β-Lactams: chemical structure, mode of action and mechanisms of resistance

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This synopsis summarizes the key chemical and bacteriological characteristics of β-lactams, penicillins, cephalosporins, carbanpenems, monobactams and others. Particular notice is given to first-generation to fifth-generation cephalosporins. This review also summarizes the main resistance mechanism to antibiotics, focusing particular attention to those conferring resistance to broad-spectrum cephalosporins by means of production of emerging cephalosporinases (extended-spectrum β-lactamases and AmpC β-lactamases), target alteration (penicillin-binding proteins from methicillin-resistant *Staphylococcus aureus*) and membrane transporters that pump β-lactams out of the bacterial cell.

Keywords: β-lactams, chemical structure, mechanisms of resistance, mode of action

Historical perspective

Antimicrobials must be understood as any kind of agent with inhibitory or killing properties to a microorganism. Antibiotic is a more restrictive term, which implies the natural source of the antimicrobial agent. Similarly, underlying the term chemotherapeutic is the artificial origin of an antimicrobial agent by chemical synthesis [1]. Initially, antibiotics were considered as small molecular weight organic molecules or metabolites used in response of some microorganisms against others that inhabit the same 'habitat' and that compete for the same nutrients. However, recently, some authors and, particularly, Davies [2] are trying to open a discussion suggesting that in the environment, the majority of these compounds play important roles in the modulation of metabolic function in natural microbial communities. So, according to this theory, antibiotics are important chemical messengers, acting in cell–cell communication in a microbial ecosystem as signaling molecules [3].

Alexander Fleming first noticed the antibacterial nature of penicillin in 1928. When working with another bacteriological problem, Fleming observed a contaminated culture of *Staphylococcus aureus* with the mold *Penicillium notatum*. Fleming remarkably saw the potential of this unfortunate event. He discontinued the work that he was dealing with and was able to describe the compound around the mold and isolates it. He named it penicillin and published his findings along with some applications of penicillin [4]. However, it was not until 1940 that the first clinical trial with penicillin was undertaken against a streptococci infection in a mice model. It was then that the first β-lactam antibiotic was discovered. In fact, penicillin's chemical structure was described later, by Hodgkin *et al.* [5], by means of X-ray crystallography. Fig. 1 is one the pictures used by Sir Alexander Fleming Nobel, in the Award lecturer in 1945, and shows a circle free of *S. aureus* around the *P. notatum* (mold).

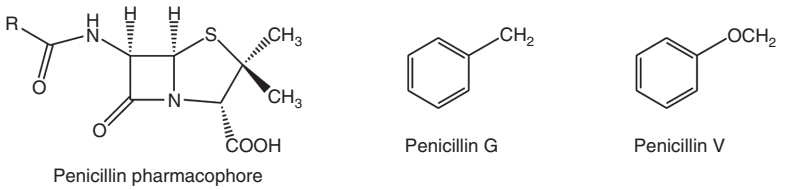

Fig. 1. Chemical structure of penicillin G and V.

Of the β-lactam antibiotics that are currently available, all feature the reactive β-lactam ring system, a highly strained and reactive cyclic amide. There are five relevant ring systems, including the penam, penem, carbapenem, cefem and monobactam ring structure.

Penams

Penams are a large group of β-lactams that include penicillins. Therefore, penicillins possess a basic bicyclic structure, 6-aminopenicillanic acid or 6-APA. This structure is composed of an enclosed dipeptide formed by the condensation of L-cystein and D-valine, resulting in the β-lactam ring and in the thiazolidinic ring [6].

The reactive nature of the β-lactam ring system makes penicillins (penams) and related compounds susceptible to a variety of degradative processes.

At acid environments and room temperature, the β-lactam ring is reconfigured: beginning with the protonation of the β-lactam nitrogen, followed by the nucleophilic attack of the remaining lateral chain carbonyl. The intermediate oxazolin ring will originate a new imidazol and, thus, form penillic acid [6]. This process has some clinical interest due to stomach acidity. So, in order to be able to administrate orally, these compounds have to be protected from acid mediums. Finally, treatments in acid environments with high temperatures or with mercury chloride may disrupt the β-lactam ring, originating tiazolodin and penicillinamine, among other products. As it acts as a chelating agent to heavy metals, penicillinamine can be also used after intoxication with those heavy metals in rheumatoid arthritis and even in other autoimmune disorders [7].

The original penicillins were produced by fermentation and were often mixtures of various β-lactams, such as penicillins G and V (Fig. 1). The availability of 6-APA has allowed the creation of hundreds of synthetic and semisynthetic penicillins.

In addition to chemical degradation, many bacteria produce a group of enzymes specifically designed to degrade and inactivate β-lactams. These enzymes are collectively known as penicillinases. By far the most prevalent type of penicillinase is the β-lactamase, which directly attacks and disrupts the β-lactam bond, inactivating the antibiotic [8]. There are also a variety of acylases that have been isolated from bacteria. These enzymes cleave the acylamino side-chain of the antibiotic, a modification that also inactivates the molecule.

The first molecule synthesized was methicillin, which differs from benzylpenicillin in the substitutions at positions 2' and 6' of the benzene ring by methoxy groups, causing steric hindrance around the amide bound [9].

Molecules that also have been developed and that are similar to methicilin (in terms of steric hindrance) are nafcillin, quinacillin and ancillin. These molecules are included in the group I of synthesized penicillins. Another group of synthesized penicillins is characterized by the presence of an isoxazol pentacycle in the 'ortho' position of the benzene ring, the stability of which against hydrolysis by penicillinases is increased by substitution at position 5' of a methyl group (oxacillin). The introduction of a chlorine atom at position 2' (cloxacillin) or two fluorine atoms at positions 2' and 6' (dicloxacillin) and fluorine atom at position 6' (floxacillin) in the benzene ring increases its stability against hydrolysis. However, these modifications cause a general decrease in the effectiveness of β-lactams, and almost all clinically available β-lactamase-resistant penicillins are less potent than the parent molecules.

Nowadays, there are also some compounds available, such as clavulanic acid, tazobactam and sulbactams, which can bind to β-lactamases irreversibly and, thus, inactivate them. There are pharmacological formulae that result from the combination of penicillins with β-lactamase inhibitors [10]. This will be discussed later in the mechanisms of resistance to β-lactams.

Ampicillin and amoxicillin belong to a group of penicillinase-sensitive, orally active antibiotics in which the phenylacetic acid moiety is replaced by a phenylglycine in the D-configuration. These antibiotics have a broader spectrum than penicillin G, but are quite susceptible to β-lactamase. They are often given with clavulanic acid to avoid enzymatic degradation.

Another result of the reactivity of β-lactam is the formation of allergenic haptens *in vivo*. Nucleophilic hydroxyl (OH^-) or sulphydryl (SH^-) groups on certain

proteins can react with the β-lactam ring system, creating a covalent penicillin–protein conjugate that can induce an allergic response, thus accounting for the inherent allergenicity of these antibiotics. About 6–8% of the population is allergic to β-lactam antibiotics [11].

Cephems

Since 1970s, cephalosporins, the major representative group of cephems, have been among the most potent and most widely used anti-infective agents. They are well tolerated and their development has paralleled that of the penicillins. Because of their importance, it is essential to classify cephems in order to allow their optimal use [12]. Numerous classifications have been proposed: chemical, biological, microbiological, pharmacokinetic and immunological. The chemical and microbiological aspects of cephems classification will be described below in more detail.

Unlike penicillins, in which the initial agent in the series was marketed with little structure–activity relationship development, cephalosporins required a great deal of refinement before a clinically useful agent was discovered [13]. The original *Cephalosporium acremonium* culture was discovered in a sewer outlet in Sardinia (1954), and Newton *et al.* [14] isolated cephalosporin C, a weak antibiotic compound that showed some activity against penicillin-resistant cultures. Chemical removal of the cephalosporin C side-chain forms 7-aminocephalosporanic acid or 7-ACA, which, like its congener 6-APA, was used as a synthetic starting point for most of the cephalosporins available today. It is now more economically feasible to produce 7-ACA from penicillin G in a seven-step synthesis, rather than to incur the cost of large-scale fermentation of cephalosporin C [15].

The metabolism of cephalosporins is analogous to those described for penicillin. In terms of their chemical mechanism, cephalosporins are very similar to penicillins, forming a covalent bond with peptidoglycan synthetases (PBPs) and causing cell lyses. Susceptible cephalosporins can be hydrolyzed by β-lactamases, and in fact some β-lactamases are more efficient at hydrolyzing cephalosporins than penicillin itself. Allergic reactions are not as common in this chemical class as in the penicillin class [11,16].

Chemical classification

Chemically, cephems can be classified into five different classes: cephalosporins; cephamycins; oxa-1-cephems; carba-1-cephems; and miscellaneous (Fig. 2). Cephalosporins, according to their side-chain at position 7, may be non-α-substituted cephalosporins, α-substituted cephalosporins and oxyimino-cephalosporins (Fig. 3).

Microbiological classification

Traditionally, cephalosporins are divided into first-generation, second-generation, third-generation, fourth-generation and fifth-generation, according to their antibacterial activity (Table 1). They differ in their antimicrobial spectrum, β-lactamase stability, absorption, metabolism, stability and side-effects. First-generation members have narrowed or limited activity when compared with third-generation, fourth-generation or fifth-generation broader spectrum cephalosporins. The structure–activity features responsible for the various properties in the penicillins (oral activity, β-lactamase stability, etc.) are similar with the cephalosporins [17].

First-generation cephalosporins First-generation cephalosporins are very active against Gram-positive cocci, except enterococci and methicillin-resistant staphylococci, and moderately active against some Gram-negative rods primarily *Escherichia coli*, *Proteus*, and *Klebsiella*. Anaerobic cocci are often sensitive, but *Bacteroides fragilis* is not.

Cephalexin, cephradine and cefadroxil are absorbed from the gut to a variable extent and can be used to treat urinary and respiratory tract infections. Other first-generation cephalosporins must be injected to give adequate levels in blood and tissues. Cefazolin is a choice for surgical prophylaxis because it gives the highest (90–120 μg/ml) levels with every 8-h dosing. Cephalothin and cephapirin in the same dose give lower levels. None of the first-generation drugs penetrate the central nervous system, and they are not drugs of first choice for any infection [18].

Second-generation cephalosporins The second-generation cephalosporins are a heterogeneous group. All are active against organisms covered by first-generation drugs, but have extended coverage against Gram-negative rods, including *Klebsiella* and *Proteus*, and not against *Pseudomonas aeruginosa* [19]. Some (not all) oral second-generation cephalosporins can be used to treat sinusitis and otitis caused by *Haemophilus influenzae*, including β-lactamase-producing strains.

Cefoxitin and cefotetan are not cephalosporins but cephamycins. Often, cephamycins are considered as second-generation *cephlosporins* for its clinical utility. They are particularly active against *B. fragilis* and, thus, are used in mixed anaerobic infections, including peritonitis or pelvic inflammatory disease [18].

Third-generation cephalosporins Third-generation cephalosporins have decreased activity against Gram-positive cocci, and enterococci often produce super-infections during their use. Most third-generation cephalosporins are active against staphylococci, but ceftazidime is only weakly active [19]. A major advantage of third-generation drugs is their enhanced activity against Gram-negative rods [20]. Whereas second-generation drugs tend to fail against *P. aeruginosa*, ceftazidime or cefoperazone may

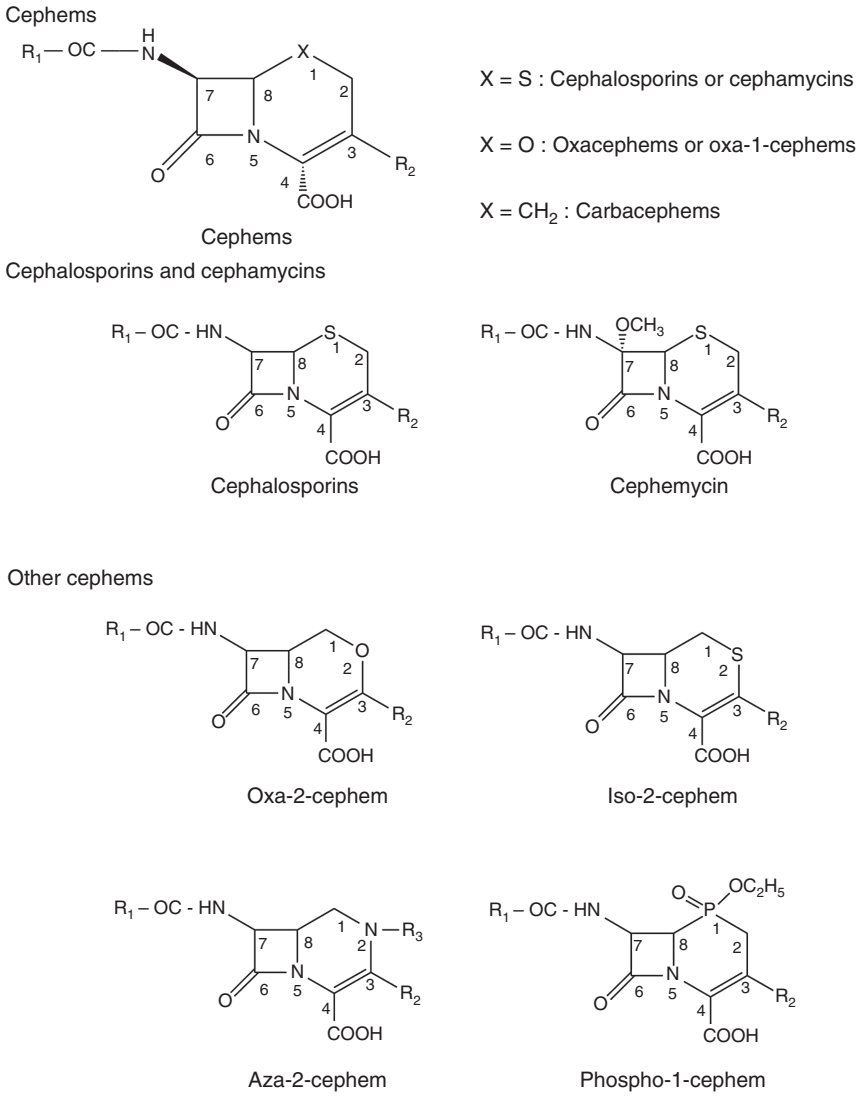

Fig. 2. Chemical structures of the five main groups of cephems. The presence of a sulfur atom at position 1 indicates the group of cephalosporins except when an α-7-methoxygroup is present, which indicates a cephamycin. The existence of a central atom at position 1 indicates an oxa-1-cephem if it is an oxygen and a carbacephem if it is a carbon atom. The four structures shown are examples of the fifth group.

succeed. Thus, third-generation drugs are very useful in the management of hospital-acquired Gram-negative bacteremia. In immunocompromised patients, these drugs are often combined with an aminoglycoside. Ceftazidime may also be lifesaving in severe melioidosis (*Pseudomonas pseudomallei*) infection.

Another important distinguishing feature of several third-generation drugs is the ability to reach the central nervous system and to appear in the spinal fluid in sufficient concentrations to treat meningitis caused by Gram-negative rods. Cefotaxime, ceftriaxone or ceftizoxime given intravenously is normally the choice

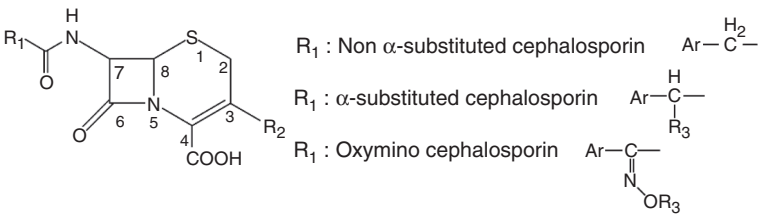

Fig. 3. Chemical features of the three main cephalosporin structures.

Table 1. Major group of cephalosporins according to their antimicrobial activity.

First-generation	Second-generation	Third-generation	Fourth-generation	Fifth-generation
Cephalothin	Cefamandole	Cefotaxime	Cefepime	Ceftobiprole
Cephapirin	Cefuroxime	Ceftizoxime	Cefpirome	Ceftaroline
Cefazolin	Cefonicid	Ceftriaxone		
Cephalexin[a]	Ceforanid	Ceftazidime		
Cephradine[a]	Cefoxitin[b]	Cefoperazone		
Cefadroxil[a]	Cefmetazole[b]	Cefixime[a]		
	Cefminox[b]	Ceftibuten[a]		
	Cefotetan[b]	Cefdinir[a]		

[a]Oral cephalosporins; all the others are parental cephalosporins. [b]Besides being cephamycins (chemical classification), they are usually included in the microbiological classification as second-generation cephems.

for management of Gram-negative bacteria sepsis and meningitis [21].

Fourth-generation cephalosporins Cefepime and cefpirome are the only fourth-generation cephalosporins in the market. They have enhanced activity against *Enterobacter* and *Citrobacter* species that are resistant to third-generation cephalosporins. Cefepime has activity comparable with that of ceftazidime against *P. aeruginosa*. The activity against streptococci and methicillin-susceptible staphylococci is greater than that of ceftazidime and comparable with that of the other third-generation compounds [22].

Fifth-generation cephalosporins Fifth-generation cephalosporins were developed in the laboratory to specifically target against resistant strains of bacteria. Particularly, ceftobiprole is effective against methicillin-resistant *S. aureus* (MRSA). Until this drug was introduced, this strain of *Staphylococcus* was impossible to contain. Other drugs in this class include cefotetan and cefoxitin, used against anaerobic Gram-negative bacilli. This class of drugs is ineffective against enterococci bacteria. Ceftaroline is a new oxyimino-cefalosporine that is also effective against MRSA [23], but ineffective against extended-spectrum β-lactamase (ESBL) producers or active AmpCs. However, ceftaroline has showed to be effective against broader spectrum β-lactamases (ESBLs and AmpCs) in synergism with amikacin [24].

Other β-lactams

Monobactams

Monobactams have a monocyclic β-lactam ring and are resistant to β-lactamases. They are active against Gram-negative rods, but not against Gram-positive bacteria or anaerobes. The first drug to become available was aztreonam [19]. Patients with immunoglobulin-E-mediated penicillin allergy can tolerate it without reaction and, apart from skin rashes and minor aminotransferase disturbances, no major toxicity has been reported. Super-infections with staphylococci and enterococci can occur [25].

Carbapenems

These drugs are structurally related to β-lactam antibiotics. Imipenem, the first drug of this type, has good activity against many Gram-negative rods, Gram-positive organisms and anaerobes. It is resistant to some β-lactamases, but is inactivated by dihydropeptidases in renal tubules. Consequently, it is administered together with a peptidase inhibitor such as cilastatin [19].

Imipenem (Fig. 4) penetrates the body tissues and fluids well, including cerebrospinal fluid. Imipenem may be indicated for infections caused by microorganisms resistant to other drugs. Nevertheless, *Pseudomonas* species rapidly develop resistance to this drug, and the concomitant use of an aminoglycoside is, therefore, required. However, this procedure may not delay the development of resistance [25].

Meropenem (Fig. 4) is similar to imipenem in pharmacology and antimicrobial spectrum of activity. However, it is not inactivated by dipeptidases and is less likely to cause seizures than imipenem [19].

Antimicrobial resistance to β-lactam

Since the discovery of the first antibiotic, penicillin, by Alexander Flemming in 1928, until now enormous changes in this field have occurred. First of all, the use of antibiotic was a medical revolution like no other in the

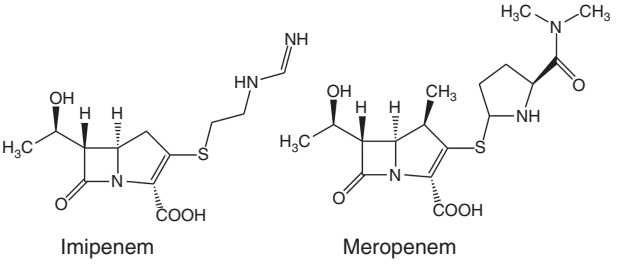

Fig. 4. Chemical structure of two carbapems: imipenem and meropenem.

treatment of infectious diseases [8]. Nevertheless, a rapid appearance of a great number of bacteria presenting acquired resistance was observed, thus resulting in therapeutic failures. Six years after the introduction of benzylpenicillin in the market, for example, the frequency of staphylococci resistance in British hospitals increased from less than 10% up to 60% and today is over 90% at world level [26].

Antibiotic mode of action and resistance

β-Lactams are a group of antibiotics that have specificity for bacteria. Bacteria are prokaryotic and, hence, offer numerous structural and metabolic effects that differ from those of the eukaryotic cells such as the animal or human host. There are several possible targets for antibiotics [27,28]. Generally speaking, we can group the mechanisms of action of antibiotics into five categories (Fig. 5): inhibition of cell wall synthesis; impairment cytoplasmic membrane; inhibition of nucleic acid synthesis; inhibition of protein synthesis; and metabolic antagonist action. In general, there are four basic mechanisms (Fig. 5) by which resistance to drug may occur in bacteria: alteration of the antimicrobial target that can be due to the complete loss of affinity or simple reduction of it; reduction in the amount of the antimicrobial that reaches the target by entrance reduction caused by a decrease permeability due to porin mutation or by an exit increase caused by the pumping out by an efflux transporter; the presence of an enzymatic mechanism that totally or partially destroys the antimicrobial molecules; and the development of an alternative metabolic pathway involving precursors [28–30].

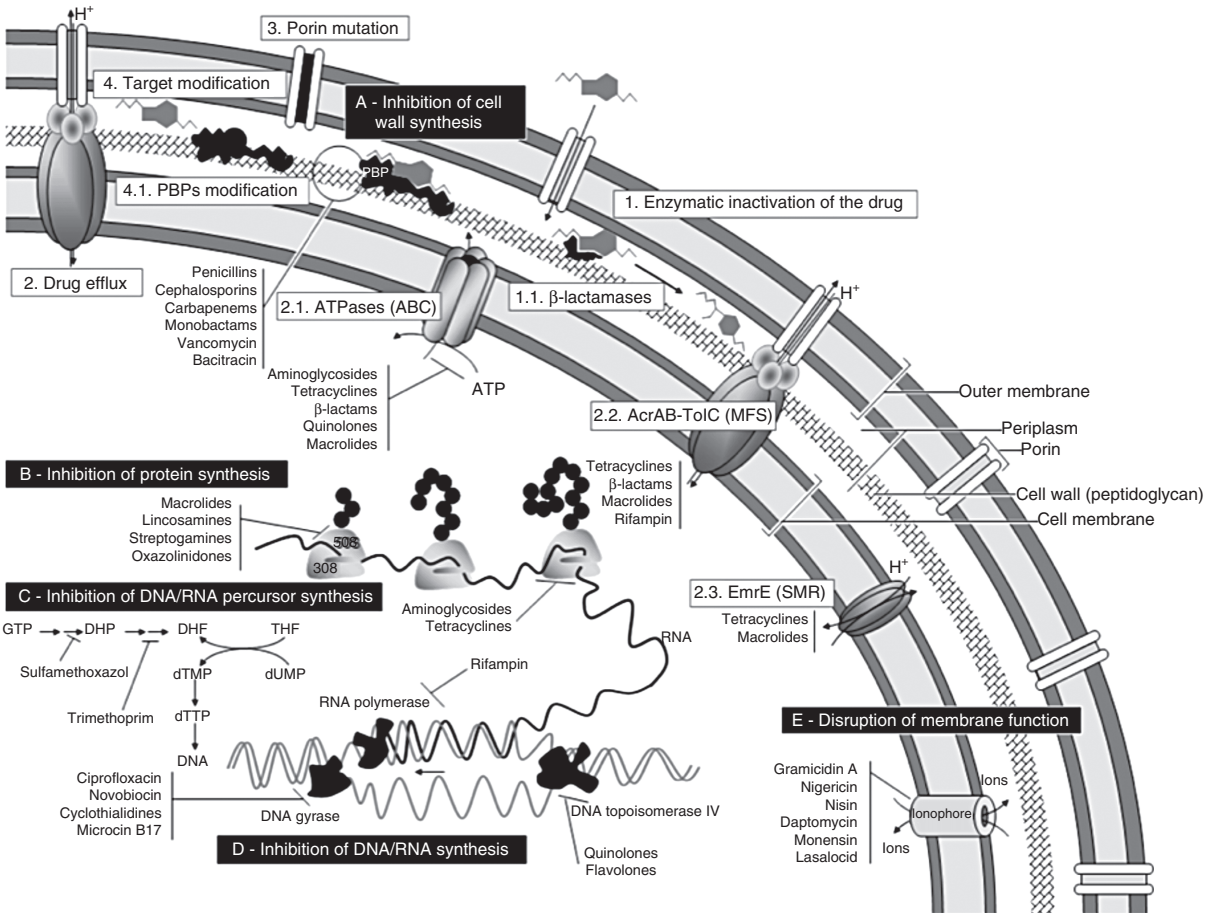

Fig. 5. Mechanisms of antimicrobial action and resistance in Gram-negative organisms. This picture represents a Gram-negative bacteria cell. Black boxes represent mechanisms of drug action and white boxes represent mechanisms of resistance. Below each box there are several examples of drugs presenting those types of mechanisms. The main mechanisms of antimicrobial action can be divided into five major classes. (a) Those who act in the cell wall synthesis; (b) those who act in the protein translation; (c) those who act in metabolic precursor biosynthesis; (d) those who act in the molecular genetics processes (replication, transcription); and (e) those who disrupt membrane function and permeability. Some of the mechanisms of resistance are represented here by numbers. (1) Enzymatic inactivation of the drug by the presence of β-lactamases (1.1); (2) presence of an enhanced efflux pump, whether it is by an active transport system involving ATPases (2.1) or rather if it is driven by proton motive force (2.2, 2. 3); (3) porin mutation obstructing the drug entrance; and (4) target modification of the drug, such as the mutation in the penicillin binding proteins (PBPs).

β-Lactamases

The most widespread mode of clinical resistance development to β-lactam antibiotics is the expression of β-lactamases that hydrolyze the antibiotic. It is estimated that $30 billion is the annual economic loss to the US population from disease caused by β-lactamase-producing resistant bacteria [27].

Serine β-lactamases

β-Lactamases hydrolyze the four-membered β-lactam ring in both penicillin and cephalosporin classes of antibiotics as well as the carbapenem series. They thereby destroy the antibacterial activity by deactivating the chemical properties of the drug molecule, which is the chemically reactive acylating group for modifying the active site serine side-chains in the PBPs. β-Lactamase activity was detected a few years before clinical use of penicillins in humans, indicating its presence in soil bacteria that combat the natural product penicillins [31].

There are many different types of β-lactamases and several different systems have been proposed to classify them. The functional classification was first attempted by Bush and collaborators [32] in 1989 and then improved in 1995. The structural classification proposed by Ambler [33] in 1980 suggests four distinct molecular classes: A, B, C and D based on the amino acid sequencing.

Ambler β-lactamases molecular classes A, C and D are active site serine enzymes, with architectural and mechanistic similarities to the PBPs, suggesting evolution from PBPs. In the A, C and D classes of β-lactamases, the same type of penicilloyl-*O*-Ser enzyme covalent intermediates are formed as in the catalytic cycle of PBPs, which attack and open the β-lactam ring and become self-acylated. There is no such covalent penicilloyl enzyme intermediate in the catalytic site of the zinc-dependent, class B β-lactamases, which enables the failure of class B β-lactamases to be inhibited by certain drugs [34].

It has been argued that PBPs may have evolved into β-lactamases independently to generate the different orientations of the active site residues in the class A, C and D β-lactamases [33].

The first plasmid-mediated β-lactamase in Gram-negative bacteria, TEM-1, was described in the early 1960s. The TEM-1 enzyme was originally found in a single strain of *E. coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM. Being plasmid-mediated and transposon-mediated, the spread of TEM-1 was facilitated to other species of bacteria. Within a few years after its first isolation, the TEM-1 β-lactamase spread worldwide and is now found in many different species of members of the family *Enterobacteriaceae, P. aeruginosa, H. influenzae* and *Neisseria gonorrhoeae* [29]. The TEM-1 and related TEM-2 β-lactamases, prevalent in Gram-negative bacteria such as *E. coli* and Klebsiella *pneumoniae*, are encoded on transposable elements and move rapidly through these populations [35].

Extended-spectrum cephalosporins such as ceftazidime and cefotaxime were developed to combat resistance provided by TEM-1 and related β-lactamases. In turn, subsequent widespread cephalosporin use is thought to have selected for sequential mutants in the TEM β-lactamases, producing hydrolytic enzymes that have improved affinity for these lactam scaffolds and consequent extended-spectrum β-lactam resistance. Many variants of TEM β-lactamases have been isolated and sequenced. Another common plasmid-mediated β-lactamase found in *K. pneumoniae* and *E. coli* is SHV-1 (for sulphydryl variable). SHV-1 β-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae*, but is usually plasmid mediated in *E. coli* [36].

Extended-spectrum β-lactamases The class A plasmid-encoded, broad-spectrum β-lactamases, TEM-1, TEM-2 and SHV-1, of Gram-negative bacilli hydrolyze penicillins and narrow-spectrum cephalosporins, but not extended-spectrum cephalosporins, aztreonam (the monobactam) and the carbapenems, imipenem and meropenem. However, variants of these enzymes are capable to destroy the four-member β-lactam ring of the extended-spectrum cephalosporins, that is, third-generation cephalosporins, and thus called extended-spectrum cephalosporinases or ESBLs [37].

By definition, ESBLs are plasmid-encoded β-lactamases that not only hydrolyze the third-generation cephalosporins but also penicillins and narrow-spectrum cephalosporins, but not the cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (e.g. imipenem, meropenem and ertapenem), which are inhibited by clavulanic acid [38].

Although ESBLs are inhibited by clavulanate, sulbactam and tazobactam, hyperproduction of these enzymes can result in resistance to β-lactam/β-lactamase combinations as well. The only β-lactam antibiotics that are reliably stable to the ESBLs are the carbapenems imipenem and meropenem [32]. Organisms carrying ESBLs are frequently resistant to other classes of antimicrobial drugs, such as aminoglycosides, trimethoprim/sulfamethoxazole and tetracyclines, as a consequence of additional resistance genes linked to the ESBL *bla* genes. In addition, these isolates are also commonly resistant to the fluoroquinolones [38].

ESBLs were first reported in the early 1980s in Europe. Since that time, ESBLs have been identified worldwide. The number of different types of ESBLs has steadily

increased, and also their prevalence. Another fast-growing group of non-TEM and non-SHV was first reported in a *E. coli* strain isolated from the fecal flora of a laboratory dog that was being used for pharmacologic tests in 1986 in Japan and another strain was then isolated in 1986 in Germany from a clinical isolate and was called CTX-M-1 due to its particular affinity to cefotaxime [39].

The β-lactam resistance emergence began even before the first β-lactam penicillin was developed. These enzymes are numerous, and they mutate continuously in response to the heavy pressure of antibiotic use, leading to the development of ESBLs. Examples are the mutated *TEM, SHV* and *CTX-M* genes, mainly found in strains of *E. coli* and *K. pneumonia,* respectively [35].

The difference between TEM and SHV and CTX-M enzymes is their affinity for ceftazidime and cefotaxime. TEM and SHV have more affinity and lower catalytic constant (k_{cat}) to cefatazidime than to cefotaxime, both third-generation cefalosporins. CTX-M enzyme in opposition presents higher catalytic efficiency to cefotaxime than to ceftazidime [30].

CTX-M enzymes have become the most prevalent type of cefotaximases found during the past 5 years among ESBL-producing bacteria isolated in certain European and South American countries [40]. The CTX-M β-lactamases, now described in more than 50 different types, can be divided into five groups based on their amino acid identities: CTX-M1, CTX-M2, CTX-M8, CTX-M9 and CTX-M25 [39].

The CTX-M enzymes originated from the *Kluyvera* spp. of environmental bacteria, usually having higher activity against cefotaxime than ceftazidime (although certain types also inactivate ceftazidime), and are associated with mobile elements such as ISEcp1 [41]. The epidemiology of organisms producing CTX-M enzymes is very different from those that produce TEM-derived and SHV-derived ESBLs. CTX-M enzymes are not limited to nosocomial infections caused by *Klebsiella* spp., and their potential ability to spread beyond the hospital environment serves to exacerbate public health concerns. *E. coli* is most often responsible for producing CTX-M β-lactamases and seems to be a true community ESBL pathogen [42].

AmpC β-lactamases The two types of β-lactamases that are causing most of the increasing multidrug resistance (MDR) seen in Gram-negative bacillary pathogens are class A ESBL and the class C enzymes, namely the chromosomal-encoded AmpC β-lactamases [43]. AmpC β-lactamases are widely well distributed and are expressed constitutively at very low levels. These enzymes generally do not contribute to β-lactam resistance, but in some organisms (*Serratia marcescens*, *Citrobacter freundii*, *Morganella morganii*, *Providencia stuartii*, *Acinetobacter calcoaceticus* and especially *Enterobacter cloacae*) they can be induced under certain circumstances such as the presence of a β-lactam or mutations on regulatory genes *ampR*, *ampD* and *ampG* involved in the expression of AmpC β-lactamases [44].

Zinc β-lactamases

β-Lactamases of class B have a binuclear zinc cluster in the active site, but are commonly known as metallo-β-lactamases or MBLs. Unlike the class A, C and D β-lactamases, which open the β-lactam ring via covalent acyl enzyme intermediates, described above, the class B

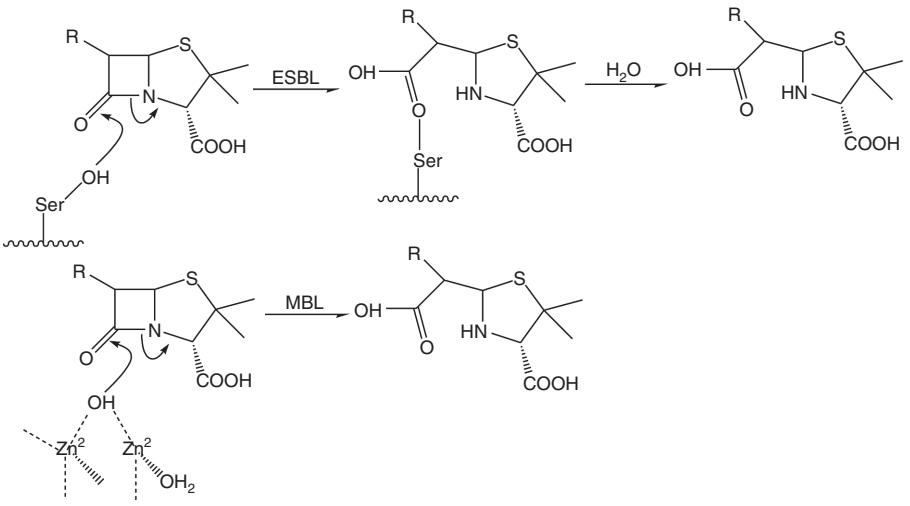

Fig. 6. Hydrolytic β-lactam ring opening and deactivation by serine proteases. For example, by extended-spectrum β-lactamases (ESBLs) (picture on top) and by metallo-proteases (e.g. metallo-β-lactamases or MBLs) (picture on bottom). Mechanism mediated by A, C and D serine enzymes (on top) involves a covalent penicilloyl enzyme intermediate, whereas class B zinc-dependent MBL (on bottom) carries out direct attack by water.

β-lactamases use zinc to activate a water molecule and catalyze its direct addition to the β-lactam ring (Fig. 6).

The MBLs of type B are thought to be the major subclass of hydrolases that destroy the carbapenem antibiotics such as imipenem (thienamycin) and mer-openem. The widespread use of carbapenems in Japan has probably been instrumental in selecting the IMP-1 version of the zinc β-lactamase first seen in *Ser. marcescens* and *P. aeruginosa* [45]. The carbapenemases have been described as a clinical concerning issue for pseudomonal infections, but the more acute carbapenem resistance problems in *P. aeruginosa* are efflux mechanisms [31].

β-Lactams' target alteration

The most important example of target alteration to β-lactams is the case of MRSA. By definition, the presence of *mecA* gene is responsible for methicillin resistance phenotype in staphylococci [46]. Originally, *S. aureus* has four PBPs (PBP 1, 2, 3 and 4). The *mecA* gene encodes a modified PBP of 78 kDa, designated PBP2a or PBP2', which is a peptidoglycan transpeptidase, which differs from *S. aureus* endogenous PBP. This peptidoglycan transpeptidase PBP2a retains its normal enzymatic activity. However, the PBP2a differs because it has the recognition site for β-lactam modified. Thus, when the PBPs are linked to β-lactams and become inactive, except that PBP2a by being insensitive to various β-lactams, including methicillin. Therefore, despite being linked to methicillin, the PBP2a can also promote cell wall synthesis [47].

The regulation of expression of *mecA* and the consequent production of PBP2a that confers resistance to methicillin in MRSA is processed and mediated by an operon. Thus, the complex regulator of *mecA* gene consists of three genes, *mecR1*, *mecA* and *mecI*. The exterior domain of the protein is also a MecR1 PBP. So when the β-lactam binds covalently to the PBP domain, in this case the fragment MecR1, transmembrane signaling is initiated, resulting in the release of a cytoplasmic fragment with MecR2 in the interior domain. This fragment of MecR2 will subsequently cleave the protein into two fragments with intact MecI relieving, thus, the repression of the *mecA* gene, resulting in the synthesis of PBP2a [27].

Permeability changes to β-lactams

One of the mechanisms of resistance to β-lactam is the permeability change in outer membrane. This alteration on permeability can be due to the presence of efflux proteins or to the alteration or loss of porins. The presence of efflux proteins in the cell wall of both Gram-negative and Gram-positive has been known as one of the causes of the pumping of some unrelated agents such as antibiotics, organic solvents, dyes and detergents. Generally, a wide range of structurally dissimilar compounds have also been identified, and these have become known as MDR exporters or MDR efflux pumps [48].

There are two major types of efflux pumps, ATP-dependent transporters and those that are secondary transports driven by proton motive force (PMF). Among PMF transporters, there are presently four main families: resistance nodulation division (RND), the major facilitator superfamily, the small MDR family and the MDR and toxic compound extrusion (MATE) family [49].

Both the proton and sodium ion gradients have been identified as the energy source for substrate transport for MATE family transporters [50]. Another important group of MDR pumps is the ATP-binding cassette (ABC) family that is not driven by PMF but is ATP-dependent. The ABC transporters are more important for clinical resistance in eukaryotic cells such as the resistance to chemotherapy presented by tumor cells [51], parasites [52] and some opportunistic fungi [53].

The RND-type pump is the one most thoroughly studied in Gram-negative bacteria. It is located in the cytoplasmic membrane of the bacteria, working together with a membrane fusion protein (MFP) that spans through the periplasmatic space and an outer membrane efflux protein (OEP). These three proteins (RND–MFP–OEP) form a complex that can move a substrate (e.g. an antibiotic) from the interior of the bacterium to the exterior. The best characterized of those complexes in *E. coli* is the AcrAB–TolC complex in which AcrB is the RND, AcrA is the MFP and TolC is the OEP [54].

It has been described that increased levels of multiple antibiotic resistance (*mar*) locus expression in relation to the presence of some efflux pumps, such as AcrB [53] and porin losses [55]. Thus, genetic regulation of AcrAB–TolC system seems to be complex and, besides *mar* locus, it seems to be involving the oxidative stress machinery, such as superoxide dismutase (*sox*Slocus) and *Rob*-binding proteins acting as transcriptional regulator, SdiA, and AcrR among others [56].

AcrAB–TolC system has also been found in some clinical important issues involving Gram-negative bacilli. In *Salmonella enterica,* it has been recently reported that the system AcrAB–TolC may have some importance in pathogenesis. There is also recent evidence that AcrAB may system be involved in the cell basic metabolism, as it participates in the intracellular regulation of the levels of coenzyme A in *E. coli* [57].

Regarding antibiotics in *E. coli* strains and other *Enterobacteriaceae*, mutation of either TolC or AcrA/B proteins, display hypersensitivity to quinolones, tetracyclines, tigecycline, eritromycin, novobiocin, among others [58]. Also, it has been demonstrated in *P. aeruginosa* that a similar pump (MexEF-OprN) has as substrates

some β-lactamase inhibitors (clavulanate, cloxacillin and BRL42715) [59]. However, few evidence until now has shown little involvement of AcrAB–TolC or other efflux systems in *E. coli* as a mechanism of resistance to β-lactams. A pilot study was published by Källman *et al.* [60] suggesting an efflux mechanism to cefuroxime resistance. More studies are needed to enlighten this issue.

Conclusion

In the last decades, antimicrobial resistance has gone from being an interesting scientific observation to a reality of great medical importance. There are no new antibiotics being developed by the pharmaceutical industry and for some pathogens fifth-generation cephalosporins are the ultimate drug. Massive usage of antibiotics in clinical practice resulted in resistance of bacteria to antimicrobial agents.

The introduction of the β-lactam antibiotics was met with the emergence of altered targets, such as PBP2a, resulting MRSA and antibiotic inactivating by β-lactamases. Some of these new β-lactamases, such as ESBLs and AmpCs, result from simple point mutations in existing *β-lactamase* genes that lead to a changed substrate profile. Also, these resistance genes have been borrowed from the chromosomally encoded genes that occur naturally in some species to conjugative plasmids increasing their spreading ability among other species, becoming an emerging public health concern.

Better understanding of the chemical structure for new drug development, of the mechanisms of antibiotic resistance and their expression would allow us to develop therapeutic, screening and control strategies that are needed to reduce the spread of resistant bacteria and their evolution.

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Conflicts of interest

There are no conflicts of interest.

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