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Review Article

The Development of β-Lactam Antibiotics in Response to the Evolution of β-Lactamases

Sabiha Y. Essack[1,2]

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β-Lactam antibiotics, *viz.,* penicillin, penicillin derivatives, cephalosporins, cephamycins, carbapenems, monobactams, and monocarbams, are the most widely used of all antimicrobial classes by virtue of their high efficacy and specificity and the availability of several derivatives. The expression of one or several β-lactamases (β-lactam antibiotic-inactivating enzymes) represents the most widespread and the most clinically relevant resistance mechanism to these antibiotics. The development of β-lactam antibiotics has thus been a continuous battle of the design of new compounds to withstand inactivation by the ever-increasing diversity of β-lactamases. This article traces antibiotic development in response to the evolution of β-lactamases.

KEY WORDS: penicillin; cephalosporins; cephamycins; carbapenems; β-lactamases.

INTRODUCTION

Since the advent of the antibiotic era with sulphonamides in the 1930s, medical science has witnessed the successful therapeutic application of numerous classes of antibiotics (1), including penicillins, cephalosporins, tetracyclines, aminoglycosides, chloramphenicol, macrolides, glycopeptides, monobactams, carbapenems, quinolones, dihydrofolate reductase inhibitors, and streptogramins (2).

Although antibiotics have been effective in the treatment of infections, infectious diseases remain the leading cause of death globally as a result of both new and emerging diseases, but more importantly, as a result of the increasing prevalence of antibiotic resistant pathogens (3–5).

Resistance is an inevitable consequence of selective pressures imposed by the widespread use and misuse of antibiotics (6,7). Antibiotic resistance adversely affects both clinical and financial therapeutic outcomes in terms of higher morbidity and mortality rates, longer durations of hospitalization, increased health care costs, and the administration of expensive and/or toxic alternative drugs (8).

In no instance is the problem of antibiotic resistance or its consequences more evident than with the β-lactam antibiotics (9), which are the most widely used of all groups of antimicrobials, constituting 50% of all systemically used antimicrobials (10) by virtue of their high efficacy and safety profile (11) and the availability of several derivatives.

β-LACTAM ANTIBIOTIC STRUCTURES

Penicillin, penicillin derivatives, cephalosporins, cephamycins, carbapenems, monobactams, and monocarbams are classified as β-lactam antibiotics (10). All possess an essential four-membered lactam ring that may be fused to form bicyclic ring structures or may exist as isolated rings (12). Several natural and synthetic β-lactams have been described since the discovery of penicillin in 1928.

Table I depicts the generalized structures of β-lactams antibiotic classes. The types of substitutions attached to the basic nucleus (the particular "R" group) determine the activity of a particular β-lactam compound, e.g., substitutions at the 7-α position of cephalosporins confer increased stability against β-lactamases although many also decrease the antibiotic activity against some organisms. The replacement of sulphur with oxygen in the nucleus may increase biologic activity. The chemical structure of a β-lactam compound is thus a compromise between biologic activity, β-lactamase stability, and toxicity (12).

MECHANISM OF ACTION

β-Lactam antibiotics interfere with the final stage of cell wall (peptidoglycan) synthesis (13) by inhibiting the bacterial enzymes, transpeptidases, and carboxypeptidases that catalyse the reactions of peptidoglycan synthesis (7). These enzymes, commonly called penicillin-binding-proteins (PBPs), crosslink the peptidoglycan polymers (14). Peptidoglycan is an essential component of the bacterial cell wall. It protects the organism from osmotic rupture, determines cell shape, and is integral to cell growth and division. Its net-like structure, composed of saccharide chains crosslinked by peptides, maintains cell integrity and viability (10). It must thus remain physically continuous during the bacterial cell cycle (15). Inhibition of PBPs causes bacteriolysis by yielding a wall unable to withstand osmotic forces (16). Bacteriolysis is accelerated by the action of autolysins destroying the existing cell wall (10,17).

[1] School of Pharmacy and Pharmacology, University of Durban-Westville, Private Bag X54001, Durban, 4000, South Africa.

[2] To whom correspondence should be addressed. (e-mail: sessack@pixie.udw.ac.za)

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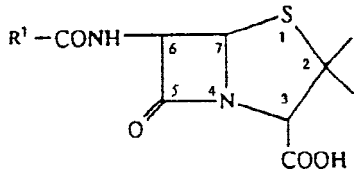
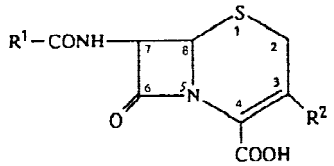
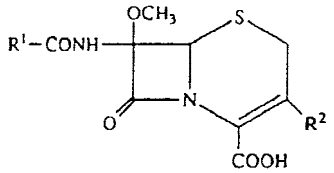
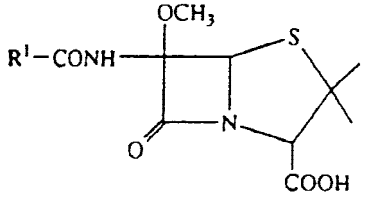
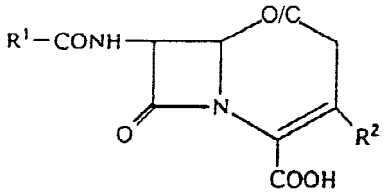
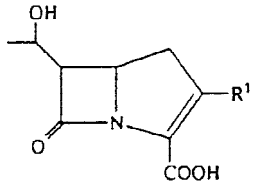
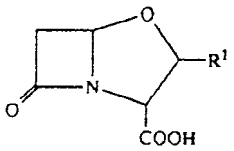
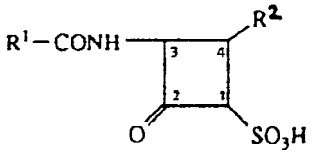
Table I. Generalised Structures of β-Lactam Antibiotic Classes

Class	Structure	Notes
Pencillins/ penams		Consist of a thiazolidine ring fused to the β-lactam ring to which different acyl side chains are attached (22).
Cephalosporins		Possess a dihydrothiazine ring fused to the β-lactam ring (22).
Cephamycins		Essentially ceph-3-ems with a methoxy group replacing the 7-α hydrogen atom on the cephalosporin nucleus (22).
Amidino penicillins		First non-classical β-lactams produced semi-synthetically from 6-amino-penicillanic acid (APA) (38).
Oxacephems and carbecephems		Represent a second group of non-classical β-lactams in which an oxygen or carbon atom (respectively) replaced sulphur in the β-lactam ring (38).
Carbapenems		Resemble penicillins, except that the five-membered ring has the sulphur atom replaced by a carbon and has a double-bond between carbon 2 and 3 (10). Olivanic acids and thienamycin were the first carbapenems discovered. *Streptomyces olivaceus* was found to produce olivanic acids and thienamycin isolated from *Streptomyces cattleya* was discovered during a soil-screening for antibiotics inhibiting cell wall synthesis (44).
Clavams		Clavulanic acid, is the only naturally occurring compound containing an oxygen molecule at position 1 (22).
Monobactams		Monocyclic β-lactam molecules (22) initially isolated from the eubacteria *Chromobacterium violaceum* (44).

Adapted from (10).

RESISTANCE MECHANISMS TO β-LACTAM ANTIBIOTICS

The efficacy of β-lactam antibiotics is dependent on accessibility to its targets, the degree of resistance to enzymatic inactivation by β-lactamases, and the ability of β-lactam to inhibit the target PBPs. Altering one or a combination of these parameters may result in resistance (18). Resistance mechanisms in order of clinical importance are detailed in the sections below.

Enzymatic Inactivation of Antibiotics

The greatest single cause of resistance to β-lactam antibiotics is antibiotic-inactivating enzymes, the β-lactamases,

which efficiently catalyse the irreversibly hydrolysis of the amide bond of the β-lactam ring resulting in biologically inactive products (19). Over 250 β-lactamases have been documented, varying in their encodement (whether plasmid or chromosomally mediated), level of production (whether constitutive or inducible), substrate profiles, inhibition profiles, molecular mass, isoelectric points, amino acid sequences, and molecular structure (12,20,21).

Factors influencing this mechanism include the quantity of β-lactamase produced, the affinity of the enzyme for the antibiotic, the rate of hydrolysis of the antibiotic, and, the location of the β-lactamase. β-Lactamases in Gram-positive organisms are exocellular enzymes excreted into their immediate environment (22). β-Lactamases are localized in the periplasmic space between the outer and cytoplasmic membranes in Gram-negative bacteria, where they attempt to maintain the local antibiotic concentration below the bactericidal threshold (23).

Inaccessibility to the Target Site

Diffusion to the membrane-bound target PBPs may be impeded by the outer components of the cell wall in Gram-negative bacteria. The outer membrane acts as a barrier to hydrophobic compounds in general and a barrier to hydrophilic compounds that exceed a low molecular weight. The former is because of the nature of the lipopolysaccharide with associated hydrophilic polysaccharide chains in its outer leaflet (12). Low molecular weight hydrophilic molecules penetrate into the periplasmic space across transmembranous hydrophilic protein channels, the porins (24). Lipopolysaccharide alterations and porin modification or loss results in diminished permeability. Decreased permeability confers only moderate increases in resistance but may act synergistically with the expression of β-lactamases or active efflux (25) to confer elevated levels of resistance (12). Efflux systems consist of cytoplasmic membrane proteins that extrude drugs using the proton-motive force (25).

Alteration of the Target Site

The development of PBPs with reduced affinities for β-lactam antibiotics is a major resistance mechanism in the absence of β-lactamases (7). PBP modification is the sole cause of resistance in pneumococci (26) and other haemolytic streptococci (27), and, together with impermeability, a major factor of intrinsic resistance in *Neisseria* spp. (7,28) and *H. influenza,* which do not produce β-lactamases (29,30). Resistance has also been reported in *Acinetobacter* spp. (31,32).

Resistance mediated by alterations in the target PBPs may theoretically occur by the reduced affinity of the target PBP for a β-lactam antibiotic, acquisition of a resistant PBP, or increased target PBP number (12).

β-LACTAM ANTIBIOTICS CLASSES

The expression of one or several β-lactamases represents the most clinically relevant (33), the most widespread, and frequently the most efficient bacterial mechanism devised to escape the lethal action of β-lactam antibiotics (21).

The development of β-lactam antibiotics has thus been a continuous battle of the design of new compounds to withstand inactivation by an ever-increasing diversity of β-lactamases (34). The development of β-lactam antibiotics in response to β-lactamases is described below with each consecutive class indicated in bold.

Penicillins

Benzylpenicillin, a **biosynthetic penicillin**, was the first penicillin to be used clinically. It dramatically diminished the prevalence of streptococci (the greatest threat to hospital patients before 1941) as nosocomial pathogens. However penicillin-resistant *Staphylococcus aureus* were identified as early as 1942. Their prevalence as nosocomial pathogens rose dramatically, reaching pandemic proportions by the end of the 1940s. Resistance disseminated by the clonal spread of strains with plasmids carrying genes for the production and regulation of an inducible class A penicillinase (35).

The susceptibility of benzylpenicillin and its congeners to inactivation by staphylococcal penicillinases led to the search for penicillinase stable β-lactam antibiotics. The addition of selected side chains to the 6-APA nucleus resulted in **isoxazolylpenicillins**, such as methicillin (restricted to parenteral use because of its acid instability), and acid-stable compounds, such as cloxacillin, oxacillin, and flucloxacillin, which remain in widespread use. These compounds are used primarily for the treatment of staphylococcal infections (10).

Broad-spectrum **aminopenicillins** contain an amino group in the α-position of the side chain yielding penicillins with a broadened antibacterial spectrum (36). Ampicillin, the first semi-synthetic penicillin with activity against Gram-negative bacteria, was introduced clinically shortly after the isoxazolylpenicillins. Species intrinsically susceptible to ampicillin, such as *Escherichia coli,* soon acquired transferable plasmids carrying β-lactamase genes encoding enzymes that hydrolysed ampicillin and other β-lactam antibiotics. An *E. coli* strain isolated in Athens in 1963 was the first identified producer of TEM-1 (37). Transposons carrying the TEM-1 gene spread worldwide. Numerous different plasmid-mediated β-lactamases with different specificities against aminopenicillins have since disseminated amongst clinical isolates of *Enterobacteriaceae* and *Pseudomonas* spp. particularly in hospitals. The most prevalent of these enzymes are TEM-1 and SHV-1, which occur most frequently in *Enterobacteriaceae,* whereas PSE-1 predominates in *Pseudomonas aeruginosa* (38–41).

Ureidopenicillins such as azlocillin, mezlocillin, and the piperazine penicillin, piperacillin, have heterocyclic groups substituted on the α-amino group (10). This increases activity against Gram-negative bacteria primarily because it increases affinity for PBP-3. The chemical structure also reduces the propensity to induce class C β-lactamases in organisms such as *Enterobacter* spp., *C. freundii, Serratia* spp., indole-positive *Proteus* spp., and P. *aeruginosa.* Ureidopenicillins are active against *Klebsiella* spp. *in vitro,* but activity is lost when heavy inocula are tested (42). Organisms that are resistant to ampicillin because of the acquisition of β-lactamases also tend to be resistant to the ureido and piperazine penicillins (10).

The addition of a carboxylic (**carboxypenicillins**), sulfamic, or sulphonic acid on the carbon atom of the acyl side chain of the benzylpenicillin nucleus markedly increases activity against *P. aeruginosa,* stabilizing these antibiotics against the chromosomal AmpC β-lactamase produced by the organism (10,43). Carbenicillin has some activity against am-

picillin-resistant indole-positive *Proteus* spp. and *Enterobacter* spp., but *Klebsiella* spp. are generally resistant (10).

The **amidino penicillins** have alkyl groups on the amidino nitrogen atom (43). Mecillinam and its oral ester pivmecillinam have low activity against Gram-positive bacteria, although almost all rapidly growing fermentative Gram-negative bacilli, such as *E. coli, Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., and *Proteus* spp. are susceptible. *Pseudomonas* spp., *B. fragilis,* and *H. influenza* are resistant (10).

The **non-classical 6-α-methoxypenicillin**, temocillin, is the only penicillin with complete stability to hydrolysis by transferable β-lactamases of Gram-negative bacteria and by the AmpC chromosomal enzymes (43).

Cephalosporins

Cephalosporin C, the original member of the cephalosporin class of β-lactam antibiotics, contains a side chain derived from D-α-aminoadipic acid condensed with a dihydrothiazine β-lactam ring system (7-aminocephalosporanic acid), which renders it resistant to staphylococcal penicillinase (44).

The **first-generation cephalosporins** were introduced into clinical practice in the mid-1960s and were stable to the β-lactamases known at the time. They permeated the outer membrane of Gram-negative bacilli more rapidly than penicillins. However, clinical isolates with diminished permeability emerged, nosocomial infection due to Gram-negative bacilli became more prevalent, and these organisms displaced *S. aureus* as predominant nosocomial pathogens. *K. pneumoniae*-carrying plasmids encoding TEM-1 in addition to multiple antibiotic resistant genes became endemic in hospitals. Isolates rarely implicated in clinical resistance (such as *S. marcescens* and *Acinetobacter* spp.) emerged as a result of the hyperproduction of class C cephalosporinases (35,45).

The **second-generation cephalosporins**, cefamandole and cefuroxime, have increased activity against Gram-negative microorganisms (46). They were stable to hydrolysis by plasmid-mediated β-lactamases and were more stable than cefoxitin to the chromosomal class C cephalosporinases of several *Enterobacteriaceae* when used clinically (35).

Resistance to second-generation cephalosporins arose as a result of hyperproduction of the species-specific class A chromosomal β-lactamase of *Klebsiella oxytoca* because of promoter mutations (47,48), hyperproduction of β-lactamases in *Enterobacteriaceae* because of regulator gene mutations (49,50), the production of inducible chromosomal β-lactamases by *Pseudomonas* spp. (35), and the hyperproduction of class C β-lactamases (51,52).

Third-generation cephalosporins are generally less active than first-generation cephalosporins against Gram-positive cocci but are much more active against *Enterobacteriaceae,* including the β-lactamase-producing strains (44). The aminothiazolyl and iminomethoxy groups are substituents in third-generation cephalosporins (43), yielding greater stability to the chromosomal class C β-lactamases together with an increased spectrum of activity. Different derivatives were introduced in the classes in an attempt to increase antibacterial spectrum and to improve pharmacokinetic and pharmacodynamic characteristics (35). Third-generation cephalosporins vary in their ability to induce β-lactamases, but none are as effective inducers as the cephamycins, clavams, or carbapenems (43).

Hyperproduction of the species-specific class A chromosomal β-lactamase of *K. oxytoca* conferred a unique resistance phenotype to third generation cephalosporins with high minimum inhibitory concentrations (MICs) of ceftriaxone, moderate MICs of cefotaxime, and low MICs of ceftazidime (35). Hyperproduction of class C β-lactamases conferred clinically relevant resistance to third-generation cephalosporins, as well as cephamycins, monobactams, β-lactamase inhibitors, sparing carbapenems only (51,52). The discovery of *Klebsiella* isolates resistant to oxyiminocephalosporins in 1983 (53) marked the beginning of a major new era in the history of resistance to β-lactam antibiotics mediated by extended-spectrum β-lactamases (ESBLs). Mutations in the structural genes of plasmid-mediated TEM, SHV, and OXA β-lactamases (35) and to a lesser extent in the PER (54) and CTX enzymes enhanced their affinity for third-generation cephalosporins and monobactams, albeit to varying degrees (55).

Fourth-generation cephalosporins contain a positively charged quaternary nitrogen atom at C-3, resulting in increased activity (compared to the third-generation cephalosporins) against β-lactamase derepressed mutants of *P. aeruginosa* and other enteric bacteria (56).

The fourth-generation cephalosporins, cefepime and cefpirome, have the 7-amino-thiazolyl groups (10). Cefepime is stable to hydrolysis by the more common chromosomal and plasmid-mediated β-lactamases, and it has poor affinity for inducible chromosomally mediated cephalosporinases. ESBLs hydrolyze cefepime to a lesser extent than third-generation cephalosporins, and although cefepime was found to have activity against ceftazidime-resistant Gram-negative bacteria such as *E. aerogenes* and *K. pneumoniae* (57), it is markedly prone to an inoculum effect (54). Hyperproduction of class C enzymes also confers resistance to these agents (35).

Cephamycins

Cephamycins, or α-methoxycephalosporins, resemble cephalosporins structurally but have a methoxy group at C-7 of the β-lactam ring of 7-aminocephalosporanic acid. The semisynthetic product, cefoxitin, exhibits a broad spectrum of activity and is highly resistant to hydrolysis by β-lactamases (58) by virtue of the 7-α-methoxy group. The methoxy group and other substituents on the 7-α position protect the β-lactam ring from attack (36). The discovery of cephamycins resulted in the development of compounds such as cefoxitin, cefotetan, latamoxef, cefbuperazone, and cefmetazole (59).

Compounds containing 7-α-methoxy groups are excellent inducers of chromosomally mediated β-lactamases and result in the selection of derepressed mutants, particularly among *E. cloacae* and *C. freundii* (43). Plasmids have acquired genes determining class C β-lactamases with cephamycinase activity (60). This has paved the way for dissemination among Gram-negative pathogens (59).

The **oxacephem**, latamoxef is highly resistant to β-lactamases due to its 7-α-methoxy group and is very effective against Gram-negative aerobes and anaerobes. It is, however, inactive against staphylococci. Flomoxef (a derivative of latamoxef with a difluoromethylthio-acetamide group at C-7) is an oxacephem with activity against both Gram-negative and Gram-positive pathogens. Both compounds are stable against ESBLs but are labile to cephamycinases (61).

Monobactams

Monobactams have a single β-lactam ring structure. The first members of this class were norcardins, but the only clinically used monocyclic β-lactam is aztreonam. The activation of the β-lactam ring in aztreonam is via a sulfonic acid substituent at C-1 while the C-3 side chain is identical to that of ceftazidime (10).

The monobactam nucleus has weak antibacterial activity and requires molecular substitution around it to realize its antibacterial potential. Side-chain substitution results in compounds with primarily Gram-positive, primarily Gram-negative, or broad-spectrum activity (62). Aztreonam has primarily Gram-negative activity.

Monobactams are labile to the class A chromosomal β-lactamase of *K. oxytoca* (48), class C enzymes (51,52), and ESBLs (35).

The 1-sulfonic residue of monobactams may be replaced with a phosphonate to yield a **monophospham**, or, with *N*-sulphonylated carbonyl amino moieties to yield **monocarbams** (63). Monophosphams have less intrinsic antibacterial activity but are more stable to β-lactamases (43).

Carbapenems

Carbapenems are 1-carbapen-2-em 3-carboxylic acids with substituents at the C-2 and C-6 positions (36). The two carbapenems, imipenem and meropenem, currently in clinical use have a simple *trans*-configured 6-hydroxy ethyl group conferring considerable β-lactamase stability compared with the *cis*-configured aminoacyl groups carried by most other β-lactam antibiotics. The compounds differ in their C-2 substituents with meropenem being 4- to 8-fold more active against Gram-negative bacteria but marginally less active against Gram-positive organisms (10).

The carbapenems have the broadest spectrum of activity of all β-lactam antibiotics. The excellent activity of imipenem is as a result of its high affinity for PBP-2 (an essential protein in cell wall synthesis in Gram-negative bacteria), its high affinity for critical PBPs of Gram-positive species, and its great β-lactamase stability (64). Both imipenem and meropenem are effective β-lactamase inhibitors as well. Although potent inducers of AmpC β-lactamases, their stability ensures the retention of clinically useful activity (65).

Biapenem, a newer carbapenem, has excellent activity against a wide range of bacterial pathogens and has high stability to serine β-lactamases. Although labile to metallo β-lactamases, hydrolysis rates of biapenem are lower than those of imipenem and meropenem for enzymes from *Bacteroides fragilis* and *Stenotrophomonas maltophilia* (66).

Plasmid-mediated carbapenem-hydrolysing metallo β-lactamases were identified in Japan (67,68) and have disseminated to *K. pneumoniae* (69).

Carboxypenams

The introduction of carboxyl substituents at C-2 in carboxypenams T-5575 and T-5578 in addition to the modification at C-6 confers greater antibacterial activity and stability to β-lactamases. T-5575 has a spectrum of activity similar to aztreonam but is stronger against most Gram-negative bacteria. It has also shown potent activities against ceftazidime-resistant *Enterobacter cloacae, C. freundii,* and *P. aeruginosa* (70).

Both compounds have poor affinity against Gram-positive bacteria. They are stable against a range of β-lactamases and have high affinities for PBP-3 of *E. coli* and *P. aeruginosa* (70).

Trinems

Trinems are a new class of β-lactam antibiotics containing a tricyclic nucleus as the main structural feature. The first trinem to be fully developed was sanfetrinem, a highly potent agent with a broad spectrum of activity against a wide range of Gram-positive and Gram-negative bacteria (excluding *Pseudomonas* spp.), aerobes, and anaerobes by virtue of its stability to the β-lactamases produced (71,72).

GV 129606 is a new parenteral trinem combining broad-spectrum activity with high potency and stability against the most common clinically relevant β-lactamases. It possesses a very broad antibacterial spectrum (including *Pseudomonas* spp.), is superior to any other penicillin or cephalosporin, and is comparable to meropenem (72).

β-Lactamases responsible for resistance to commonly used antibiotics are shown in Table II.

β-LACTAMASE INHIBITORS

Two major groups of clinically important β-lactamase inhibitors are clavulanic acid and the penicillanic acid sulphones, sulbactam and tazobactam. Clavulanic acid has been combined commercially with amoxicillin and ticarcillin, sulbactam with ampicillin and, in some countries, with cefoperazone, and tazobactam with piperacillin (10).

Clavulanic Acid

Clavulanic acid is a naturally occurring β-lactamase inhibitor derived from *S. clavuligerus* (73). It inhibits many class A β-lactamases, including staphylococcal penicillinase, ESBLs, the most prevalent plasmid-mediated β-lactamase of Gram-negative bacilli (TEM-1), and the chromosomal enzymes from *B. fragilis, P. vulgaris,* and *Citrobacter diversus* (74). It has slight activity against class C chromosomal β-lactamases (10).

Clavulanic acid can penetrate bacterial cell walls and can therefore inactivate both extracellular and intracellular β-lactamases (73), although it is generally a more potent inhibitor of cell-free enzymes (75). Its mechanism of action varies with the particular β-lactamase inhibited, but it generally acts as a competitive and often irreversible inhibitor (73).

Clavulanic acid can act as a cephalosporinase inducer. Studies have shown it to be a good inducer of the *E. cloacae, P. aeruginosa,* and *Proteus rettgeri* β-lactamases (12).

Sulbactam

Sulbactam is a penicillanic acid sulphone with a mechanism of inhibition similar to that of clavulanic acid. It is a weak antibacterial agent, relatively active against *N. gonorrhoea* (12), and most isolates of *Acinetobacter* spp. and *Bacteroides* spp. but usually has low activity against most others such as *E. coli* (10). Sulbactam inhibits ESBLs and penicillinases, although it is less efficient than clavulanic acid. Its in-

Table II. β-Lactamase-Mediated Resistance to Commonly Used B-Lactam Antibiotics

Class	Example/Prototype	β-Lactamase-Mediated Responsible for Resistance
Biosynthetic penicillins	Benzylpenicillin	Staphylococcal penicillinase (35)
Isoxazolyl penicillins	Cloxacillin, oxacillin, flucoxacillin, methicillin	Staphylococcal β-lactamases (10)
Aminopenicillins	Ampicillin	TEM-1 and SHV-1 in *Enterobacteriaceae* and PSE-1 in *Pseudomonas aeruginosa* (39–41)
Ureidopenicillins	Azlocillin, mezlocillin, piperacillin	As for aminopenicillins (10)
First-generation cephalosporins	Cephalothin, cephaloridine, cephazolin, cephradine, cefroxadine, cefradroxil, cefatrizine, cephalexin	TEM-1 and the hyperproduction of class C cephalosporinases (35,45)
Second-generation cephalosporins	cephamandole, cefuroxime, cefonicid, ceforanide, cefotiam	Hyperproduction of species-specific class A chromosomal β-lactamase of Klebsiella *oxytoca* (47,48), hyperproduction of classic TEM and SHV β-lactamases in *Enterobacteriaceae* (49,50), the inducible expression of chromosomal β-lactamases by *Pseudomonas spp.* (35), and the hyperproduction of class-C β-lactamases. (51,52)
Third-generation cephalosporins	cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, cefixime, ceftibuten, cefpiramide, cefsulodin	Hyperproduction of species-specific class A chromosomal β-lactamase of Klebsiella *oxytoca* (35), hyperproduction of class-C β-lactamases (51,52) and the expression of extended-spectrum β-lactamases (ESBLs) (35).
Fourth-generation cephalosporins	Cefipime	ESBLs, specifically SHV-5 hyperproduction, hyperproduction of class-C β-lactamases (35)
Cephamycins	Cefoxitin, cefotetan, latamoxef, cefbuprazone, cefmetazole	Inducible chromosomally mediated class-C β-lactamases, plasmid-mediated class-C β-lactamases (59)
Monobactams	Aztreonam	Class-A chromosomal β-lactamase of *K. oxytoca* (48), class-C β-lactamases (51,52), and ESBLs (35)
Carbapenems	Imipenem, meropenem, biapenem	Plasmid-mediated metallo β-lactamases identified in Japan (67) have disseminated to *K. pneumoniae* (69)

hibitory power for TEM-1 is reported to be weak, particularly if the β-lactamase is overproduced (76). Sulbactam has been reported as a poor inducer of most cephalosporinases with the exception of those in a particular *P. vulgaris* strain (12).

Tazobactam

Tazobactam, a penicillanic acid sulphone, is an irreversible β-lactamase inhibitor with activity against a wide range of β-lactamases including some chromosomal cephalosporinases of *Providencia stuartii*. It possesses no inherent antibacterial activity (77) and has not been found to be a good inducer of cephalosporinases (12).

Tazobactam is equipotent to clavulanic acid (78). However, its viability against SHV-derived ESBL producers is controversial. Certain studies (79,80) have reported that *E. coli, K. pneumoniae,* and their transconjugants expressing SHV-2, -3, -4, and –5 were typically resistant to inhibition by tazobactam while Livermore (78) quoted another study that reported good susceptibility to the combination.

A number of new penicillanic acid sulphones, such as Ro 48-1220, a 2-β alkenyl penicillanic acid sulphone (81), GD 40, a 6α-halo-2 β-chloromethyl sulphone (82), and the sodium salt of the 7-((2)-(2′-pyridyl)methylene) cephalosporanic acid sulphone (83) are presently under investigation. All these compounds compare favorably with the inhibitors presently used clinically.

The emergence and subsequent prevalence of inhibitor resistant β-lactamases has been observed since 1991 (84). Hyperproduction of classical plasmid-mediated β-lactamases overwhelming the inhibitor has also been implicated as a resistance mechanism (58).

Brobactam

6-β-Bromopenicillanic acid, brobactam, is an efficient inhibitor of β-lactamases produced by both Gram-positive and Gram-negative bacteria (85). It is a powerful reversible β-lactamase inhibitor prepared from 6-β-aminopenicillanic acid (86). It potently inhibits the more common plasmid-mediated enzymes, such as TEM-1, TEM-2, and SHV-1. The OXA-type enzymes are also susceptible to inhibition by brobactam as are the broad spectrum chromosomally-mediated enzymes found in *Klebsiella aerogenes* and the chromosomal cephalosporinases of *P. vulgaris* and *P. rettgeri* (85).

Brobactam has been used clinically with ampicillin. This combination compares favorably with other orally administered β-lactam antibiotics presently used in clinical practice (85).

Other Inhibitors

Penems such as BRL42715 (87) and SYN-1012 are potent β-lactamase inhibitors but certain pharmacokinetic properties precluded their development for clinical use (88).

Ro 48-1256 is a bridged monobactam inhibitor, which inhibits class C β-lactamases but lacks appreciable antibacterial activity of its own (89).

A number or mercapto-acetic acid thiol esters have been

identified as metallo β-lactamase inhibitors with free mercapto-acetic acid functioning as a competitive inhibitor (90).

CONCLUSION

Penicillinase-resistant penicillins, broad-spectrum penicillins, and first-generation cephalosporins were the first line of defense against bacterial infections for more than 20 years before β-lactamase-mediated resistance in Gram-negative bacteria became a serious problem. In response the pharmaceutical industry introduced novel classes of β-lactam antibiotics, *viz.,* cephamycins, oxyimino-cephalosporins (second-, third-, and fourth-generation cephalosporins), carbapenems, monobactams and clavam, and penicillanic acid sulfone inhibitors (35). Their clinical use has, however, resulted in the selection of diverse β-lactamases with ever-increasing spectra of antibiotic substrates (55).

There are presently 340 documented β-lactamases, a testament to the genetic pliability of bacteria. Knowledge of the types and hydrolytic spectra of enzymes prevalent in particular health care environments may thus guide the choice of appropriate therapy (55).

The correlation of resistance phenotypes with the expression of particular β-lactamase types has been considered to allow the prediction of an isolate's enzyme-mediated resistance mechanism from its antibiogram inferred from MIC or zone determinations. This is advantageous in that the antibiogram reported to the clinician can be usefully edited once a resistance mechanism has been inferred. Further antibiotics meriting testing may be predicted. For example, a *Klebsiella* isolate resistant to penicillins, narrow-spectrum cephalosporins, ceftazidime, and aztreonam but susceptible to cefotaxime and cefoxitin probably expresses a ceftazidime-preferring ESBL. Clinical experience has shown that the producers of these enzymes fail to respond to cefotaxime and that the isolate should be reported resistant to it. The inference of this mechanism also suggests that it is futile to test further extended-spectrum β-lactam antibiotics but that carbapenems and β-lactamase inhibitor combinations may merit further testing. If the isolate was also resistant to cefoxitin and cefotaxime, the inferred resistance mechanism would be the production of a plasmid-mediated AmpC β-lactamase, in which case there would be no need to test and β-lactamase inhibitor combinations (78).

However, prediction based on antibiograms fails with isolates producing exceptionally large or small amounts of enzyme. The level of β-lactamase expression varies widely among different isolates because it is a function of gene copy number and type of promoter. Such isolates may behave anomalously especially with β-lactamase inhibitor combinations. Prediction also fails when isolates have multiple resistance mechanisms or express more than one β-lactamase type. The latter is an increasing problem in developing countries and intensive care units (78).

The identification of a particular resistance phenotype and by extension the putative β-lactamase involved, was designed to assist in the choice of therapeutic strategies. However, the emergence of such complex organisms carrying multiple and diverse β-lactamases has several implications. First, irrespective of the presence of AmpC enzymes, the expression of multiple β-lactamases increases the likelihood that β-lactamase inhibitors will be overwhelmed (78). Second, the complexity constrains the value of "interpretative reading," which is the basis of the rules introduced to interpret antibiogram data. Third, surveillance of the molecular basis of antimicrobial resistance becomes progressively more complicated, with the increasing possibility that some resistance mechanisms present in an isolate will be masked by others. These are grave concerns in view of the emphasis placed on the need for good quality surveillance of resistance and its causes.

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