Chapter 1

Antibacterial Drugs — From Basic Concepts to Complex Therapeutic Mechanisms of Polymer Systems

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Additional information is available at the end of the chapter

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Abstract

Infections caused by diverse bacteria represent a major problem that threats the health of humans. This stimulates the scientists to find new solutions for treating these diseases by clarifying the interactions of antibacterial compounds with the biological medium. In this context, the chapter presents some basic concepts regarding the antibacterial drugs. The synthesis routes of novel compounds and specific design techniques with polymer materials are described in correlation with the *in vitro* and *in vivo* activity of antibacterial substances. Essential data about the mechanism of action, selected *in vivo* efficacy and mechanisms of resistance to the most used antibacterial drugs are reviewed.

Keywords: Antibacterial, drugs, classification, synthesis, polymers

1. Introduction

In the past decades, many research findings were directed towards biomedical sciences in an attempt to give a solution to the present health issues caused by microorganism infections. Infectious diseases represent a considerable factor of human morbidity and mortality for most of human existence. The introduction of antimicrobial materials into general clinical use is one of the most successful approaches in chemotherapy, considerably contributing to the control of infections [1]. The existing antimicrobials in the clinical investigations have provided an array of choices when treating many types of infectious diseases. However, treatment options for patients are limited because the bacterial resistance

evolved more rapidly than the antibacterial drug development. Clinical results are reporting increasing rates of *in vitro* resistance among previously susceptible organisms and the occurrence of intrinsically resistant microorganisms as pathogens in immunocompromised hosts [2, 3]. To reduce the development and spread of antimicrobial resistance, the preservation of current antimicrobials through their appropriate use becomes mandatory. This motivates scientists to focus on discovery and production of new chemical substances that destroy pathogenic microorganisms with minimal damage to host tissues [4]. Thus, the design of novel classes of antibacterial drugs is redirected on synthesis of compounds with a completely novel mechanism of action [5].

For a deeper understanding of the interactions occurring between the antibacterial drugs and the human beings, in this chapter the basic concepts regarding these bioagents, like definition and classification, are presented. The latter involves the presentation of the main principles, which were taken into consideration to define the effectiveness of an antibiotic. The classical and modern synthesis routes of the main antibacterial drugs are further discussed regarding the main categories of antimicrobial agents. The implications of the preparation methods in designing new therapeutic systems based on polymer materials are analyzed in accordance with the advantages and disadvantages of such delivery devices. A short review on the *in vitro* and *in vivo* activities of antibacterial substances is performed by highlighting the beneficial treatments, and also the secondary effects caused by some delivery systems. Essential data about the mechanism of action, selected *in vivo* efficacy and mechanisms of resistance to the most used antibacterial drugs is presented.

2. Definition and classification of antimicrobial drugs

An antibacterial drug represents a chemical substance derived from a biological source or produced by chemical synthesis that is able to destroy or to inhibit the development/growth of bacteria.

Antibacterial drugs are commonly classified by considering the following criteria:

- **Targeted pathogens:** This category involves antibacterial, antiviral, and antifungal drugs. This grouping may be further subdivided because antibacterial drugs also include urinary antiseptics and anti-mycobacterial drugs. Antimicrobial drugs, especially antibacterial drugs, are strictly classified into chemotherapeutic agents (synthetic chemicals), and antibiotics, produced from living organisms, usually fungi. However, 'antibiotic' is often used loosely to mean all antibacterial drugs;
- **Chemical structure**: Examples are penicillins and cephalosporins;
- **Source**: Natural (mainly fungal sources), semi-synthetic (chemically-altered natural compound), and synthetic (chemically designed in the lab). The “natural” antibiotics arise from fungal sources. Organisms become resistant faster to the natural antimicrobials because they have been pre-exposed to these compounds in nature. Natural antibiotics are often more toxic than synthetic ones. Semi-synthetic drugs appeared as alternative to

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decrease toxicity and increase effectiveness of the first category. Synthetic drugs have an advantage that the bacteria are not exposed to the compounds until they are released. They are prepared to exhibit greater effectiveness and less toxicity. There is an inverse relationship between toxicity and effectiveness starting from natural to synthetic antibiotics (Figure 1);

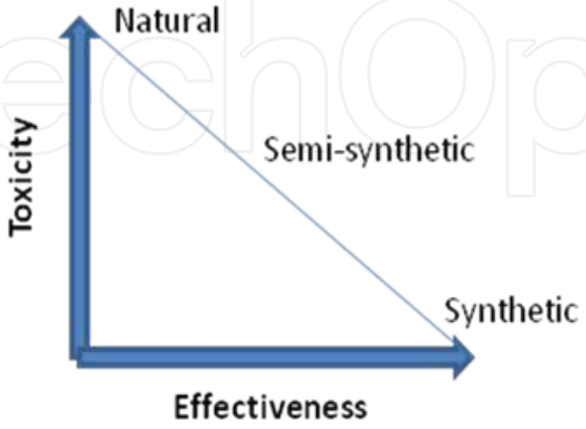

Figure 1. Toxicity *versus* effectiveness for antimicrobial drugs derived from different sources

- Mechanism of action: The antibacterial drugs are designed to have a complex mechanism of action by considering some essential factors which render to the obtained drug-specific features. One can classify antibacterial drugs by taking into account the following:
- *The effect on bacterial growth*
  - bacteriostatic
  - bactericidal drugs
- *The targeted site*
  - drugs that inhibit bacterial wall synthesis or activate enzymes that destroy the cell wall
  - drugs that enhance cell membrane permeability (causing leakage of intracellular material)
  - drugs that determine lethal inhibition of bacterial protein synthesis
  - drugs that generate nonlethal inhibition of protein synthesis
  - drugs that inhibit bacterial synthesis of nucleic acids
  - antimetabolites (disruption of specific biochemical reactions---decrease in the synthesis of essential cell constituents)
  - inhibitors of viral enzymes
- *The target specificity*

- the broad-spectrum drug affects a wide range of disease-causing bacteria, including both Gram-positive and Gram-negative bacteria
- the narrow-spectrum antibacterial drug, which acts against specific families of bacteria. For example, ampicillin is a widely used broad-spectrum antibiotic.

For a better understanding of the different types of antimicrobial drugs, Figure 2 displays the main classes of these substances and their applications.

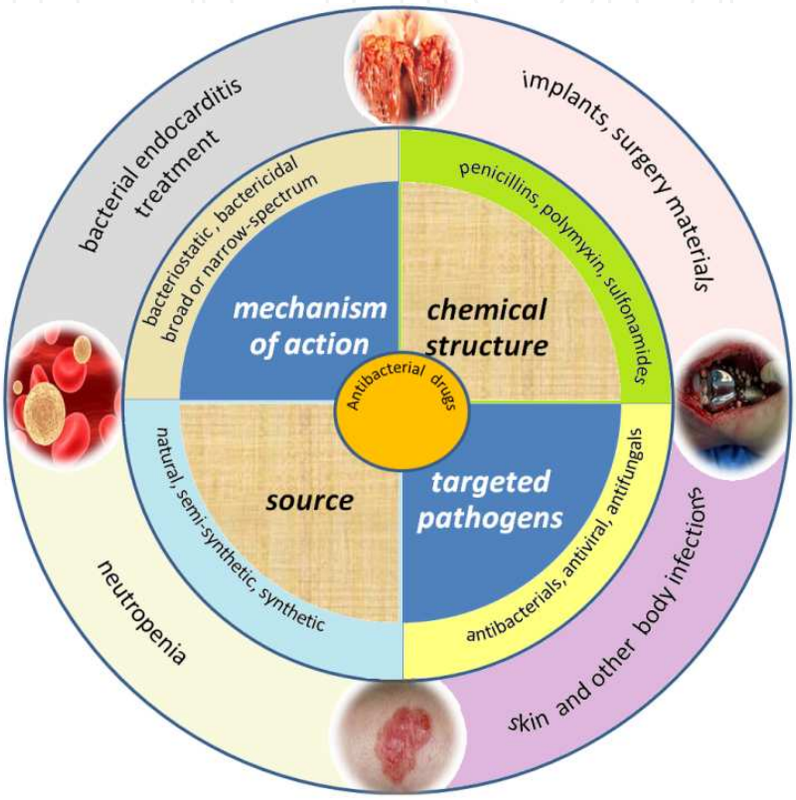

Figure 2. Classification of antimicrobial drugs and their applications

Antibiotics act by inhibiting the basic life-sustaining processes in the microorganism. In order to minimize toxicity, the targets of antibiotics must be selective. However, all antibiotics are toxic to some degree. Selective toxicity should be focused on harming the bacteria, not the host. Selection of the appropriate antibiotic depends on the following parameters:

- Knowledge of organism's natural resistance
- Pharmacological properties of the antibiotic toxicity, binding, distribution, absorption achievable levels in blood, urine
- Previous experience with same species
- Nature of patients underlying pathology
- Patient's immune status

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Evaluation of susceptibility focuses mainly on the interaction of antimicrobial agents, the organisms, and their resistance mechanisms. The resistance to antimicrobial drugs is based on several mechanisms, including the following:

- Microbes may generate drug-metabolizing enzymes (such as penicillinase)
- Microbes may cease active uptake of certain drugs
- Microbial drug receptors may suffer change resulting in reduced antibiotic binding and action
- Microbes may produce compounds that antagonize drug actions

Susceptibility tests are essentially artificial measurements that include *in vitro* response, approximate range of effective inhibitory action and reflect possible error equivalent to one tube dilution. The only true measure of bacterial response to an antibiotic drug is the clinical response of the patient (outcome or *in vivo* response).

The bacteria are innately resistant to some antibiotics since they lack a target site or are impermeable to the antibiotic. Resistance spreads between bacteria in three physical ways: conjugation (by direct contact), transduction (by phages), and transformation (uptake of free DNA). The resistance is acquired by spontaneous mutation and conjugation. This could be the results of two aspects as follows:

- Utilization of antibiotics that promote the emergence of drug-resistant microbes
- Suprainfection: A new infection that occurs during the treatment of a primary infection

Delaying the emergence of resistance can be achieved by taking into account the following solutions:

- Use antimicrobial agents only when required
- Utilization of narrow-spectrum antibiotics whenever possible
- Novel antibiotics should be kept for cases in which older drugs are harmful or no longer effective

It is very important that patients should be instructed to strictly follow their prescription particularly during the entire curing treatment period even though symptoms may subside before the full course has been completed. Otherwise, it is possible that interruption of the medication could lead to fail of the treatment and other drugs should be included in the cure. However, there are some disadvantages of antibiotic combinations as follows:

- Enhanced risk of toxic and allergic reactions
- Possible antagonism of antimicrobial effects
- Increased risk of suprainfection
- Selection of drug-resistant bacteria
- High costs

On the other hand, without doctor's advice, the patient might misuse the antibiotics leading to some issues like:

- attempted treatment of untreatable infection (viral infections);
- improper dosage;
- treatment in absence of adequate bacteriologic information
- omission of surgical drainage.

Generally, the prophylactic use of antimicrobial drugs can be divided in three categories: (1) surgery (cardiac, orthopedic, gastrointestinal tract surgery), (2) bacterial endocarditis, and (3) neutropenia. Antimicrobial drugs are also indicated in the treatment of other infections such as young women with recurrent urinary tract infection, prophylaxis against type A influenza with amantadine, lifelong prophylaxis of individuals who have had severe rheumatic carditis. The features of an ideal antibacterial drug are as follows:

- Selective target---target unique
- Bactericidal---kills the bacteria
- Narrow spectrum---does not kill normal flora
- High therapeutic index---ratio of toxic level to therapeutic level
- Few adverse reactions---toxicity, allergy
- Various routes of administration
- Good absorption
- Good distribution to site of infection
- Emergence of resistance is slow

3. Synthesis routes of antimicrobial drugs

The mechanism of action of natural antibacterial agents inspired scientists to prepare more complex structures with improved properties for the cases where the natural products fail due to the resistance occurrence. In this context, the chemical synthesis of new drugs is in continuous evolution. The development of partially or fully synthetic routes to antibacterial compounds is a strategy whose constraints (molecular size and complexity, scalability) must be re-assessed in light of advances in modern chemical synthesis, both strategic and methodological.

Natural products have been a rich source of antibacterial drugs since their discovery, but investments in this domain have been diminished over the past two decades. The main source of natural antibacterial agents is represented by plants, which are able to synthesize aromatic substances, like phenols or their oxygen-substituted derivatives [6]. Most of these substances

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are secondary metabolites that serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Major classes of antimicrobial compounds obtained from plants are the following:

- Phenolics: simple phenols (catechol, epicatechin), phenolic acids (cinnamic acid), quinines (hypericin), flavonoids, flavones, flavonols, tannins, coumarins
- Terpenoids, essential oils
- Alkaloids
- Lectins and polypeptides
- Polyacetylenes

Catechol is produced industrially by the hydroxylation of phenol using hydrogen peroxide [7]:

$$C_6H_5OH + H_2O_2 \rightarrow C_6H_4\left(OH\right)_2 + H_2O$$

This natural antibacterial agent can also be obtained by hydrolysis of 2-substituted phenols, especially 2-chlorophenol, with hot aqueous solutions containing alkali metal hydroxides. Its methyl ether derivative converts to catechol via hydrolysis of the CH_3-O bond as promoted by hydriodic acid.

Terpenoids are prepared by two metabolic pathways:

a. Mevalonic acid pathway

Many organisms manufacture terpenoids through the HMG-CoA reductase pathway, the pathway that also produces cholesterol. The reactions take place in the cytosol.

b. Non-mevalonate pathway

The 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway), also known as mevalonic acid-independent pathway, occurs in the plastids of plants and apicomplexan protozoa, as well as in many bacteria. Pyruvate and glyceraldehyde 3-phosphate are converted by DOXP synthase to 1-deoxy-D-xylulose 5-phosphate, and by DOXP reductase, (IspC) to 2-C-methyl-D-erythritol 4-phosphate (MEP). The subsequent three-reaction steps catalyzed by 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (YgbP, IspD), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (YchB, IspE), and 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (YgbB, IspF) favor the formation of 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP). Finally, MEcPP is converted to (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) by HMB-PP synthase (GcpE, IspG), and HMB-PP is converted to isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) by HMB-PP reductase (LytB, IspH). IPP and DMAPP are the end-products in either pathway, and are the precursors of isoprene, monoterpenoids (10-carbon), diterpenoids (20-carbon), carotenoids (40-carbon), chlorophylls, and plastoquinone-9 (45-carbon). Synthesis

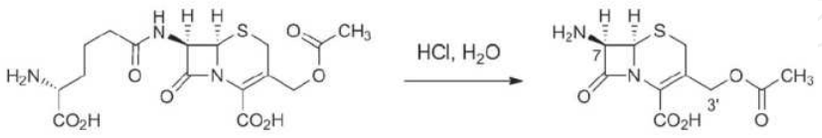
of all higher terpenoids proceeds via formation of geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate.

Lectins with certain carbohydrate specificity have been purified from various plant tissues and other organisms. On the other hand, these substances can be obtained by recombinant techniques. A sizeable quantity of a lectin (~10g) can result by large-scale fermentation. This procedure is carried out in defined pH value, temperature, optical density at the time of induction, inducer concentration, and time of expression. The most commonly used inducer is isopropyl β-d-thiogalactoside. Subsequently, the cells are harvested by centrifugation. Lectins are released from the cells by resuspension in lysis buffer followed by sonication. Further chromatographic steps are needed for purification of lectins [8]. Isolation of lectins can be accomplished by a combination of several purification techniques. Acids [9], organic solvent, [10] or salt can be used to precipitate lectins. The chromatographic methods, such as affinity chromatography, ionic exchange chromatography, hydrophobic interaction chromatography, and gel filtration are employed. An increase in the number of purification steps usually results in a lesser amount of recovery.

The need for new antibacterial agents remains high, considering the increasing rates of resistance of the drugs available on the market. The results of many of the current therapies with natural products begin to fade, even for common infections.

Semisynthetic antibacterial agents appeared as a result of the researchers efforts to modify the antibacterial substances produced in nature. Thus, semisynthesis came to the forefront of antibacterial discoveries following innovative chemical alterations naturally occurring in some compounds. In the following paragraph, the main chemical insights that helped overcome the numerous limitations of cephalosporin, tetracycline, and macrolide antibacterial drugs, are discussed.

Semisynthesis of β-lactams. It was discovered that cultures of the mold *Cephalosporium acremonium* contained one or more substances that were antagonistic to bacteria. After purification of cephalosporin C from the *Cephalosporium* culture, the 7-aminocephalosporanic acid (7-ACA) is obtained by hydrolysis of cephalosporin C under acidic conditions.



Sheme 1. Hydrolysis of cephalosporin C under acidic conditions

The parenteral cephalosporins (cephalothin) present potent activity against Gram-positive organisms but only moderate activity against Gram-negative bacteria. Improved pharmacological properties, as well as lower susceptibility to resistance mechanisms, are achieved by introducing innovative side chains at just two modifiable sites of 7-ACA---the amine function C7 and C3′.

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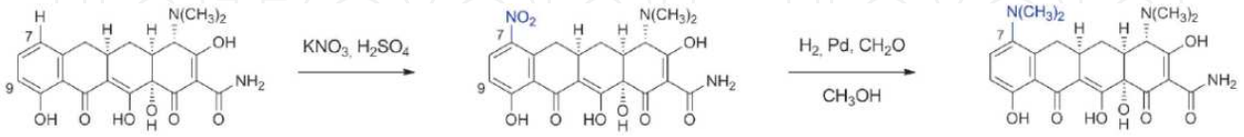
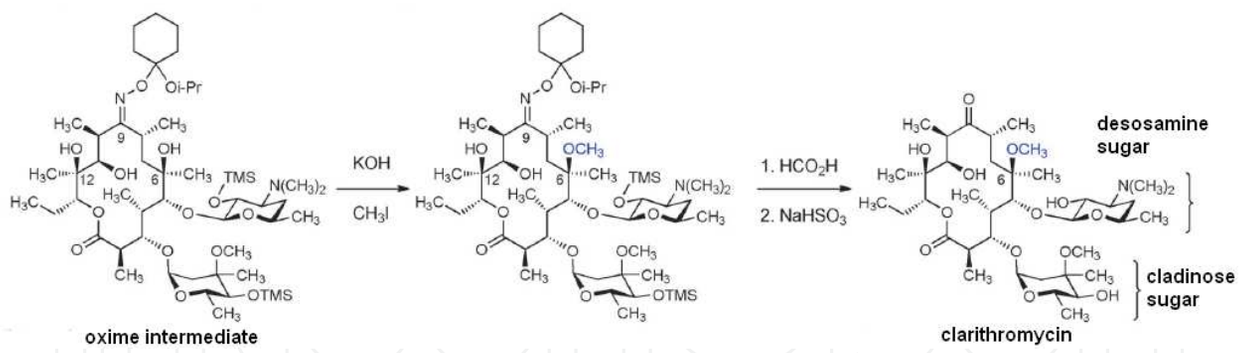
Semisynthesis of tetracyclines. The development of semisynthetic tetracyclines has been marked by a series of specific, impactful discoveries. The C6-hydroxy group of the natural products oxytetracycline, tetracycline, and 6-demethyltetracycline could be removed reductively. Minocycline was obtained from 6-deoxy-6-demethyltetracycline (sancycline) by an electrophilic aromatic substitution reaction at C7, and it presented a broader spectrum of activity than prior tetracyclines.



Sheme 2. Preparation steps of minocycline

Semisynthesis of macrolides. Erythromycin, the first macrolide antibiotic (isolated the natural product from the culture broth of the soil-dwelling fungus *Saccharopolyspora erythrea*), is effective against a variety of Gram-positive bacterial infections. However, it possesses poor oral bioavailability and short *in vivo* half-life, and is unstable under acidic conditions, leading to side effects, such as stomach pain. When it is placed in acidic conditions erythromycin decomposes by intramolecular cyclization reactions beginning with addition of the C6 hydroxy group to the C9 ketone, forming anhydrohemiketal and spiroketal derivatives. One solution is to develop a 6-step sequence from erythromycin resulting in selective capping of the C6 hydroxy substituent with a methyl group, affording the antibacterial clarithromycin.



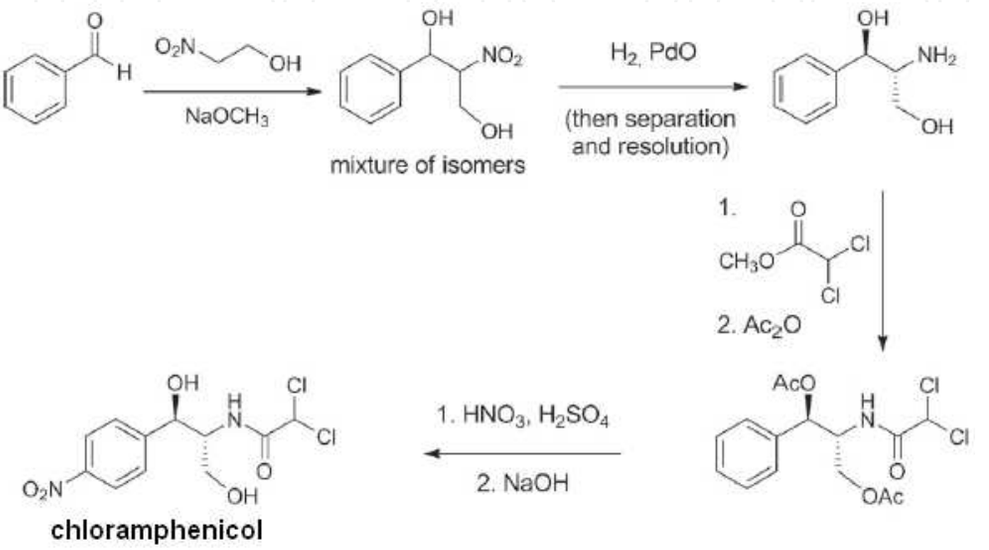
Sheme 3. Preparation stages of clarithromycin

Another innovative semisynthetic solution concerning the chemical instability of erythromycin is focused on removal of the C9 ketone from the erythromycin by a sequence comprising oxime formation, Beckmann rearrangement (ring expansion), and then hydrogenolysis of the iminoether intermediate.

Fully synthetic antibacterial drugs are an alternative solution to the other presented categories. These approaches have also led to important new classes of antibacterial agents and large numbers of approved drugs. The most appreciated examples of synthetic substances used in

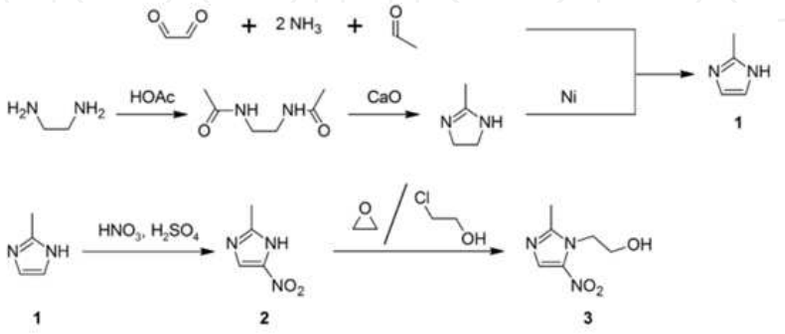
therapeutics are the chloramphenicol, metronidazole, fosfomycin, quinolones, carbapenems, and oxazolidinones.

Chloramphenicol is a natural compound that is more economical to produce on industrial scale by chemical synthesis rather than fermentation. The substitution of nitro group in chloramphenicol with methanesulfonyl group leads to an increased potency and avoids the fatal aplastic anemia.



Sheme 4. Synthesis reactions leading to chloramphenicol

Nitroimidazoles (5-nitroimidazole, metronidazole) represent effective drugs for the treatment of trichomoniasis and infections produced by a variety of anaerobic bacteria (including *C. difficile*). 2-Methylimidazole (1) may be prepared via the Debus-Radziszewski imidazole synthesis, or from ethylenediamine and acetic acid, followed by treatment with lime, then Raney nickel. 2-Methylimidazole is nitrated to give 2-methyl-4(5)-nitroimidazole (2), which is in turn alkylated with ethylene oxide or 2-chloroethanol to give metronidazole (3) (http://en.wikipedia.org/wiki/Metronidazole):



Sheme 5. Preparation steps of metronidazole

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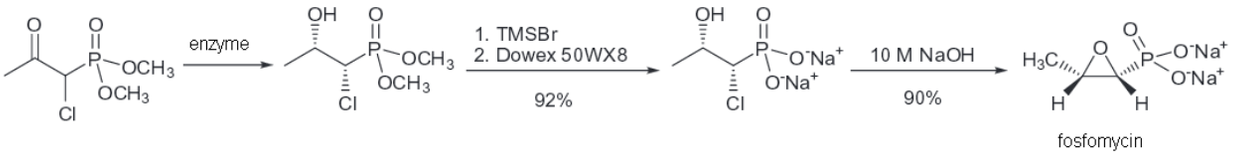
Fosfomycin is a polar and small molecule presenting bactericidal activity against both Gram-positive and Gram-negative bacteria due to disruption of cell-wall biosynthesis. This drug prescribed for urinary tract infections is conveniently administered as a single-dose treatment [11]. Fosfomycin was obtained as a secondary metabolite in a glucose-asparagine medium containing citrate for growth and L-methionine combined with L-glutamate for stimulating the production of this compound [12]. More recently, asymmetric synthetic strategy for the synthesis of (-)-fosfomycin was performed using a biocatalytic reduction as the key step [13].



Sheme 6. Synthesis strategy to obtain fosfomycin

Quinolones are more difficult to synthesize in the laboratory than sulfanilamides, but they are obtained by shorter synthetic routes. Norfloxacin is prepared by incorporation of both a fluorine atom at C6 and a piperazine substituent at C7 [8]. The resulted drug presents greatly improved Gram-negative activity and medium activity against Gram-positive bacteria. If the N1 ethyl group of norfloxacin is changed with a cyclopropyl substituent, one obtains ciprofloxacin, which can be used for treatment of respiratory tract, skin, and joint infections, including infections caused by *Pseudomonas aeruginosa*.

Carbapenems arise from the development of fully synthetic β-lactams, leading to a dramatic leap forward in the complexity of antibacterial molecules produced on an industrial scale using fully synthetic approaches. Thienamycin was the first natural "carbapenem" antimicrobial drug. It exhibits exceptional activity against both Gram-positive and Gram-negative organisms, including strains of *Pseudomonas aeruginosa* and organisms with acquired β-lactamase resistance mechanisms. In order to solve the chemical instability problems, it was tried to defer the introduction of the C2-cysteamine side-chain until late in the preparation procedure, enabling a series of analogs with structural variations in the thiol side-chain to be obtained. In the key step of the preparation, the bicyclic carbapenem core is generated in quantitative yield by rhodium-catalyzed cyclization of a diazo-keto ester. This impressive approach led to the discovery of imipenem and other derivatives (obtained by introduction of a C1-β-methyl substituent into the carbapenem core) [14].

Oxazolidinones are active against streptococci and staphylococci. In this class of antibacterial agents, linezolid is the first synthetic compound that helped in curing infections caused by Gram-positive bacteria, like MRSA and vancomycin-resistant enterococci. It is not developed by building upon a naturally occurring skeleton. Several approaches for the synthesis of linezolid have been reported in the chemistry literature [15], but the original preparation procedure developed by Upjohn [15] is lengthy, demands the use of expensive chemicals (palladium on carbon and the highly sensitive reagents methanesulfonyl chloride and n-butyllithium), and needs low-temperature conditions. Later syntheses have included an

"atom-economical" method starting from D-mannitol [16] and a route starting from (S)-glyceraldehyde acetonide (prepared from vitamin C) [15]. It was also reported the development of the "second-generation" of linezolid: a convergent, green synthesis starting from (S)-epichlorohydrin, with higher yield and a 56% reduction in total waste [8]. However, long-term use can determine serious adverse effects including bone marrow suppression. Further development of oxazolidinones is based on the x-ray crystal structure of linezolid bound to its target, the large subunit of the bacterial ribosome [17].

A schematic representation of the main synthesis routes of the antibacterial agents is displayed in Figure 3.

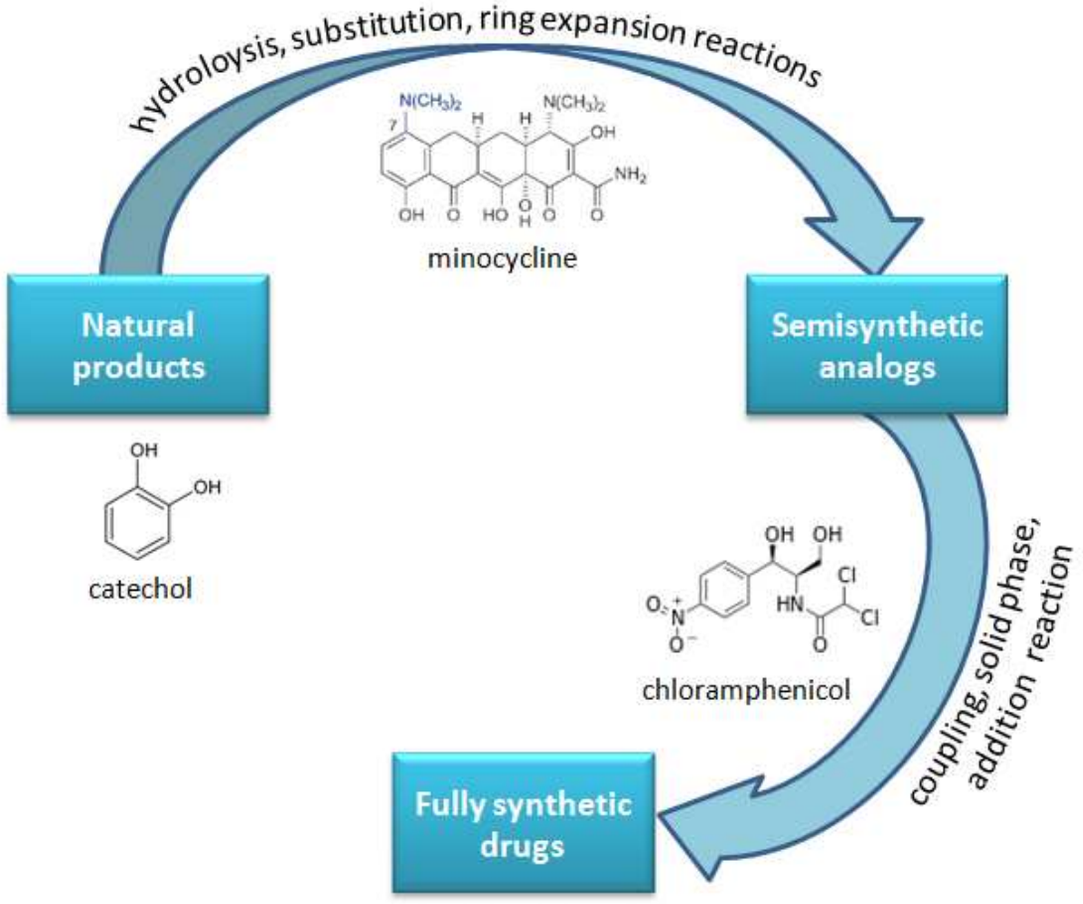


Figure 3. The schematic representation of the main synthesis routes of the antibacterial agents

4. Design of therapeutic systems based on polymers

The design of therapeutic polymer systems with antibacterial action is very complex and it involves special polymerization reactions or chemical functionalization for binding the drugs. Literature reports [18, 19] highlight the influence of some parameters, such as molecular

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weight, type and degree of alkylation, and distribution of charge on the bactericidal action of antimicrobial polymers. Considering the working principles of antimicrobial macromolecular systems, the design procedures can be divided in three categories (Figure 4), as follows:

- Polymer biocides
- Biocidal polymers
- Biocide-releasing polymers

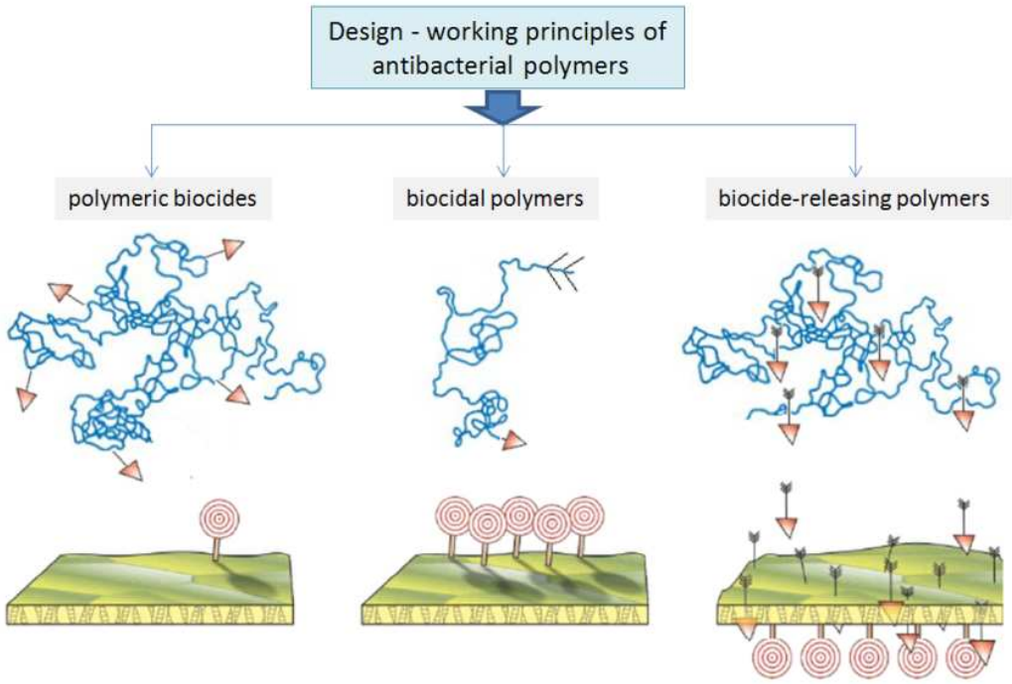

Figure 4. Schematic representation of the design possibilities of antibacterial polymers based on the working principles of macromolecular systems

Polymeric biocides are macromolecules constituted of bioactive repeating units, namely polymers, represent multiple interconnected biocides, which act similarly to the monomers. They can be designed by synthetic routes that yield macromolecules with antimicrobial activity. Their function is not always understood. For instance, polymers and copolymers prepared from 2-methacryloxytroponones are able to kill bacteria. The design of this category of antimicrobial polymers is based on the concept that biocidal groups attached to a polymer act in the same way as analogous low molecular weight compounds, demonstrating that the repeating unit is a biocide. Taking into account the sterical hindrance exerted by the macromolecular backbone, polymeric biocides are expected to be less active than the respective low molecular weight compounds. Literature [20] shows possibility to polymerize antibiotics maintaining their activity at the polymer backbone, e.g., by copolymerization of methacrylate-modified norfloxacin and PEG-methacrylates. Also, polymers with side groups based on hydrophobic quaternary ammonium functions can be considered as polymeric biocides.

The *biocidal polymers* preparation steps require the embodiment of the active principle in the whole macromolecule. It does not necessarily demand the use of antimicrobial repeating units. Microbial cells generally carry a negative net charge at the surface due to their membrane proteins. These charges are prepresented by teichoic acids in case of Gram-positive bacteria, and phospholipids at the outer membrane in case of Gram-negative bacteria. Therefore, polycations are attracted and if they have a proportionate amphiphilic character, they are able to destroy the cytoplasmic membrane, resulting in cell death. The most antimicrobial polycations contain quaternary ammonium [21] and phosphonium [22], tertiary sulfonium [23], and guanidinium functions. Biocidal macromolecules that do not contain biocidal repeating units present antimicrobial activity that originates from the whole molecule. The biocidal repeating units are not required if the polymer backbone exhibits a hydrophobic character. On the other hand, polymers with a hydrophilic polymer backbone demand a hydrophobic region parallel to the backbone, which is provided by the hydrophobic side groups. Polymeric biocides are efficient when cationic groups are placed along the polymer backbone, but the good results are obtained also for polymers having only one biocidal end group. These polymers are obtained by cationic ring-opening polymerization of 2-alkyl-1,3-oxazolines and terminating the macromolecule with a cationic surfactant. The advantage of this preparation technique is the controlled introduction of a specific group at one end and another group at the opposite end of the macromolecules by selecting a suitable initiator and termination agent.

Biocide-releasing polymers do not act through the actual polymeric part, but the latter represents a carrier for biocides that are transferred through different mechanisms (like diffusion) to the infected area. Such polymers are usually the most active systems, because they can release their biocides close to the cell in high local concentrations. The biocide-releasing macromolecules were prepared for the first time through polymerization of salicylic acid subjected to degradation, which enables the controlled releasing of salicylic acid. Several types of biocidal polymers have been designed to release chlorine, nitric oxide, phenols, or singlet oxygen. Another class of biocide-releasing polymers is the contact-active ones, like tributyltin esters of polyacrylates.

Natural polymers, such as collagen, can serve as antibacterial drug carrier. The latter can be complexed to the polymer through direct binding of the biocide to free amino or carboxylic groups of the collagen molecule [24]. The diffusion is the main process that assures the release of the drug after implantation or injection. The design of the sponge and the drug incorporation by colyophilization allow the uniform distribution of the drug within the spongy matrix. This also ensures an equal drug dose applied per square centimeter of the treated surface. Pore size of natural polymer sponges can be controlled by the lyophilization process [24].

On the other hand, synthetic copolymers, like epoxy-functional poly(dimethylsiloxane) can be processed to favor the attachment (i.e., tethering) of levofloxacin [25]. The reaction occurred via ring-opening of epoxy groups by the carboxylic acid group of levofloxacin, the tether produced was an ester-functional tether. Compared to a control coating generated by simply blending levofloxacin into a polysiloxane, the samples with tethered levofloxacin moieties presented uniform distribution of levofloxacin, higher initial kill, and sustained antimicrobial surface activity.

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The antimicrobial agents can be introduced into polymer microparticles. The co-precipitation of $CaCO_3$ and silver nanoparticles (SNPs) in the presence of poly(sodium 4-styrenesulfonate) leads to a system that enables sustained release of biocide [26]. Microbiological tests confirmed the effectiveness of these microparticles as an antibacterial agent. This designed material can be stored as a dry powder and subsequently re-suspended in water without the risk of losing its antimicrobial activity.

Plasma is widely used as a tool for polymer functionalization with biocide agents. SNPs-based antibacterial coatings can protect eukaryotic cells from SNPs-related toxic effects, while preserving antibacterial efficiency [27]. A system of SNPs containing n-heptylamine (HA) polymer matrix was deposited by plasma polymerization and covered by a second HA layer. Even so, the antibacterial activity is maintained to planktonic bacteria living in the near surroundings of the material. The SNPs-based materials also revealed antibacterial effect on adhered bacteria [28, 29]. Their number was significantly reduced compared to pure HA plasma polymer and the physiology of the bacteria was affected. The number of adhered bacteria is directly diminished with thickness of the second HA layer.

5. *In Vivo* and *In vitro* activity of antibacterial substances

Bacterial agents can cause both superficial and serious systemic diseases, generating infections anywhere, including in hospitals. Infections involve the formation of *in vitro* or *in vivo* bio-clusters on implanted devices, such as catheters or prosthetic heart valves. These bacterial agents formed *in vitro* on different bio-materials consist of micro-colonies which are resistant to a range of antibacterial agents, through several mechanisms of resistance [30, 31]. Microorganisms can be organized as biofilms, including pathogens, thus offering a means to protect themselves against antimicrobial agents. Several mechanisms have been proposed to explain this phenomenon of resistance within biofilms, such as:

- delayed penetration of the antimicrobial into the biofilm extracellular matrix;
- slowing of growth rate of organisms within the biofilm
- physiologic changes brought about by interaction of the organisms with a surface.

Implications of biofilm formation are that alternative control strategies must be devised both for testing the susceptibility of the organisms within the biofilm and for treating the established biofilm to alter its structure. A number of testing protocols have been developed. The effective treatment strategies include antimicrobials or other agents that can penetrate and kill the biofilm organisms, or treatments that can disrupt or target specific components of the biofilm matrix [32].

Recent studies on bacterial and fungal species suggest that extensive and striking interactions occur between the prokaryotic and eukaryotic cells [33]. Also, their possible mechanisms of resistance vary with the nature of the administered antimicrobial agent, are not fully understood, and are grouped in literature taking into account [34] the following parameters:

- Restricted penetration of drugs through the micro-colonies
- Phenotypic changes resulting from a decreased growth rate or nutrient limitation
- Nature of resistance genes induced by contact with a surface
- Number of 'per-sister' cells considered responsible for resistance

The methods of selection of drugs to be considerate in case of combined devices are provided by Venn diagram---a diagram (Figure 5) that shows all possible logical relations between finite collections of different sets, including the pharmaceutical device (pharmacokinetics, pharmacodynamics, biopharmaceutics, therapeutic dose), medical device (type and class of device, manufacturing process, testing requirements), and combination device perspective (local drug effects, controlled release combined manufactured methods, business drivers, clinical concerns) [33].

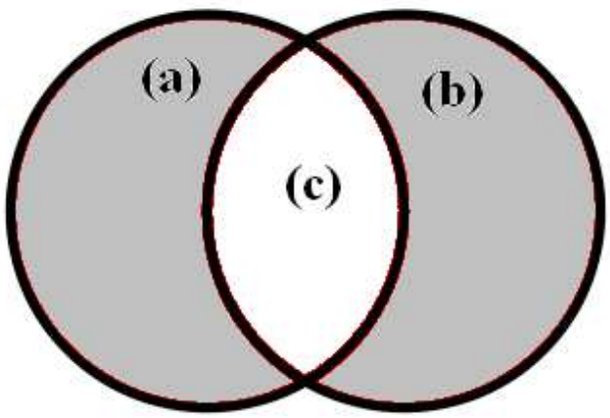

Figure 5. The Venn diagram for perspective of devices and combined devices: (a) pharmaceutical device; (b) medical device; and (c) combination device

The considerable interest exists in combination devices that include the type of peripheral stents, orthopedics, indwelling catheters, dental implants, surgical meshes, wound dressings, ophthalmic implants, sutures, and even artificial organs [35, 36]. The physicochemical properties of the drugs used in combination devices, as well as their location in vivo or in vitro, include the knowledge of their antibacterial activity.

Table 1 presents the beneficial effects of treatment with antimicrobial agents against various types of bacteria, associated as biofilms.

However, besides the beneficial treatments, there are some secondary effects produced by antimicrobial drugs. The fluoroquinolone class of antibacterial drugs that determine adverse events include central nervous system toxicity, phototoxicity, and in some cases electrocardiographic changes. Some side-effects of the quinolones are class effects, and cannot be modulated by molecular variation. These include gastrointestinal irritation and arthropathy.

Tested bacteria	Antibacterial drug	
Escherichia coli	Cefazolin, ciprofloxacin, clindamycin, gentamicin, oxacillin, penicillin, vancomycin	[37]
Escherichia coli	Latamoxef	[38]
Pseudomonas aeruginosa	Cefazolin, ciprofloxacin, clindamycin, gentamicin, oxacillin, penicillin, vancomycin	[37]
Pseudomonas aeruginosa	Cefuroxime, piperacillin, tobramycin, vancomycin	[39]
Staphylococus aureus	Cefazolin, ciprofloxacin, clindamycin, gentamicin, oxacillin, penicillin, vancomycin	[37]
Staphylococus aureus	Cefazolin, cefuzonam, cephalexin, gentamicin, ofloxacin, tosufloxacin	[40]
Staphylococus aureus	Cefazolin, cefuroxime, bioluminescence ciprofloxacin, erythromycin, gentamicin, novobiocin, penicillin G, phosphomycin, rifampin, tobramycin, vancomycin	[41]
Staphylococus aureus	Fleroxacin, gentamicin, oxacillin, vancomycin	[42]
Staphylococus aureus	Cefuroxime	[39]
Staphylococus aureus	Vancomycin, teicoplanin	[43]
Staphylococus epidermidis	Ciprofloxacin, quinupristin/dalfopristin Vancomycin	[44]
Staphylococus. epidermidis	Ciprofloxacin	[45]
Staphylococus epidermidis	Cefuroxime, piperacillin, tobramycin, vancomycin	[39]
Staphylococus epidermidis	Amikacin, levofloxacin, rifampin, teicoplanin	[46]
Streptococcus pneumoniae	Vancomycin, teicoplanin	[43]

Table 1. Response to antibiotics of different bacteria associated as biofilms

Moreover, gastrointestinal prokinetics, such as metoclopramide, cisapride and levosulpiride, are widely used for the management of functional gut disorders. Recent investigations revealed that cisapride (a partial 5-HT4 receptor agonist) can generate dose-dependent cardiac adverse effects, including lengthening of the electrocardiographic QT interval, syncopal episodes, and ventricular dysrhythmias.

Determination of the minimum inhibitory concentration, based on the activities of antibiotics against planktonic bacteria, is the standard assay for antibiotic susceptibility testing. In this context, the Calgary Biofilm Device is well recognized as a technology for the rapid and reproducible assay of biofilm susceptibilities to antibiotics, for the rational selection of antibiotics effective against microbial biofilms, and for the finding of new effective antibiotic compounds [8]. On the other hand, the antibiotic tolerance is defined as the ability of bacteria to survive but not grow in the presence of antibiotics. It is known that adherent bacteria on

solid surfaces already have tolerance to antibiotics and depend essentially on the different stress conditions on antibiotic tolerance [47]. The rise in antibiotic resistance among pathogenic bacteria and the declining rate of novel drug discovery motivate recent studies to find new classes of antibacterial agents and novel drugs, in order to maintain the ability to treat infectious diseases---especially those caused by multidrug-resistant organisms [48]. Research concerning the activity and design of cationic antimicrobial peptides and their mimics has produced several antimicrobial compounds with good antibacterial activity and elucidated trends of increasing activity and specificity. Applications are numerous, including antimicrobial surfaces [49] and conjugates in targeted therapy [50].

The interest in cationic antimicrobial peptides opens new perspectives in antimicrobial drug design. The Hunter-killer peptides represent a novel class of targeted peptides that have demonstrated remarkable efficacy in several basic proof-of-principle paradigms including therapeutics for cancer [51], arthritis [52], prostate reduction [53], and obesity [54]. There are also reported new hunter-killer "nanostructures/nanospheres" and second-generation Hunter-killer peptides derivatives, which are protected from proteolytic degradation and adjust their "modulation of the absorption, distribution, metabolism, and excretion" properties [55]. This technology uses similar way to encapsulate anticancer proteins, such as the small globular proteins. Development and evaluation of the next generation of Hunter-killer peptides with improved modulation of the absorption, distribution, metabolism, and excretion properties are studies currently underway.

Host-defense peptides are present components of the innate immune system across all organisms. These molecules have been widely studied for their activities, such as antimicrobial, antitumor, mitogenic, and chemical signaling properties. It is known that oligo-acyl-lysyls are synthetic mimics of host-defense peptides known to exert antibacterial activity both in cultures and in animal models of disease [56]. Data obtained with representative bacteria, including the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*, shows that the oligo-acyl-lysyls potency is affected by pH changes and subsides essentially throughout a wide range of salt concentrations and temperature, whereas antistaphyloccocal activity is vulnerable. Also, in biomedical strategy concerning host-defense peptides, several short synthetic oligomers such as methacrylate, arylamide foldamers, and oligo-acyl-lysyls have attracted particular attention due to their lower cost and rapid structural optimization capabilities [57]. Although a number of compounds that demonstrate broad-spectrum antimicrobial activities *in vitro* have been identified, robust/safe *in vivo* activity has been a great challenge for most published peptidomimetics [58]. As a result of the appearance of multidrug-resistant bacteria, antimicrobial peptides have emerged as one of the leading prospects for drug development. Antimicrobial peptides are retained in a wide range of organisms as a defense mechanism against a broad array of microbial targets. These peptides vary in size, sequence, and efficacy, allowing a good interaction with the charged bacterial membrane [59]. In this context, to mimic the amphiphilic nature of antimicrobial peptides, arylamide foldamers were prepared, that demonstrated bactericidal activity against both Gram-negative and Gram-positive strains, without many of the drawbacks of natural antimicrobial peptides [60].

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The emergence and spread of antibiotic resistance represent an alarming concern in clinical practice. From this reason, the antimicrobial agents are used to cover materials and medical devices as a prophylaxis to prevent bacteria from growing or for therapeutic uses. A range of silver-coated or impregnated dressings are commercially available for use. The antibacterial activities of silver-coated/impregnated dressings were compared against common burn-wound pathogens, namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*, *Acinetobacter baumannii.* The rapidity and extent of killing of these pathogens evaluated under *in vitro* conditions shows that silver-impregnated dressings exert bactericidal activity, particularly against Gram-negative bacteria, including *Enterobacter* species, *Proteus* species, and *Escherichia coli*. Therefore, the incorporation of silver for dressings or as coating on medical products plays an important role in the domain of antimicrobial agents [61].

On the other hand, it is known that infection is the most common cause of biomaterial implant failure in modern medicine [62]. Adhesion and subsequent surface growth of bacteria on biomedical implants and devices cause the formation of a biofilm, in which the so-called ''glycocalyx'' embeds the infecting bacteria offering protection against the host immune system and antibiotic treatment. Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* are the predominant infecting organisms, followed by Gram-negative bacilli like *Escherichia coli* and *Pseudomonas aeruginosa*. A possible approach to prevent biomaterial-centered infections is to render the biomaterial surface antimicrobial properties by functionalization with, e.g., quaternary ammonium groups, which are widely known as disinfectants. Quaternary ammonium-functionalized surfaces have a high positive surface charge, and thus exert a strong adhesive force on negatively charged bacteria which are proposed to inhibit surface growth of bacteria. The main mechanisms of action of antibacterial drugs (Figure 6) can be classified as follows:

- Inhibition of cell wall synthesis (amoxicillin, cefalexin, oxacilin)
- Inhibition of protein synthesis (chloramphenicol, clarithromycin, erythromycin)
- Inhibition of nucleic acid synthesis (ciprofloxacin, norfloxacin, novobiocin)
- Inhibition of metabolic pathways (sulphanilamide, trimethoprim)
- Interference with cell membrane integrity (cerulenin, triclosan)

Several *in vitro* and *in vivo* studies show that methicillin-resistant *Staphylococcus aureus* (MRSA) remains a leading cause of bacterial infections worldwide, ranging from minor skin and soft tissue infections to more severe conditions such as bacteremia and infective endocarditis [63]. The molecules currently under pre-clinical and clinical investigation that are active against MRSA, with special emphasis on their mechanism of action are grouped as follows:

- Molecules acting on peptidoglycan biosynthesis and the cell membrane (lipopeptides, lipoglycopeptides, cell membrane inhibitors)
- Protein synthesis inhibitors (oxazolidinones, aminomethylcyclines, aminoglycosides, peptide deformylase inhibitors)

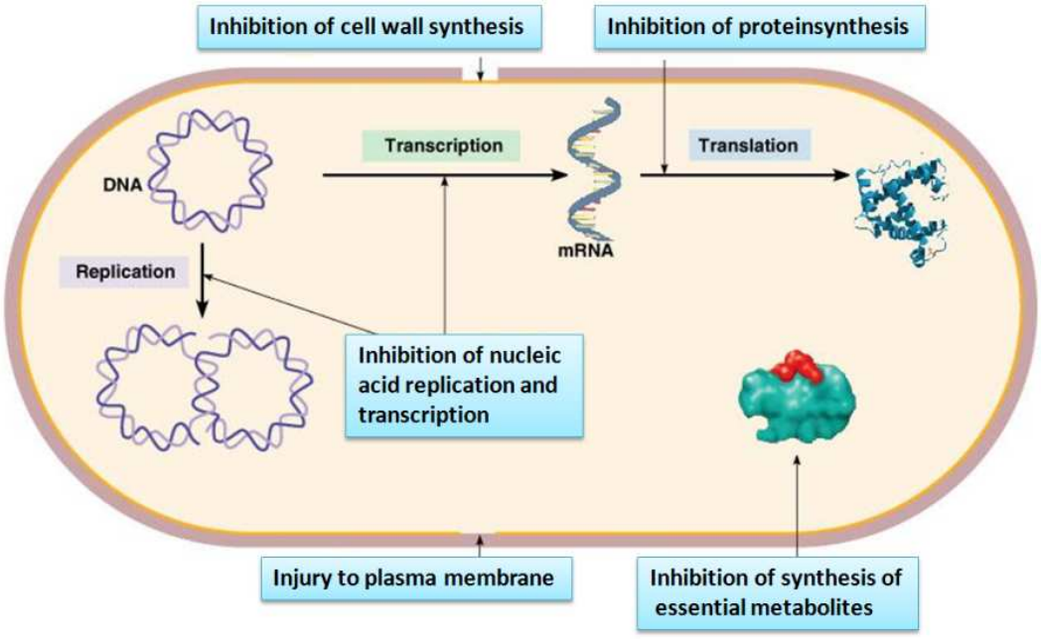

Figure 6. The main action mechanisms of antibacterial drugs

- DNA synthesis inhibitors (fluoroquinolones, dihydrofolate reductase inhibitors)
- Fatty acid synthesis inhibitors

As can be seen here, drug discovery and development against MRSA is a highly active field. The wide-ranging clinical spectrum of infections is mirrored by the discovery of chemical classes active against it [63]. Literature offers the opportunity to obtain a new drug with antibacterial, antifungal, antiviral, and antiparasitic potential from natural products by extraction process. Cos et al. [64] presents a scheme of testing organisms for antibacterial, antifungal, antiviral, and antiparasitic screening *in vitro*. Irrespective of the adopted plant collection strategy, a critical step is the processing of the plant material that will be used in the process of screens. Infections caused by different species continue to increase in frequency and severity [65]. The number of enzymes and the number of organisms that exhibit cross-resistance to some classes of antimicrobials is growing, generating an increase of researches in antibacterial substances domain.

6. Conclusion and future perspectives

The treatment of infections caused by bacteria remains an actual problem that still raises problems and maintains a vivid interest of both scientists and pharmaceutical companies for finding new and innovative solutions. These health issues should be approached on multiple levels, starting from synthesis, design, and the mechanisms of action. The research directed towards the preparation of new antibacterial compounds must be based on deeper understanding of the resistance mechanisms. More promising antibacterial drugs with novel

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mechanisms of action should be developed and new types of targets must emerge. There is a great need for enhancing the precision of targeting the pathogens and limit the misuse of antimicrobials and other practices that accelerate the emergence of novel resistance mechanisms. So, specific microbial target enzymes will continue to attract attention for novel antimicrobial discovery. A starting point is given by peptide deformylase, but its unstable character delays its utilization on industrial level. Advances in deciphering the genome of a several microbes will further help the discovery of agents against them by providing a wider selection of potential targets.

Another emerging trend for the future is the increasing correlation between infections and other diseases. For example, infection with *Chlamydia pneumoniae* is a determining factor in the pathogenesis of atherosclerosis. Administration of specific drugs was found to be protective against atherosclerosis complications, thus reducing the disease progression in as yet unspecified ways. This type of significant correlation will be taken into increasing account by antibacterial drug producers in the future, as the repercussions of infection, and therefore treatment, expand beyond the microbe itself.

On the other hand, although there is a major progress on designing scientifically sound and feasible clinical trials, more efforts must be made for making new therapies available to meet patient needs. On the other hand, novel strategies, like targeting host infection response pathways or anti-infective antibodies, offer an insight into the anti-infective drug discovery pipeline of the future.

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