactam antibiotics is the ability of bacteria to express β-lactamases. These enzymes render the antibiotic ineffective by hydrolytic cleavage of the β-lactam moiety of the drug; the product of the enzymic reaction lacks antibiotic property entirely. A few review articles have appeared in the literature recently on β-lactamases [**1-6**]. In the present review we will approach the subject somewhat differently by presenting a brief historical perspective, the mode of action of β-lactam antibiotics, the emergence of resistance and various approaches that are pursued to combat bacterial resistance to β-lactam antibiotics.



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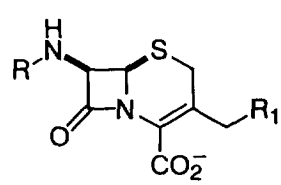

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The antibacterial activity of penicillin was discovered serendipitously by Alexander Fleming in 1929 [**7, 8**]. The pioneering work of Howard

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Florey, Ernst Chain and colleagues set in motion the initial experiments in purification, structural studies, and the first clinical trials of this antibiotic [9]. It is remarkable that before the widespread clinical use of penicillin, cases of resistant *Staphylococci* were already in existence [10]. Subsequently, penicillinase (now more commonly referred to as β-lactamase) was isolated from the clinical strains of penicillin-resistant *Staphylococcus aureus* by Kirby [11]. The resistant strains of bacteria rose quickly in hospitals, leading to nosocomial infections which are hard to treat. In a study performed in a hospital in England, 14% of the isolates were resistant to penicillin in 1946, whereas 59% of the isolates were so by 1948 [12].

The emergence of resistant strains of bacteria resulted in a twofold response to the problem. First, hospitals undertook measures and increased their efforts to control the cross-infection of resistant strains by educating the medical personnel and by surveillance. Second, scientists devoted their research efforts to the development of semi-synthetic penicillins and cephalosporins in the 1950s and 1960s, and subsequently the extended-spectrum cephalosporins, the monobactam aztreonam and carbapenems in the 1970s and 1980s. The newer variants of these antibiotics were generally resistant to the action of β-lactamases, although, as will be outlined below, β-lactamases also underwent structural alterations to keep pace with the new clinical challenge presented to pathogens by these structural variants of the parental antibiotics [13]. Extended-spectrum β-lactamases (ESBL) were soon identified which posed new challenges, in light of the fact that the breadth of spectrum of these enzymes includes some of the current mainstays in clinical treatment of infections. To put this matter in perspective, consider the case of the Gram-negative TEM-1 β-lactamase, which was first identified in *Escherichia coli* in 1963 [14]. A survey of the variants of this enzyme indicated a total of 67 such enzymes that have been identified up to July 7, 1998 [15]. These variants expand the substrate profile of the parent enzyme. It is important to note that there exist presently known β-lactamases for hydrolysis of virtually every type of β-lactam agent [3, 5].

Mode of action of β-lactam antibiotics

β-Lactam antibiotics inhibit the enzymes of bacterial cell-wall biosynthesis. Because these proteins were shown to bind to penicillins, they are referred to as penicillin-binding proteins (PBPs). These include the low-molecular-weight penicillin-binding proteins, high-molecular-weight PBPs and peptidoglycan hydrolases (PGH). This family of enzymes has been the subject of a recent review by Ghuysen and coworkers [16].

A critical reaction for bacteria catalysed by some PBPs is the final cross-linking of the cell-wall polymer, the peptidoglycan. This cross-linking reaction imparts rigidity to the cell wall that is required for the survival of bacteria. It has been suggested that the structure of the backbone of a typical β-lactam antibiotic mimics the three-dimensional structure of the terminal portion of the peptidoglycan, namely acyl-D-Ala-D-Ala [17]. As such, both peptidoglycan and penicillins bind the active site of the PBP by acylating a critical serine residue. The acyl-enzyme species with the peptidoglycan undergoes a reaction with a second peptidoglycan residue to complete the catalytic process, giving rise to the cross-linked cell wall [18]. On the other hand, the acyl-enzyme species with penicillins, and other β-lactam antibiotics, is incapable of this second step, resulting in irreversible inhibition of the PBP and incomplete biosynthesis of the cell wall, which leads to bacterial cell death.

It is known that β-lactamases, enzymes of resistance to β-lactam antibiotics, are related to

BLIP = β-lactamase-inhibitory protein.
ESBL = extended-spectrum β-lactamase.
MRSA = methicillan-resistant *S. aureus*.
PBP = penicillin-binding protein.

PBPs in an evolutionary sense [5]. In essence, a PBP had only to develop the means to deacylate the acyl-enzyme intermediate that it forms with β-lactam agents to have evolved into a resistance enzyme. That is to say, by having an acylation and a deacylation step the catalytic cycle for hydrolysis of β-lactam antibiotics would be complete and such a PBP, now a nascent resistance enzyme, would be regenerated to destroy additional molecules of antibiotic. This process has indeed happened very effectively in evolution of these resistance factors. Massova and Mobashery have recently provided insight into the mechanism of the acquisition of this hydrolytic second step in catalysis by β-lactamases, which shed light on the potential evolutionary routes taken by these enzymes in response to the challenge by the antibiotic [5].

Classification of β-lactamases and evolutionary relationship to PBPs

Classification of β-lactamases has been useful in appreciating the diversity of structure and function of these enzymes. Ayliffe classified the resistant strains of coliform bacilli in 1963 for the first time, and these strains were known to produce β-lactamase enzymes [19]. Based on limited biochemical data, Ayliffe divided 148 strains of various bacteria into three groups depending on whether a particular bacterial strain harbouring the resistant enzyme was sensitive, partially sensitive or resistant to ampicillin. A more sophisticated classification was needed to accommodate the increasing number of resistant strains and resistant enzymes. Richmond and Sykes proposed a classification for β-lactamases from Gram-negative bacteria in 1973, according to which the enzymes were categorized into five major classes based on the biochemical data [20]. They used a total of 15 known β-lactamases in that study. According to this classification, all enzymes that hydrolyse cephaloridine, a cephalosporin, at a markedly greater rate than benzylpenicillin and ampicillin (both penicillins) were grouped into class I (i.e., cephalosporinases). They were not inhibited by carbenicillin or oxacillin, and their genes were encoded chromosomally. These enzymes are produced either constitutively or inducibly. Class II β-lactamases were either constitutive or inducible chromosomal penicillinases, which hydrolyse benzylpenicillin and ampicillin, but are somewhat less effective against cephalosporins. These are competitively inhibited by cloxacillin, but not by carbenicillin. Class III enzymes have a wide specificity with approximately equal rates of hydrolysis towards penicillins and cephalosporins, consisting of the TEM-type and SHV-type β-lactamases, which are most common in pathogens. Class III enzymes resist inhibition by sulfhydryl modifiers and can hydrolyse carbenicillin, albeit slowly. Class IV enzymes possess similar substrate profile to those of class III, but they are sensitive to sulfhydryl modifiers. They are also resistant to inhibition by cloxacillin. Class V enzymes were comprised of β-lactamases that could hydrolyse penicillins, cephalosporins, were insensitive to sulphydryl-modifying reagents and also hydrolysed cloxacillin. β-Lactamases in the classes III and V are plasmid-mediated according to this classification.

Ambler proposed a different classification in 1980 based on the molecular types of β-lactamases [21]. According to this classification, there are four classes of β-lactamases: classes A, B, C and D. Penicillinases that possess critical active-site-serine residues which participate in the catalytic machinery of enzyme were considered as members of the class A. Class A enzymes are found both in Gram-positive and Gram-negative bacteria. Class B β-lactamases are metalloenzymes and are probably structurally not related to class A enzymes. Class C enzymes are also serine-dependent, however, they can hydrolyse cephalosporins well, in contrast to class A enzymes [22]. Class D β-lactamases are serine-dependent oxacillin-hydrolysing β-lactamases (OXA-type), which do not have close functional or sequence identities to enzymes of classes A and C [23].

A more recent and comprehensive functional classification of β-lactamases was proposed by Bush in 1989, which was later expanded to include all the known β-lactamases in 1995 (just under 190 enzymes) [3]. Bush's system of clas-

sification utilized the extensive kinetic data on various β-lactamases that were characterized until 1994. The strategy of this classification is based on the substrate preferences and inhibition characteristics of various β-lactamases. According to this system, four major groups are recognized. "Cephalosporinases" which are not inhibited by clavulanic acid belong to group 1. Penicillinases, including broad-spectrum penicillinases that are generally inhibited by the active-site-directed β-lactamase inhibitors, are grouped into group 2. Subgroups within group 2 viz. 2a, 2b, 2be, 2br, 2c, 2d, 2e and 2f are recognized according to their biochemical data based on rates of hydrolysis of carbenicillin, cloxacillin, extended-spectrum β-lactams ceftazidime, cefotaxime or aztreonam and inhibition profile by clavulanate. Enzymes that are inhibited by EDTA (presumably metal-dependent) are classified as group 3. Group 4 consists of β-lactamases that are not inhibited well by clavulanic acid.

It would appear that various classes of PBPs evolved from the primordial protein to give the diversity that one sees in this family of enzymes presently [5]. Subsequently, as branching points from several more modern variants of PBPs, β-lactamases have evolved into various different classes of β-lactamases [16, 18]. These evolutionary steps took place independently of one another and perhaps in parallel to each other [5]. This assertion is validated in light of recent studies on the details of the mechanisms of these enzymes [5]. These analyses indicated that at the minimum in the cases of β-lactamases of classes A, B and C, evolution took different routes to generate these catalysts capable of hydrolysis of β-lactam antibiotics to give the onset of resistance. Whereas β-lactamases of class D are the least understood of these resistance enzymes, it is likely that the enzymes of this class also have developed novel catalytic machineries that set them apart in the evolutionary scheme from the other enzymes of the same family.

Evolution of the catalytic machinery was a critical process for the acquisition of the β-lactamase activity. However, it was probably equally important that nature would restructure the active site of the nascent resistance factors such that they would not recognize the polymeric peptidoglycan as a substrate, and in essence become specialists in binding and turnover of the β-lactam antibiotics. One should bear in mind that a resistance enzyme which is tied up with the peptidoglycan would prove an ineffective resistance factor. This issue has been settled by reshaping the architecture of the surfaces of the active site structures of β-lactamases by insertions and deletions which have abolished the ability of these enzymes to recognize the peptidoglycan. The nature of these structural modifications has been discussed in some detail by Massova and Mobashery with regard to classes A and C of β-lactamases for which high-resolution crystal structures are known [5].

The cytoplasm of bacteria is enclosed by a lipid bilayer (referred to as the inner membrane in Gram-negative bacteria). The PBPs are often integral membrane proteins situated within this portion of the bacterial structure, with their active sites made available on the external surface of this membrane. As PBPs are involved in the final steps of the cell-wall biosynthesis, they assemble the cross-linked cell wall in the periplasmic space, which is adjacent to the membrane structure. As indicated earlier, cell wall imparts rigidity to the bacterial structure, a feature which is indispensable in light of the fact that bacteria are devoid of any mechanism to regulate their osmotic pressure. Whereas the nature and dimensions of the cell wall vary somewhat between Gram-negative and Gram-positive bacteria, the primary and significant difference between the two families of bacteria is that the cell wall in the former is enclosed in what is referred to as an outer membrane. This feature is entirely absent in Gram-positive bacteria. The penetration of nutrients (and antibiotics) into the Gram-negative bacteria is via channel-making proteins referred to as porins [24]. This penetration imposes certain limits on molecules, which have to do with their electrostatic properties, and not insignificantly, their size. β-Lactamases are exported to the periplasmic space to serve as vanguards against the

incoming antibiotics. The existence of the outer membrane traps these proteins in the periplasm in Gram-negative organisms, whereas they diffuse into the extracellular milieu in Gram-positive bacteria.

β-Lactams were biosynthesized by certain microorganisms to apparently give them advantage over other bacterial species for resources [25, 26]. Evolution of β-lactamases by other bacterial species provided the means for survival to non-producers of β-lactam antibiotics. These events proceeded at the evolutionary timescale. However, introduction of penicillins to the clinic, followed by that of cephalosporins, the semi-synthetic variants and also non-classical β-lactams, has compelled bacteria to find novel methods for survival in the face of the new challenges. In light of the fact that these antibiotics are being used extensively these days, they have accelerated evolution of the resistant phenotype. It is interesting to note that whereas evolution and dissemination of β-lactamases is widespread, and often facilitated by the sharing of the genetic materials from organism to organism, development of specific PBPs which resist the action of the antibiotic has been relatively rare. This is in part due to the critical functions of PBPs. The mutations which would impart to the PBPs resistance to modification by β-lactam antibiotics would potentially also impair the true function of the PBP as a biosynthetic enzyme. Nonetheless, such variant forms of PBPs have been observed [27], resulting in bacterial pathogens which are extremely difficult to treat. One such important organism is the methicillin-resistant *S. aureus* (MRSA), which is the most commonly encountered resilient bacterial pathogen causing nosocomial infections [28, 29]. MRSA resists all the currently used β-lactam antibiotics. The resistance in MRSA is mainly due to mutations in the already existing genes, leading to structural alterations in the PBPs. The mutant PBPs have lowered affinities to β-lactam drugs. Although there are more than one type of PBP that are produced, PBP2a is the most common one among MRSA. The mechanisms of methicillin resistance, the evolution of the related genes and their transmission are reviewed in detail by others [28, 29].

β-Lactamase inhibitors

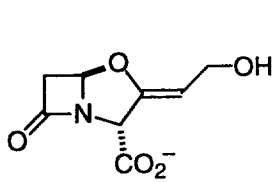
Study of bacterial resourcefulness in selection of function for survival presents yet another subject worthy of discussion. If the existence of β-lactamase in a rival bacterial population makes obsolete the biosynthetic machinery for β-lactam antibiotics in another, why not acquire an inhibitor for β-lactamases. This strategy would eliminate the function of the resistance enzyme, giving renewed value to the antibiotic (see fig. 1).

Indeed, there are examples of such a strategy from the microbial world. A "β-lactamase-inhibitory protein" ("BLIP") has been discovered in *Streptomyces clavuligerus*, which also produces the clinically used β-lactamase inhibitor, clavulanic acid [30]. BLIP, a small protein of molecular mass 17.5 kDa, was found to be a potent inhibitor of a number of β-lactamases, but it does not inhibit the PBPs except PBP type 5 [30]. The interaction of BLIP and β-lactamase was found to be noncovalent in nature, and each BLIP inhibits one molecule of β-lactamase. The binding of BLIP to the active site of TEM-1 β-lactamase appears to be governed by favourable steric and electrostatic interactions at the interface of the β-lactamase active site and the protein inhibitor [31]. There are a host of β-lactam inhibitors of β-lactamases. One could envision that the biosynthetic processes for production of the β-lactam antibiotics have been modified to create novel β-lactam entities that could function as inhibitors of the resistance enzymes [32]. Since these molecules are β-lactams themselves, the process of inhibition of the enzyme commences by acylation of the active site of the enzyme prior to deviations in the catalytic machinery which lead to inhibition of the enzyme, often irreversibly. These mechanisms for inhibition have been the subject of study for the past several years [33, 34].

Whereas the protein inhibitor of β-lactamase is of limited use in a clinical setting, the small molecule inhibitors are of considerable interest. The work on these small molecule β-lactam inhibitors commenced by the discovery in the culture medium of *S. clavuligerus* of a compound with pronounced inhibitory activity against β-lactamases [32]. Later, this metabolite

was named clavulanic acid (or clavulanate in the salt form, 3). Subsequently, other natural products like olivanic acids and thienamycin (both carbapenems) and nocardicins (monobactams) were discovered which showed β-lactamase inhibitory activity as well. As the mechanisms of action of these agents became more clear, certain synthetic inhibitors for these enzymes have been developed, of which sulbactam (4) and tazobactam (5) have found clinical use in inhibition of the β-lactamase activity.

These inhibitors by themselves often lack good antibacterial activity, but they potentiate the activity of the otherwise β-lactamase-susceptible antibacterials in combination formulations both *in vitro* and *in vivo* [**33, 35-37**]. Currently, there are four combination drugs in clinical use in the United States: amoxycillin/clavulanate (also known as Augmentin™), ticarcillin/clavulanate (Timentin™), ampicillin/sulbactam (Unasyn™) and piperacillin/tazobactam (Zocyn™). These preparations function essentially exclusively against the pathogens that harbour the class A β-lactamases.



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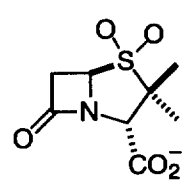

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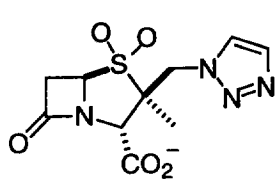

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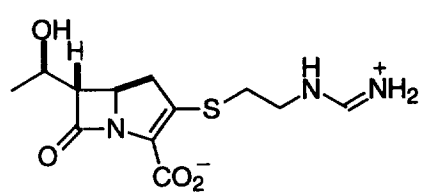

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Carbapenems, such as imipenem (6), also inhibit β-lactamases, but they are also broad-spectrum antibiotics. Imipenem was introduced into clinical use in 1985. Unexpectedly, imipenem was shown to be a substrate for a kidney zinc-dependent peptidase, renal dehydropeptidase. Imipenem is now being used in a combination formulation with cilastatin, which is a potent inhibitor of renal dehydropeptidase [**38**]. Interestingly, imipenem acylates the active-site serine in β-lactamases, but resists deacylation; as such it serves as an effective inhibitor of these enzymes [**39**]. Recently, there have been reports of carbapenem-hydrolysing β-lactamases, especially from Japan, and these enzymes are both plasmid-borne as well as chromosomal in origin [**40**]. These resistant genes may be transferred to other bacterial species in the future, compromising the utility of imipenem against these organisms.

As stated earlier, the mechanisms of the processes of inhibition of β-lactamases by these compounds have been the subject of recent study [**41-43**], and the availability of X-ray structures for class A β-lactamases has also proven helpful [**44-49**]. Binding of the inhibitors in the enzyme active sites are facilitated by electrostatic and steric contributions. In essence, disruption of these interactions by mutational alteration of the active-site residues could result in resistance of the enzyme to inhibition by these molecules. It is important to emphasize that such mutations should not affect the catalytic competence of the enzyme, lest the desirable resistance phenotype to the antibiotics be lost. Therefore, the enzyme must remain active

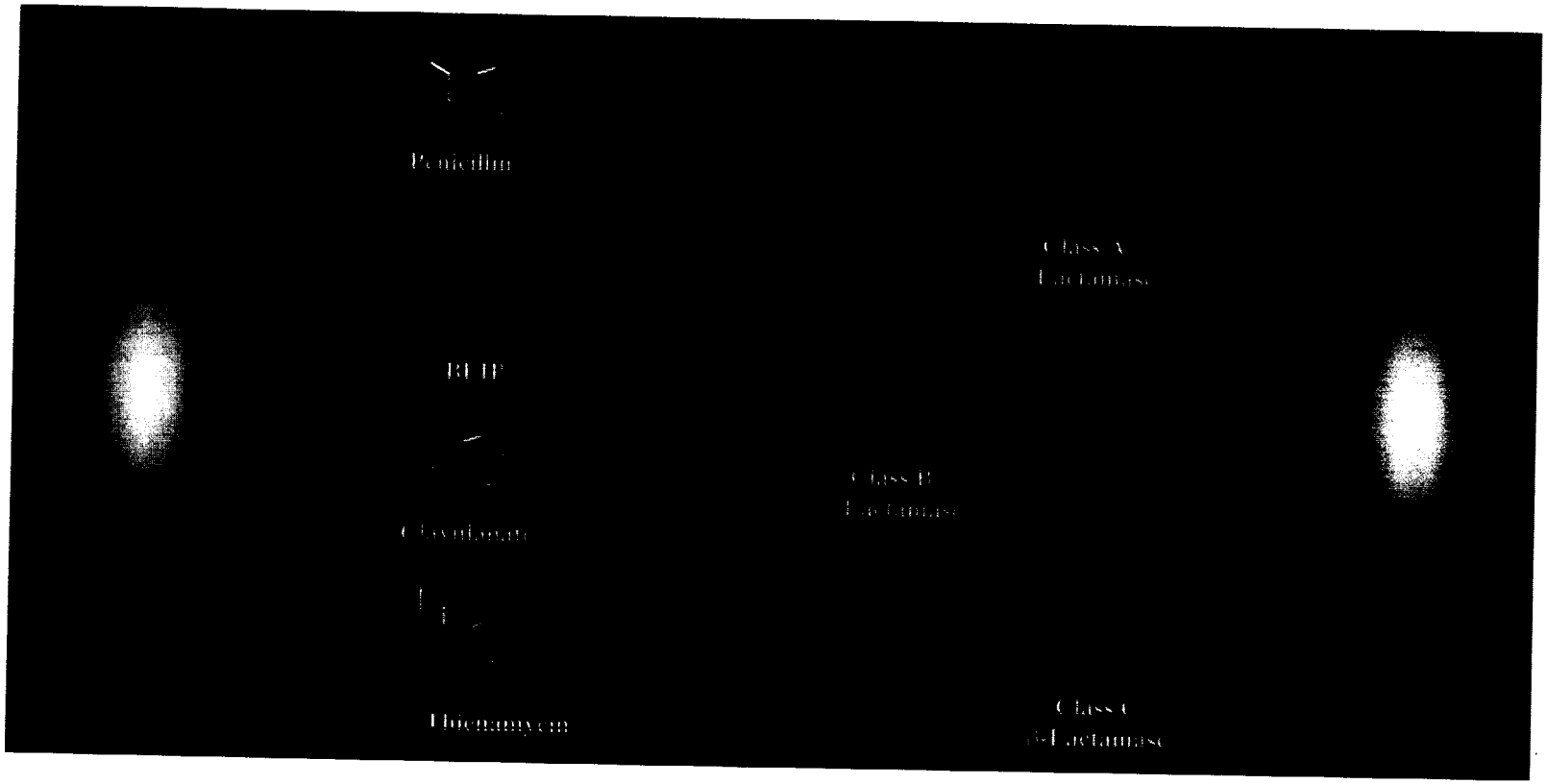

Fig. 1. A scheme of the dynamics of interactions of two coexisting bacteria.

Bacterium 1 (an organism such as *Streptomyces clavuligerus*) may produce β-lactam antibiotics (such as penicillins and cephalosporins) to potentially adversely affect the growth of bacterium 2. Bacterium 2 would produce β-lactamases, such as the typical penicillinases and cephalosporinases. Bacterium 1 would find it advantageous to produce inhibitors of such β-lactamases (BLIP, clavulanate, etc.) to preserve the utility of antibiotics. There would be a selection pressure for bacterium 2 to come up with novel β-lactamases of new types, or select for mutant variants of the existing ones. This selection pressure would favour increase in breadth of activity of the enzyme, as well as exploring the mutational means of avoiding inhibition by the inhibitors. In response, bacterium 1 would biosynthesize antibiotics which resist the action of the resistance enzymes, such as carbapenems (thienamycin would be an example; the clinical imipenem is a semisynthetic derivative of thienamycin). Bacterium 2 explores development of yet broader-spectrum enzymes, such as the class B β-lactamases. Whereas the scheme depicted here indicates "communication" between two bacteria, indeed these evolutionary developments take place in many microbial populations concurrently. The ability of these organisms to share genetic information facilitates such processes considerably, hence independent *de novo* development of catalysts (i.e., biosynthetic enzymes or resistance factors) is not often necessary in disparate organisms.

upon mutation, yet it should resist the action of the β-lactamase inhibitor. Such mutations have been discovered now both in the laboratory and in the clinic [**50, 51**]. For example, TEM-51 β-lactamase possesses an Arg244His mutation, and the enzyme does not experience ready inhibition by clavulanate, in contrast to the wild-type enzyme [**52**]. Similarly, Arg244Ser mutant variant of TEM-1 (TEM-30) resulted in attenuation of the rate of inactivation by clavulanate *in vitro* and a 128-fold increase over the wild type in minimum inhibitory concentration of ampicillin/clavulanate [**43**]. Substitutions at Asn-276 by Asp, and at Val-69 by Leu (TEM-35) or by Val (TEM-36) resulted in resistance to clavulanic acid. The mutation at Asn-276 appears to be causing resistance to clavulanic acid due to the alteration in interactions among Arg-244, Asn-276 and the carboxylate of the inhibitor [**53**]. Various other mutations and combinations of mutations in the TEM, OXA and SHV-type β-lactamases are listed and updated on the internet by Jacoby and Bush, which is a useful resource on these enzymes [**15**].

In a series of recent experiments on DNA shuffling to accelerate selection of mutant variants of TEM-1 β-lactamase, we have observed that only a set of four mutations of consequence can be generated in the gene for the TEM-1 β-lactamase to give the inhibitor-resistant phenotype [**54**]. The sites identified corresponded to those selected independently in clinical strains, so these experiments are validated by the direct outcome of the clinical utilization of the inhibitor/β-lactam combinations. Whereas it is a comfort that the number of such potential mutations are limited only to the four identified sites, the fact that the genes for these mutants are plasmid-borne and can be disseminated with ease in bacterial populations may bode poorly for the existing combination therapies in the course of time.

Clinical resistance to β-lactam antibiotics due to β-lactamases

β-Lactamases are produced by both Gram-positive and Gram-negative bacteria. The susceptibility of various strains of bacteria to diverse β-lactam antibiotics is different depending on various factors such as penetration of β-lactam drugs into the bacteria, presence of the resistance enzymes such as β-lactamases, the type of β-lactamases and mode of expression of β-lactamases, among other factors. As stated earlier, resistance due to production of β-lactamases is the most common mechanism of resistance clinically, though other factors may play a role in the case of specific antibiotics. For example, clinical isolates of *Haemophilus influenzae*, *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter freundii*, *Providencia* spp., *Morganella morganii*, *Serratia* spp. and *Pseudomonas aeruginosa* show resistance to β-lactam antibiotics primarily by production of β-lactamase [**55**]. However, altered cell-wall structure, as well as decreased transport of the drug across the membrane are also reasons for the resistance. The majority of *H. influenzae* isolates produce the TEM-1 β-lactamase (>90% of isolates), while other types of β-lactamases, such as ROB-1 and TEM-2 were also identified in this organism. These β-lactamase-producing strains are resistant to ampicillin and amoxicillin, but are sensitive to a combination of β-lactamase inhibitor/β-lactam drug. Among the strains of *E. coli* and *Proteus mirabilis*, the TEM-1 β-lactamase is the cause of resistance to β-lactam antibiotics and in the case of *Klebsiella pneumoniae*, it is the SHV-1 β-lactamase [**56**]. It was reported that more than 30% of the community-isolated and 40-50% of the hospital-isolated *E. coli* strains are resistant to amoxicillin – cause for concern, as this effective drug would appear to be largely compromised [**27**]. Resistance to β-lactamase inhibitor/β-lactam drug combination is also widely seen in clinical isolates of these species due to high levels of β-lactamase production. While the production of the TEM-1 β-lactamase in higher levels is one reason, another factor for resistance to β-lactamase inhibitors is mutations in the β-lactamase gene (the so called IRTs—inhibitor resistant TEMs; one such mutant has been seen for the SHV β-lactamases). There are about 61 different types of TEM and 12 types of SHV β-lactamases identified worldwide to date [**15**]. The newer forms of β-lactamases are often termed extended-spectrum β-lactamases, because they can hydrolyse expanded-spectrum cephalo-

sporins and the monobactam aztreonam. For example, *E. coli* and *K. pneumoniae* isolates have shown resistance to cefoxitin, as well as extended-spectrum cephalosporins. This resistance was shown to be due to the production of plasmid-mediated AmpC β-lactamase (class C, *vide infra*) [**56**]. In *K. pneumoniae* various variants of TEM and SHV-type enzymes were found to confer resistance against the expanded-spectrum cephalosporins. *Serratia* spp. are generally considered non-pathogenic. However, recently, clinical isolates of *Serratia marcescens* which were resistant to monobactams and expanded-spectrum cephalosporins were characterized in Italy [**57**]. In these isolates, three types of β-lactamases were produced, two of which are plasmid-mediated. *Bacteroides fragilis* is an anaerobic bacterium present in the human gastrointestinal system. Under certain conditions, as in immunocompromised patients or in the damaged tissues during surgery or wounds, etc., this organism can become infectious [**58, 59**]. More than 90% of *B. fragilis* strains produce β-lactamases, a certain number of which are capable of hydrolysing cefoxitin and imipenem, which are normally stable to the challenge of many β-lactamases. Recently, a clinical isolate of *B. fragilis* producing a metallo-β-lactamase was identified in the United Kingdom, which was resistant to metronidazole, co-amoxiclav (amoxicillin and clavulanate) and imipenem, simultaneously [**59**]. *B. fragilis* KSB 1468/90 survived in the presence of cefoxitin or imipenem, and the β-lactamase inhibitors clavulanate, sulbactam and tazobactam did not have any effect on potentiating the antibiotic [**58**]. This strain produced a metallo-β-lactamase and later, the gene for this enzyme was found to be transferable to other organisms.

Most of the aforementioned β-lactamases are plasmid-borne and could be produced in large quantities. However, certain species of bacteria such as *Enterobacter* spp., *C. freundii*, *Providencia* spp., *M. morganii*, *Serratia* spp., among others, have inducible chromosomally encoded AmpC β-lactamase [**55**]. Induction of this resistance-causing enzyme is not as common as the expression of other plasmid-mediated enzymes; however once selected, these bacterial strains cannot be treated by expanded-spectrum cephalosporins and aztreonam. Enterobacter and several species of *Enterobacteriaceae* are not very susceptible to newer cephalosporins. These organisms, including *Enterobacter cloacae*, possess cephalosporinase type-1 (class C), which effectively protects the bacteria from cephalosporins.

Some Gram-positive bacteria, such as *S. aureus*, Enterococci and Streptococci, are important pathogens which have reemerged in the recent past with resistant strains [**55, 60, 61**]. A majority of them produce β-lactamases in high quantities and release them into the surrounding extracellular milieu. *S. aureus* is a well-known pathogen which has developed resistance to a wide range of antibiotics over time, especially to β-lactam antibiotics. *S. aureus* produces a β-lactamase that can hydrolyse typical penicillins. Subsequently, methicillin, oxacillin and nafcillin were used to treat the penicillin-resistant *S. aureus*. However, by the late 1970s, MRSA were widespread. Interestingly, resistance to methicillin is not due to β-lactamase production, but due to the alterations in the PBPs, conferring resistance to most of the β-lactam antibiotics including cephalosporins, carbapenems and penems (*vide supra*) [**60**]. Today, more than 95% of *S. aureus* strains are resistant to penicillins [**27**].

Enterococcus faecalis and *E. faecium* are important nosocomial pathogens accounting for at least 95% of infections caused by enterococci around the world. A combination of a β-lactam such as ampicillin and an aminoglycoside are usually used to treat enterococcal infections [**61**]. Production of β-lactamases (in <0.2% of isolates) as well as altered PBPs by *Enterococcus* spp. are the major reasons for the development of resistance to β-lactams in these organisms. Among the *Streptococci* spp., *S. pneumoniae* is probably the most common pathogen which causes pneumonia, otitis media (middle ear infection) and bacterial meningitis. Penicillin resistance in *Pneumococci* is common, and according to a study in 1994, approximately 41 % of isolates from children in Atlanta (USA) were penicillin-resistant [**60**]. While the impact of resistant strains of *Pneumococci* is not fully understood at this time, these infections can be

treated with a carbapenem or a third-generation cephalosporin. Resistance to β-lactam drugs in *Pneumococci* is mainly due to altered high-molecular-weight PBPs that have lower affinity to β-lactam antibiotics.

Concluding thoughts

β-Lactam antibiotics remain to date the most commonly prescribed antibiotics. It is likely that they will remain in use for the foreseeable future, in light of the fact that novel antibiotics with new mechanisms of action have not been put into the pipeline in the past several years. The many desirable traits of β-lactam antibiotics were the reason for their extensive use, which in turn has facilitated selection of the resistant phenotype. A challenge in the field of β-lactam chemistry and biology has been to understand the details of the resistance mechanisms, and also those of the mechanisms of action for these antibiotics. In the past several years major hurdles in these efforts have been cleared, aided in part by the availability of crystal structures for both β-lactamases and PBPs. These efforts have produced an understanding of the interactions between these target proteins and the antibiotics at the molecular level, and should serve as the starting point for rational design efforts for future antibiotics — both β-lactam and otherwise — which would exploit inhibition of PBPs as the means for treatment of bacterial infections. It goes without saying that "numbers" are on the side of bacteria, which undergo mutation, and subsequently selection in consequence of the challenge by any given antibacterial agent. Therefore, it is critical that knowledge of mechanisms of action and resistance be extended at the molecular level, such that a rational basis for future developments in this field can be contemplated. The importance of these efforts perhaps has never been as high as in the present, when the population is faced with the problems of immunosuppression and reliance on invasive surgical procedure to improve the patient's well-being and also with the high frequency of international travel. These factors conspire to provide proving grounds for the occurrence of resistance phenotypes, which would reduce the quality of life that we have learned to enjoy in the late twentieth century.

Key-words: β-Lactam, Antibiotic, β-Lactamase, Resistance; Review.

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Antibiotiques β-lactames, β-lactamases et résistance bactérienne

Les antibiotiques de la famille des β-lactames ont été prescrits fréquemment pour le traitement des infections bactériennes pendant plus de 50 ans. Les β-lactames, telles les pénicillines et les céphalosporines entre autres, se lient à des PBPs (penicillin-binding proteins) et inhibent ces enzymes de la biosynthèse de la paroi bactérienne. Cette inhibition conduit à la mort de la cellule bactérienne. La fonction catalytique des β-lactamases est le mécanisme le plus important de la résistance à ces drogues. Ces enzymes catalysent l'hydrolyse de la fraction β-lactame de ces antibiotiques, par quoi l'activité antibactérienne est perdue. Les PBPs et les β-lactamases sont apparentées entre elles sur le plan de l'évolution. Dans ce rapport nous soulignons le développement de β-lactames et d'inhibiteurs des β-lactamases nouveaux utilisés en clinique. Ces développements sont discutés dans le contexte des manifestations cliniques des bactéries résistantes.

Mots-clés: β-Lactame, β-Lactamase; Antibiorésistance; Revue.

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