# Mechanisms of Resistance: Useful Tool to Design Antibacterial Agents for Drug - Resistant Bacteria

J.K. Savjani*, A.K. Gajjar and K.T. Savjani

*Institute of Pharmacy, Nirma University of Science and Technology, S.G.Highway, Chharodi, Ahemedabad-382481, Gujarat, India*

**Abstract:** Drug-resistant bacteria are now a global health threat. In the last 5 years the WHO, The House of Lords (UK), the Centre for Disease Control (USA) and many more agencies have presented reports on the scale of this problem. Microorganisms multiply very rapidly and have adapted to fill almost every available environmental niche (Rapidly growing species of bacteria under ideal conditions of growth can multiply in about 20 minutes). All members of the chemically related β-lactam class act at the same phase in cell wall synthesis; as a result, a bacterial cell resistant to one agent is often resistant to all other analogues. The beta-peptide has two promising characteristics that distinguish it from traditional antibiotics. Firstly, bacteria may have trouble developing resistance to the beta-peptide since bacterial defenses may not recognize its unnatural amino acids. Secondly, the magainins that the beta-peptides mimic have been around for millions of years, yet bacteria have not become resistant to them. All classes of antibiotics are subject to resistance by an efflux mechanism mediated by more than one type of pump within the same organism. The bacterial cell may have a membrane pump capable of pumping a class or several classes of antibacterial agents back out of the cell. Other mechanisms of drug resistance include destruction of beta-lactam ring by β-lactamases, impermeability of the drug into the bacterial cell wall, alteration of targets within the bacterial cells and the by-pass mechanism (bacterial cell may have acquired an alternative mechanism for achieving the essential function).

**Key Words:** Antibacterial, antibiotic, antimicrobial, mechanism of drug resistance, bacterial resistance.

## INTRODUCTION

Resistance has been defined as the temporary or permanent ability of an organism and its progeny to remain viable and/or multiply under conditions that would destroy or inhibit other members of the strain. Bacteria may be said to be resistant when they are not susceptible to a concentration of antibacterial agent used in practice. Traditionally, resistance refers to instances where the basis of increased tolerance is a genetic change, and where the biochemical basis is known; the basis of bacterial resistance to antibiotics is well known, but that of resistance to antiseptics, disinfectants and food preservatives is less well understood [1]. Resistance to antimicrobial agents is a major global health problem. In the last 5 years the World Health Organization, the European Union, The House of Lords (UK) and the Centre for Disease Control (USA) and many more agencies have presented reports on the scale of this problem. There is already evidence that antibacterial resistance is associated with an increase in mortality. For the person taking the antibiotic, there is always a risk of a side effect from the antibiotic. Every use of antibiotic increases the rate at which bacteria become resistant to antibiotics. If bacteria resistant to antibiotics develop in one person there is a very high chance that they are passed onto that person's family and friends and to other people.

*Address correspondence to this author at the Institute of Pharmacy, Nirma University of Science and Technology, S.G.Highway, Chharodi, Ahemedabad-382481, Gujarat, India; Tel: +91-2717-241900-04; Fax: +91-2717-241916; E-mail: jignasasolanki@rediffmail.com

## MECHANISMS OF ANTIBACTERIAL ACTION

Molecular mechanisms of the action of antibiotics must be recalled for a better understanding of bacterial resistance mechanisms. There are four main mechanisms of antibacterial action, as shown in Fig. (**1**).

### (1) Inhibition of Synthesis of Microbial Cell Wall

The enzyme targets for β-lactam antibiotics are penicillin binding proteins (PBPs), located on the external face of the cytoplasmic membrane. These antibiotics inhibit interpeptidic bond formation, which is the last step of peptidoglycan synthesis, through binding to the enzymes transpeptidases and DD-peptidases (D-Ala-D-Ala peptidases). The resultant acylenzyme complex has no physiological activity, leading to a bactericidal effect [2].

Glycopeptide antibiotics (vancomycin and teicoplanin) also act at the last step of peptidoglycan synthesis, through inhibition of peptidoglycan polymerization.

Fosfomycin acts at the first step of peptidoglycan polymerization by inhibiting pyruvyl transferase, an intracytoplasmic enzyme which is involved in the synthesis of the UDP-N-acetylmuramyl pentapeptide (NAMP) precursor. Fosfomycin crosses the cytoplasmic membrane through two systems of active transport. Bacitracin also acts early in the peptidoglycan synthesis process, through inhibition of the phospholipid dephosphorylation which is required for the synthesis of the peptidoglycan longitudinal chain.

1389-5575/09 $55.00+.00 

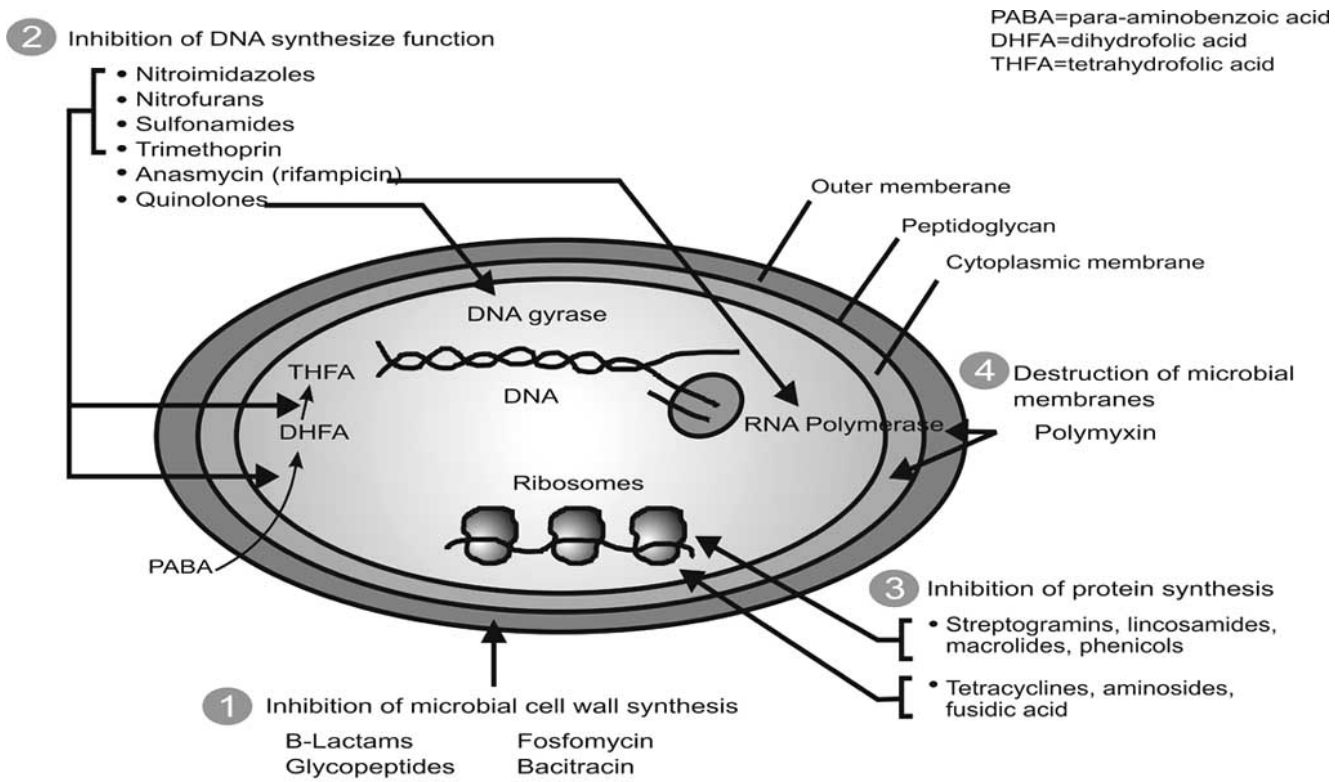



**Fig. (1).** The four main mechanisms of antibacterial action.

### (2) Inhibition of DNA Synthesis or Function

Rifampicin belongs to the ansamycin family. It is a hydrophobic antibiotic that blocks RNA polymerase, which transcribes DNA into mRNA, leading to a bacteriostatic effect. Its selective toxicity for bacteria relies on its absence of activity on eukaryote cells.

Quinolone drugs induce rapid inhibition of DNA synthesis and then bacterial death through binding to DNA gyrase, a topoisomerase, thus forming a DNA-enzyme-quinolone complex which inhibits the enzyme function causing bacterial cell death [3]. DNA gyrase is not only required for DNA compaction, but also for the replication and transcription process. Recently, quinolone drugs have been shown to inhibit DNA topoisomerase IV, a second bacterial topoisomerase [4].

Sulfonamides and trimethoprim inhibit the synthesis of folates, by inhibition of the enzyme dihydrofolate synthase. Folic acid is required for the synthesis of purines and pyrimidines, the major constituents of nucleic acids, thus leading to reduction or cessation of bacterial growth.

### (3) Inhibition of Synthesis of Bacterial Proteins

Aminoglycoside antibiotics inhibit DNA replication initiation by binding to 16S ribosomal RNA, a component of the ribosomal 30S subunit. The steps involved in translation are altered, resulting in inhibition of translation. Further, whatever proteins are synthesized are of the abnormal kind due to numerous reading mistakes [5].

Tetracyclines have bacteriostatic activity by inhibiting the elongation step of translation. They bind to the 30S ribosomal subunit, thus preventing fixation of a new aminoacyl-tRNA on ribosomal site A. They have a broad spectrum of activity.

Macrolides, streptogramin and lincosamide antibiotics bind to the ribosomal 50S subunit, thus resulting in inhibition of peptide elongation through blockage of either peptide transfer or translocation steps [6].

Chloramphenicol binds to the 50S subunit and prevents the amino acid of the aminoacyl-tRNA complex from binding normally to the ribosome. It thus inhibits peptide transfer from the site P to site A. It too displays a broad spectrum of activity.

### (4) Lesion or Destruction of Microbial Membranes

Polymyxin antibiotics bind to lipid membranes, which induce structural alterations and lead to rapid bacterial death.

## RESISTANCE AND MICROBIAL DIVERSITY

There is an important distinction between Intrinsic resistance and Acquired resistance. When an antimicrobial is discovered, it is usually the case that, it is active against some species or genera of microorganisms but not active against others. Those species or genera against which the agent has no activity from the outset are intrinsically resistant. It has been the experience with essentially every antimicrobial agent developed that after a period of use resistance to the antimicrobial is observed in species that were originally fully sensitive. This is acquired antimicrobial resistance.

Microorganisms multiply very rapidly and have adapted to fill almost every available environmental niche. Rapidly

growing species of bacteria under ideal conditions for growth can replicate their DNA and divide into 2 cells in about 20 minutes. Bacteria have an extraordinary diversity of mechanisms to increase the genetic diversity of a population of bacteria.

## EMERGENCE OF ANTIBIOTIC RESISTANCE

It is essential to have knowledge of the mechanism of the development of antibiotic resistance by bacteria to enable the design of better molecules capable of circumventing the resistance strategy of the bacteria. The most frequent mechanisms by which the bacteria become resistant to antibiotics include -

(1). **Inactivating Enzymes**: The first central mechanism by which bacteria can be resistant to an antibiotic is by enzymes that modify the drug in question.

(2). **Altered Target:** Alteration of the drug-binding site is another mechanism by which bacteria become resistant to drugs. The site targeted by the antibacterial agent may be in a form that is not sensitive to the action of the agent. The drug is no longer capable of reacting with it.

(3). **Impermeability:** Defects of antibiotic penetration into the bacteria.

(4). **Antibiotic Efflux Pumps:** Antibiotic efflux pumps are a common way resorted to by the bacteria to resist the action of numerous classes of antibiotics.

Examples of Mechanisms of microbial resistance to antibiotics

Table **1** shows a few examples of antibiotics, which can be inactivated by bacteria by various mechanisms.

## INACTIVATING ENZYMES

### Mechanism of Bacterial Resistance to β-Lactam Antibiotics

The most important biochemical mechanism of inactivation of β-lactam antibiotics is by the enzyme penicillinases. There are two general types of penicillinases: β-lactamases

**Table 1. Mechanisms of Microbial Resistance to Antibiotics**

| Class of antibiotics | Example | Mechanism of microbial resistance |
|---|---|---|
| Aminoglycosides | Gentamycin, Kanamycin, Tobramycin | Target alteration<br>Antibiotic inactivation |
| Ansamycins | Rifampicin | Active efflux<br>Target alteration |
| β-lactam | Penicillins, Methicillin | Reduction in cellular permeability<br>Target alteration<br>Antibiotic inactivation<br>Antibiotic sequestration by a protein |
| Phenicols | Chloramphenicol | Reduction in cellular permeability<br>Active efflux<br>Antibiotic inactivation |
| Glycopeptides | Vancomycin, Teicoplanin | Target alteration |
| Macrolides | Erythromycin, Clarithromycin | Active efflux<br>Target alteration<br>Antibiotic inactivation |
| Quinolones and Fluoroquinolones | Nalidixic acid, Norfloxacin | Target alteration (DNA gyrase)<br>Defect of diffusion through outer membrane<br>Active efflux |
| Sulfonamides<br>Trimothoprim | Co-trimoxazole | Active efflux<br>Alteration metabolic pathway<br>Overproduction of antibiotic target |
| Tetracyclines | Tetracycline, Minocycline | Reduction in cellular permeability<br>Active efflux<br>Target alteration<br>Alternative metabolic pathway |

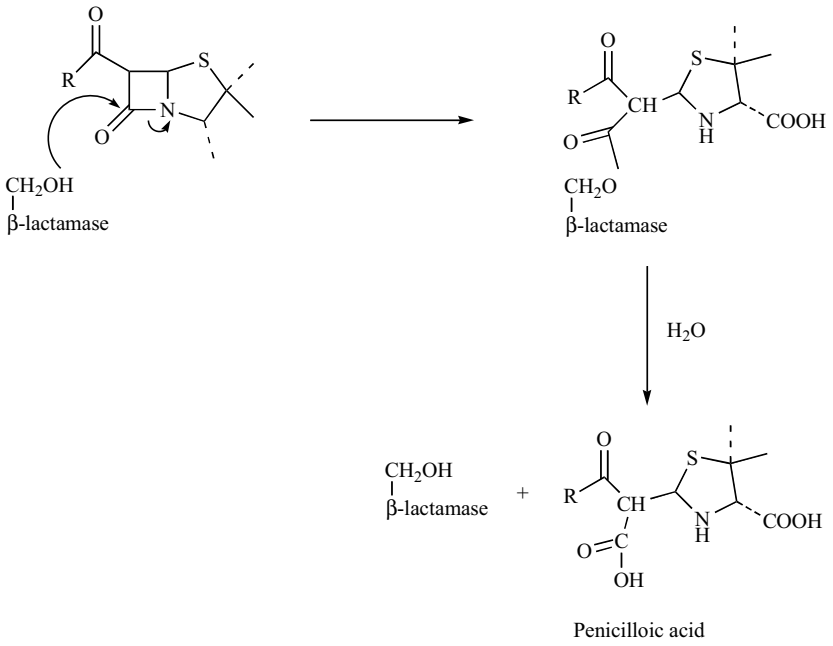



**Fig. (2).** Deactivation of Penicillin by β-lactamase enzyme.

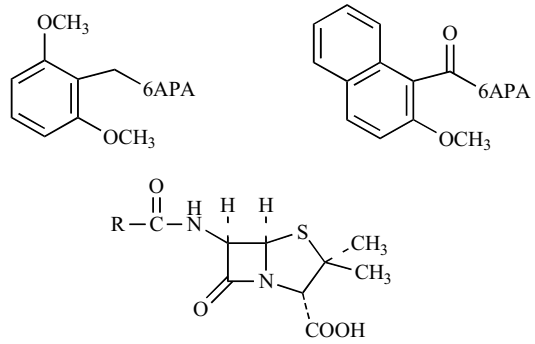
and acylases. The β-lactamases catalyze the hydrolytic opening of the β-lactam ring of penicillins to produce inactive penicilloic acid as shown in Fig. (**2**).

β-lactamase production by the cell is the most prevalent mechanism of acquired resistance to β-lactam antibiotics [7-9]. Bacteria can acquire new genes by bacteriophage transduction or by transformation. A major clinical problem is the transfer of resistance genes across genus and species lines. Synthesis of bacterial β-lactamases may be under chromosomal or plasmid R factor control and may be either constitutive or inducible depending on the bacterial species [10].

## Structural Requirement for β-Lactamases Tolerant Antibiotics

The deactivation of the β-lactam ring in penicillins and cephalosporins takes place by β-lactamase enzyme, which is produced by the resistant bacteria and causes formation of penicilloic acid. The four membered strained lactam ring is the chemically activated functionality in the drugs that acylates and irreversibly modifies the cell wall cross-linking PBPs. The lactamase producing bacteria secrete this enzymatic weapon into the periplasm to destroy β-lactam antibiotics before they can reach the PBP targets in the cytoplasmic membrane. A single β-lactamase molecule can hydrolyze $10^3$ penicillin molecules per second. So if $10^3$ enzyme molecules are secreted per resistant cell, then 100 million molecules of penicillin are destroyed every second, which is clearly an effective strategy.

Stability of the penicillins toward β-lactamase is influenced by the bulk in the acyl group attached to the primary amine in 6-amino penicillanic acid. β-lactamases are less tolerant to the presence of steric hindrance near the side chain amide bond than are the penicillin binding proteins. The aromatic ring attached directly to the side-chain carbonyl and both ortho positions substituted by methoxy groups results in β-lactamase stability. Movement of the methoxy groups to the para position or replacing one of them by hydrogen make the analogue sensitive to β-lactamase. Fig. (**3b**) shows that if ortho position is not substituted, there are more chances of hydrolysis. Putting in a methylene between the aromatic ring and 6-APA (6-amino penicillanic acid) like wise produced a β-lactamase sensitive agent. Resistance to enzyme degradation is based on differential steric hindrance [11]. Fig. (**3a**) shows that both ortho positions of the ring should be substituted, to prevent hydrolysis of the antibiotic molecule by β-lactamases.




6-aminopenicillanic acid (6APA)

**Fig. (3a).** β-lactamase resistance.

## β-Lactamase Inhibitors

The discovery of the naturally occurring, mechanism based inhibitor clavulanic acid, which causes potent and progressive inactivation of β-lactamases had created renewed interest in β-lactam combination therapy. This interest led to the design and synthesis of additional mechanism based β-lactamase inhibitors, such as sulbactam and tazobactam.

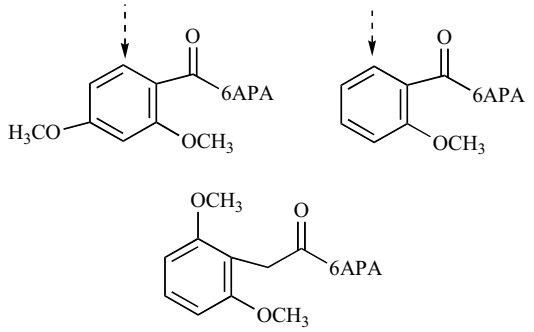



**Fig. (3b).** β-lactamase sensitive.

The chemical events leading to the inactivation of β-lactamases by mechanism-based inhibitors are very complex. There are two classes of β-lactamase inhibitors, class-I inhibitors that have a heteroatom leaving group at position 1 (e.g. clavulanic acid and sulbactam) and class II inhibitors that do not have a heteroatom leaving group at position 1 (e.g. carbapenems). Unlike competitive inhibitors, which bind reversibly to the enzyme, the mechanism-based inhibitors react with the enzyme in much the same way that the substrate does. With the β-lactamases, an acylenzyme intermediate is formed by reaction of the β-lactam with an active-site serine hydroxyl group of the enzyme. For normal substrates, the acylenzyme intermediate readily undergoes hydrolysis, destroying the substrate and freeing the enzyme to attack more substrate. The acylenzyme intermediate formed when a mechanism-based inhibitor is attacked by the enzyme is diverted by tautomerism to a more stable imine form that hydrolyzes more slowly to eventually free the enzyme (transient inhibition), or for a class I inhibitor, a second group on the enzyme may be attacked to inactivate it, as depicted in, Fig. (**4**). Because these inhibitors are also substrates for the enzymes that they inactivate, they are sometimes referred to as suicide substrates [12].

β-lactamases are deterred from β-lactam inactivation by the concurrent use of a susceptible β-lactam antibiotic with a β-lactamase inhibitor as is indicated in Fig. (**5**). Among all the groups of β-lactamases, the most important is represented by the TEM type β-lactamases, with more than 50 different TEMs. These are the most frequent β-lactamases produced by *H. influenzae*. However, these TEMs are susceptible to β-lactamase inhibitors (clavulanic acid, sulbactam, tazobactam), molecules which are related to β-lactams, but whose affinity for β-lactamases is higher than for PBPs. These have a strong inhibitory effect on β-lactamases even at low dosage, which allows the antibiotic to act on its target, leading to fairly good restoration of the activity of the antibiotic concerned.

Unfortunately, selective mutations of the TEM-encoding gene has led to β-lactamases being resistant to the inhibitors

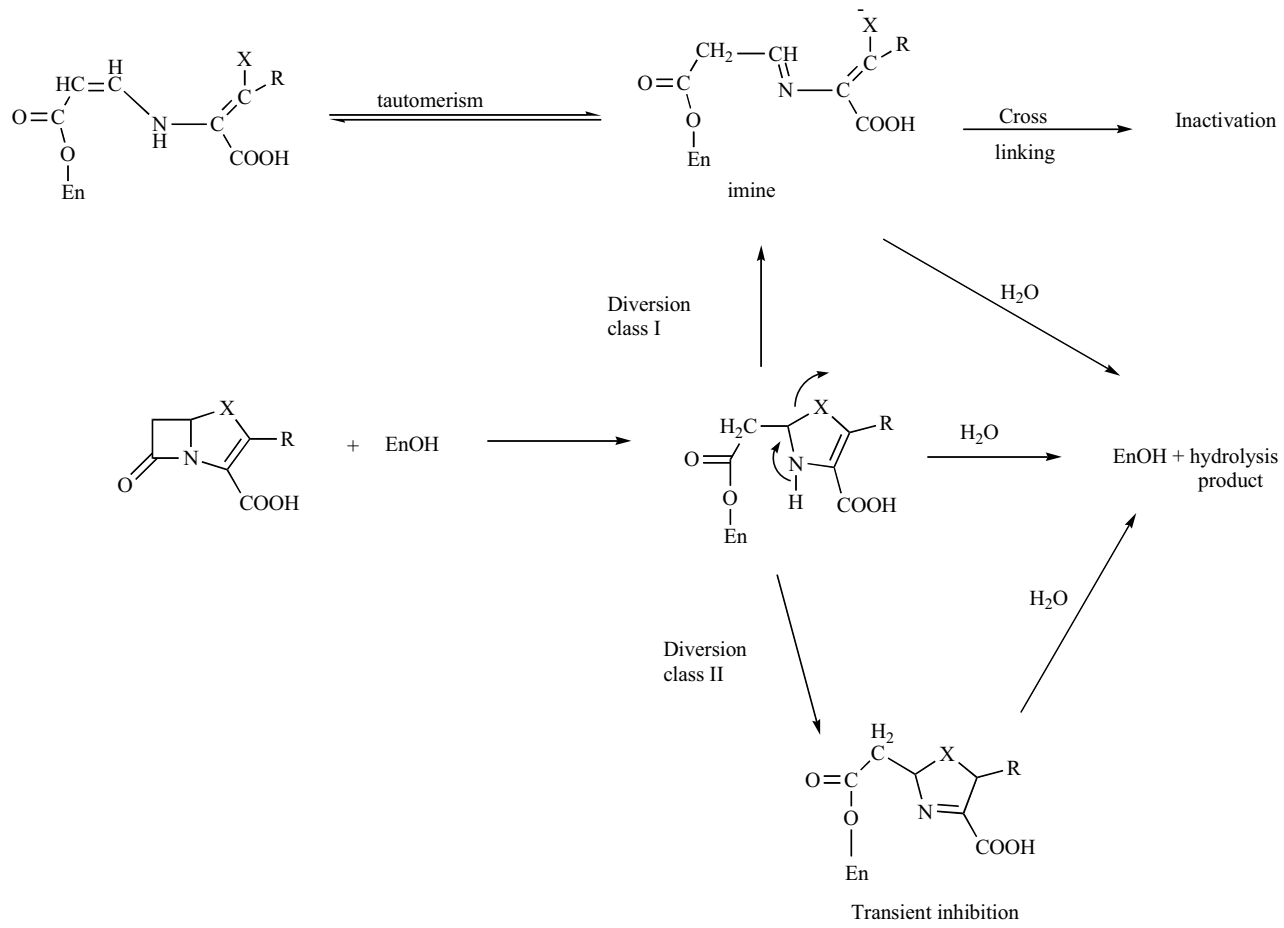



**Fig. (4).** Mechanism-based inhibition of β-lactamases.

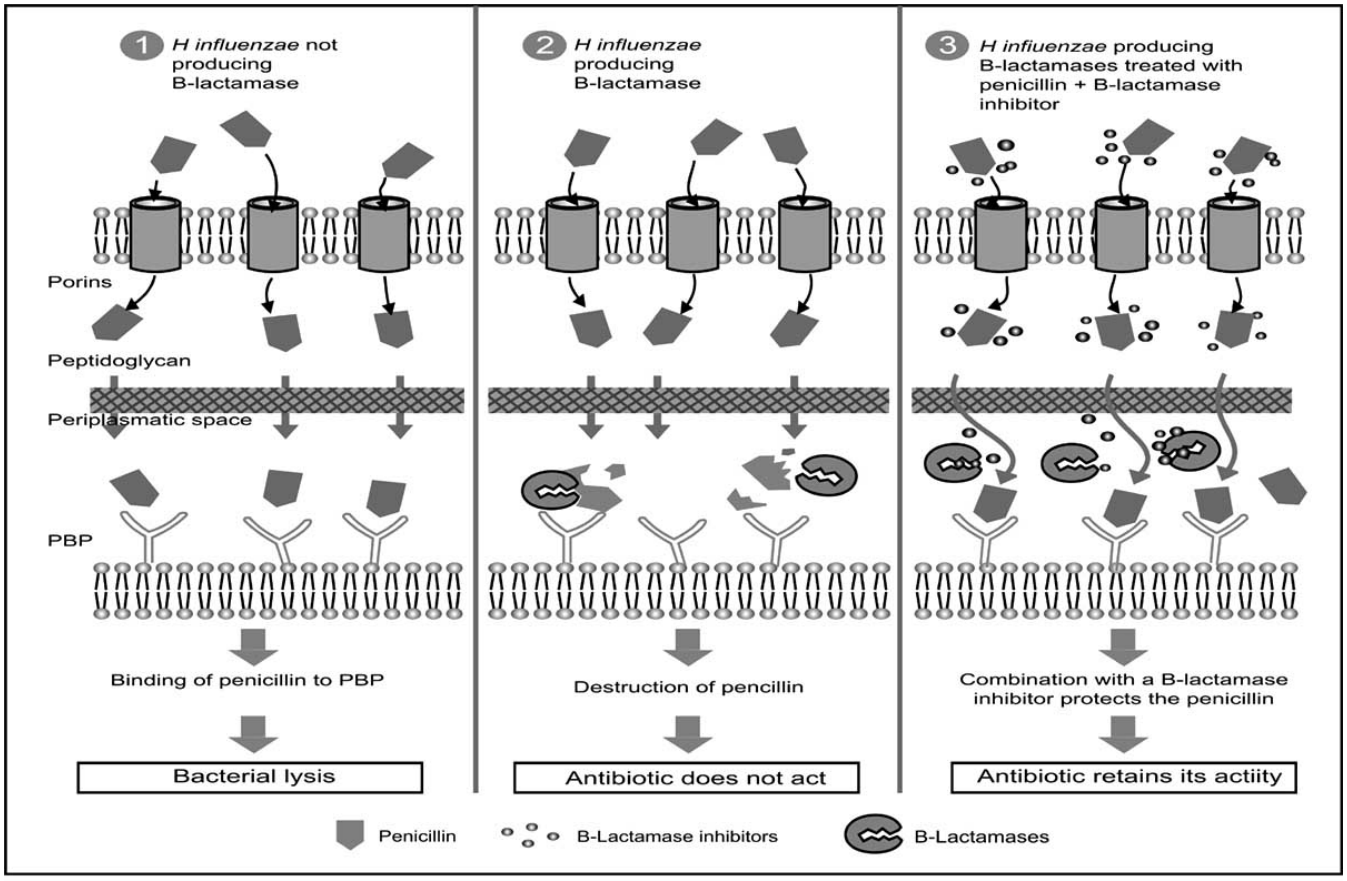



**Fig. (5).** Penicillin inactivation by β-lactamase production by *H. influenzae.*

(inhibitor-resistant TEM, or IRT), such as TEM-1 and TEM-2 [13]. The latter, present in strains of *E coli*, is very efficient and can hydrolyze up to 2000 molecules of penicillin G per second. These TEMs are responsible for penicillin resistance, even when they are associated with β-lactamase inhibitors [5]. Moreover, these β-lactamases can hydrolyze third-generation cephalosporins. Since the first description in 1965, the TEM-1 gene has easily diffused in *P. aeruginosa* in 1970, then in *H. influenzae* in 1972, and even in *N. meningitidis* in 1983. Indeed, it is part of a transposon which can integrate into plasmids specific for a large panel of bacterial genera [14]. In addition, plasmidic cephalosporinases have appeared in *Klebsiella,* which represent a real threat of epidemic hospital-acquired infections.

The strategy of using β-lactamase inhibitors in combination with a β-lactamase sensitive penicillins in the therapy for infections caused by β-lactamase producing bacterial strains to kill the organisms, met with limited success. Factors that contributed to the failure of such combinations to achieve synergy include (a) the failure of most lipophilic penicillinases resistant penicillins to penetrate the cell envelop of Gram negative bacilli in effective concentrations, (b) the reversible binding of penicillinases resistant penicillins to β-lactamase, requiring high concentrations to prevent substrate binding & hydrolysis and (c) the induction of β-lactamases by some penicillinase resistant penicillins [15].

## ALTERED TARGET

The site targeted by the antibacterial agent may be in a form that is not sensitive to the action of the agent. Resistance to antimicrobial agents can be due to a mechanism of adaptation of the cell envelope. For bactericides to be effective, they must be able to penetrate the cell envelope and attain sufficiently high concentrations at the target site to exert their antibacterial action. Hydrophilic antibacterial agents are primarily prevented from entering through the outer membrane by the lipopolysaccharide layer and the underlying phospholipids, whereas hydrophobic agents are excluded by outer membrane proteins. Certain antibiotic resistant bacterial strains either lack or overexpress certain outer membrane proteins; so the drug is no longer capable of reacting with it (ribosomes, enzymes for bacterial wall synthesis, etc) as seen in *Streptococcus pneumoniae* and beta-lactam antibiotics [16, 17].

Acquired resistance of *S. pneumoniae* to penicillin and other β-lactams relies on the presence of mosaic genes, a result of complex chromosomal recombination with genes coming from other species and encoding new PBPs as shown in Fig. (**6**).

These genes integrate into the pneumococcal chromosome as transposons and induce alterations of one out of the six pneumococcal PBPs by substitution of one or more

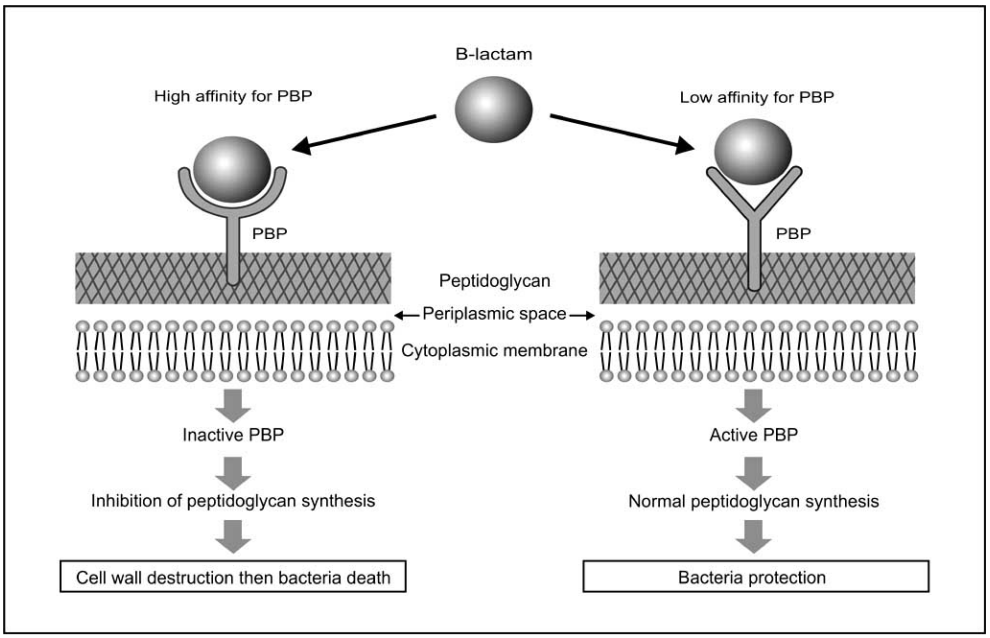



**Fig. (6).** *S. pneumoniae* resistance to β-lactams by target alteration.

amino acids, which leads to reduced affinity for β-lactams. Hence the PBPs remain functional and ensure peptidoglycan synthesis, and the cell wall is not altered. The modified strains are called *S. pneumoniae* with reduced susceptibility to penicillin (SPRSP), since their susceptibility to β-lactams is reduced to a varying extent.

## IMPERMEABILITY

Gram positive and Gram negative bacteria differ in the structure of the cell wall that extends between the outer polysaccharide capsule (if present) and inner cytoplasmic membrane. Gram positive bacteria possess a permeable cell wall that usually does not restrict the penetration of antimicrobials. Mycobacteria contain a lipid rich outer membrane of unusually low permeability which contributes to their intrinsic resistance to many agents. The outer membrane of *P. aeruginosa* presents a significant barrier to the penetration of antibiotics, restricting the rate of penetration of small hydrophilic molecules and excluding larger molecules [18]. It is assumed that small and hydrophilic antibiotics cross the outer membrane porins, whereas hydrophobic antibiotics may diffuse through the membrane directly. The porins are barrel-shaped molecules which span the outer membrane, usually associated as trimers as indicated in Fig. (**7**). A mutant of *M. smegmatis* lacking major porin MspA displayed a high level of resistance to ampicillin and cephaloridine. This

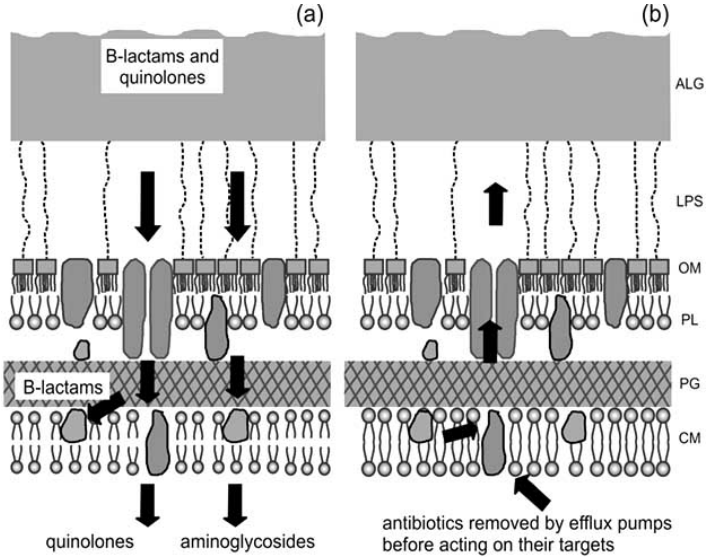



**Fig. (7).** Schematic representation of the arrangement of components in the cell wall of *P.aerusinosa*. CM=cytoplasmic membrane; OM=outer membrane; PG=peptidoglycan; LPS=lipopolysaccharide; ALG=alginate. (a) The pathways for penetration of β-lactams and quinolones through porin channels in the OM. Aminoglycosides and colistin promote their own uptake by interacting with the LPS on the outer face of the OM. (b) How efflux pumps comprise three components; an energy-dependent pump in the CM, a porin in the OM and an adapter protein joining the two membrane components.

clearly demonstrated the relationship between antibiotic activity and the outer membrane permeation [19]. The aminoglycosides and collistin do not cross the outer membrane porin channels. Instead they promote their own uptake by binding to the lipopolysaccharide (LPS) on the outer face of the membrane. This destroys the permeability barrier of the outer membrane and allows the antibiotics to penetrate through the wall to the cytoplasmic membrane. The aminoglycosides are actively transported into the cells where they interfere with protein synthesis at the ribosomes. Collistin exerts its bactericidal action through disruption of the cytoplasmic membrane. Resistance to aminoglycosides and collistin has been observed in laboratory strains of *P. aeruginosa* due to overexpression of an outer membrane protein, oprH, which protects the LPS from binding to the antibiotics [20].

All of the major classes of antibiotics used to treat *P. aeruginosa* infections have to cross the cell wall to reach their targets. *P. aeruginosa* is a type of multiresistant bacteria, resistant to β-lactams, including third-generation cephalosporins, quinolones, chloramphenicol, and cyclines. The major cause is the very low permeability of the cell wall; at least 100 times lower than *E. coli,* since *P. aeruginosa* porins are unusual and less in number. Moreover, *P. aeruginosa* is characterized by the production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase); three mechanisms which synergize with the poor permeability of the microbial cell wall. Failure of antibiotics to accumulate within the organism is due to a combination of restricted permeability of the outer membrane and the efficient removal of antibiotic molecules by the action of efflux pumps [21, 22].

## ANTIBIOTIC EFFLUX PUMPS

Bacterial cells may have a membrane pump capable of pumping a class or several classes of antibacterial agents out of the cell. This prevents the agents achieving an effective intracellular concentration. If an antibacterial agent crosses the bacterial cellular membrane, it may be eliminated from the bacteria with an active efflux pump. The bacterium develops an active efflux pump which forces the antibacterial agent out of the cytoplasm faster than it can diffuse in. Therefore, intra bacterial concentrations are too low for the drug to be effective. These efflux pumps are variants of bacterial membrane pumps that move nutrients and waste in and out of the cell. In addition, some antibiotic producing bacteria use these pumps to move the antibiotic out of the cell, into the surrounding environment. This natural mechanism protects the antibiotic producing bacterium from being killed by its own production of antibiotic substances, but kills other bacteria in the environment allowing it to grow unimpeded. Clinically relevant bacteria that use an active efflux pump as a mechanism of antibacterial resistance include *Escherichia coli* and *Shigella.* The resistance of *Escherichia coli* to fluoroquinolones is through the N-ad pump as shown in Fig. (**8**).

Efflux of antibiotics can be mediated by more than one pump in a single organism and almost all antibiotics are subject to resistance by this mechanism. Some efflux pumps selectively get rid of specific antibiotics and others, so called Multi Drug Resistance (MDR) pumps, efflux a variety of structurally diverse compounds. Five families of bacterial drug efflux pumps have been identified to date based upon the energy source they use to export their substrate and sequence similarity. Transport can either be driven by ATP hydrolysis as in case of the ATP-binding cassette (ABC) Superfamily, or pump can utilize the proton motive force (PMF). The four PMF-dependent families are, Small Multi Drug Resistance Family (SMR), Major Facilitator Superfamily (MFS), Multi drug and Toxic Compound Extrusion Family (MATE) and Resistance/Nodulation/Cell Division Family (RND). Genes encoding MDR pumps are normal constituents of bacterial chromosomes and increased antibiotic resistance is a consequence of over expression of these genes.

Efflux pumps in gram positive bacteria excrete their substrate across a single cytoplasmic membrane. This is also the case for some pumps in gram negative bacteria, and as a result their substrates are effluxed into the periplasmic space. Other efflux pumps from gram negative bacteria efflux their substrates directly into the external medium, bypassing the

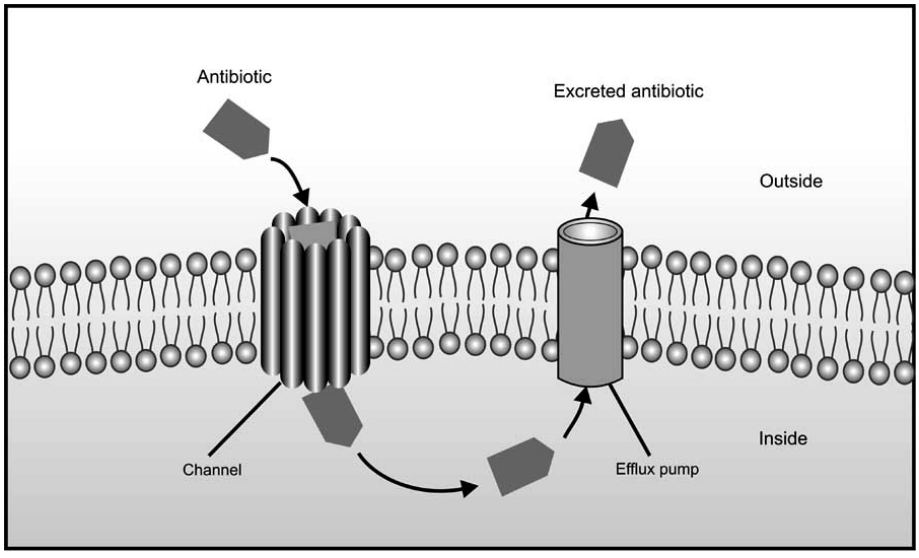



**Fig. (8).** Active efflux of antibiotic by enzymatic systems as a mechanism for *E. coli* resistance to fluoroquinolones.

periplasm and the outer membrane. These pumps are organized in complex three component structures, which traverse both inner and outer membrane. They consist of a transporter located in the cytoplasmic membrane, an outer membrane channel and a periplasmic 'linker' protein, which brings the other two components into contact [23].

**Strategies to Combat Efflux Mediated Resistance**

There are main two approaches that could be explored to combat the adverse effects of efflux on the efficacy of antibacterial agents.

***(1) Derivatization of Antibiotics that are Effluxed Minimally***

A new class of semisynthetic tetracyclines, the glycylcyclines, e.g. 9-(dimethylglycylamido) -6-demethyl-6-deoxytetracycline (DMG-DMDOT) has been developed by investigators at American Cyanamid as shown in Fig. (**9**). These compounds exhibit potent activity against a broad spectrum of gram positive and gram negative bacteria, including those that carry ribosomal protection and efflux determinants. Glycylcyclines overcome efflux-mediated resistance because they are not recognized by the transporter protein [24, 25].

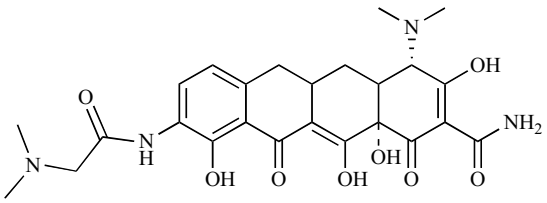



**Fig. (9).** Structure of DMG-DMDOT.

In general, attempts to modify existing classes of antibiotics to bypass gram negative efflux pumps appear to be less successful than those in gram positive bacteria. In gram negative bacteria particularly, restrictions are imposed on the structure of successful drugs; they must be amphiphilic in order to cross both membranes. It is this very property that makes antibiotics good substrates of multi drug resistance efflux pumps from gram negative bacteria. Only for antibiotics whose target is in the periplasm is this inherent, problem avoidable; for example, the multidrug efflux pump AcrAB of *Salmonella typhimurium* does not affect hydrophilic β-lactams that do not contain lipophilic side chains [26].

***(2) Development of Therapeutic Agents that Inhibit Activity of Efflux Pumps***

A key issue in this strategy is to identify antibiotics and bacteria for which the approach would be the most appropriate. While all antibiotics can be effluxed by MDR pumps, resistance mediated by them is not equally important for all in clinical settings. In general it is of particular relevance for antibiotics that are less affected by other resistance mechanisms.

In gram negative bacteria, MDR (Multi Drug Resistant) pumps that confer resistance to fluoroquinolones belong to the RND (Resistance /Nodulation /Cell Division) family and are similar on the protein level, increasing the feasibility of identifying a single inhibitor with activity against several relevant pumps. In gram positive bacteria, by contrast, resistance to fluoroquinolones is mainly due to the activity of pumps belonging to the Major Facilitator Superfamily (MFS). It is therefore less likely, that a single inhibitor will potentiate fluoroquinolones in both gram negative and gram positive bacteria. Inhibitors of three types of pump have been identified: MF Tet and NorA pumps in gram positive and RND MDR pumps in gram negatives. [27, 28].

**REPROGRAMME THE TARGET STRUCTURE**

Hydrolytic deactivation of penicillins and cephalosporins takes place by β-lactamases, cleaving the four membered, strained lactam ring, required for modification of PBPs resulting in inhibition of peptidoglycan synthesis. Other antibiotic classes, such as the aminoglycosides, do not contain such hydrolytically labile groups as in β-lactam antibiotics. These protein-synthesis inhibitors are still neutralized by deactivating enzymes but now the enzymes decorate the periphery of the aminoglycosides with three types of chemical substituents that interrupt the binding of the aminoglycoside to the RNA targets in the ribosome. Aminoglycoside resistance enzymes can be adenylyl transferases, which add AMP moieties, phosphoryl transferases, which add a phosphate group, or acetyl transferases, which acetylate the amino groups of the antibiotic as depicted in Fig. (**10**), the modified aminoglycoside products have considerably lower affinity for RNA and so do not bind and interrupt the process of protein synthesis. A resistance strategy focuses not on removal or destruction of the antibiotic but on a reprogramming or camouflaging of the target in the now resistant bacteria. In the erythromycin resistance manifolds, in addition to efflux pumps, resistant bacteria have emerged that have learned to mono- or dimethylate a specific adenine residue, A2058, in the peptidyl transferase loop of the 23S RNA component of the ribosome. This modification is carried out by a methyl transferase enzyme, Erm, that does not impair protein biosynthesis but does lower the affinity of all the members of the erythromycin class of drugs for the RNA, as well as for the pristinamycin class. The Erm mechanism is the main resistance route in drug resistant clinical isolates of *S. aureus* and is present in erythromycin producing organisms as a self immunity mechanism. An additional example of the reprogramming strategy is used by VRE (Vancomycin Resistant Enterococci) to escape from vancomycin. In VRE the vanHAX genes encode a new pathway of enzymes that reduce pyruvate to D-lactate (vanH), add D-alanine and D-lactate together to produce D-Ala-D-Lac (vanA), and then hydrolyze the normal metabolite D-Ala-D-Ala while sparing D-Ala-D-Lac (vanX) [29]. In this cell, only the D-Ala-D-Lac accumulates and serves as a substrate to be elongated and presented at the termini of the peptidoglycan strands as shown in Fig. (**11**). The reprogramming of peptidoglycan to end in D-Ala-D-Lac rather than the normal D-Ala-D-Ala has no effect on the cross linking efficiency carried out by the transpeptidating PBPs, but the switch from the amide linkage in the D-Ala-D-Ala peptidoglycan termini to the ester linkage in the D-Ala-D-Lac termini is accompanied by a 1000 - fold drop in drug-binding affinity of vancomycin and enables the VRE to grow at 1000 – fold higher levels of antibiotic [30]. Finally, penicillin resistance can arise not only by β-lactamase expression, but also by mutation of penicillin-

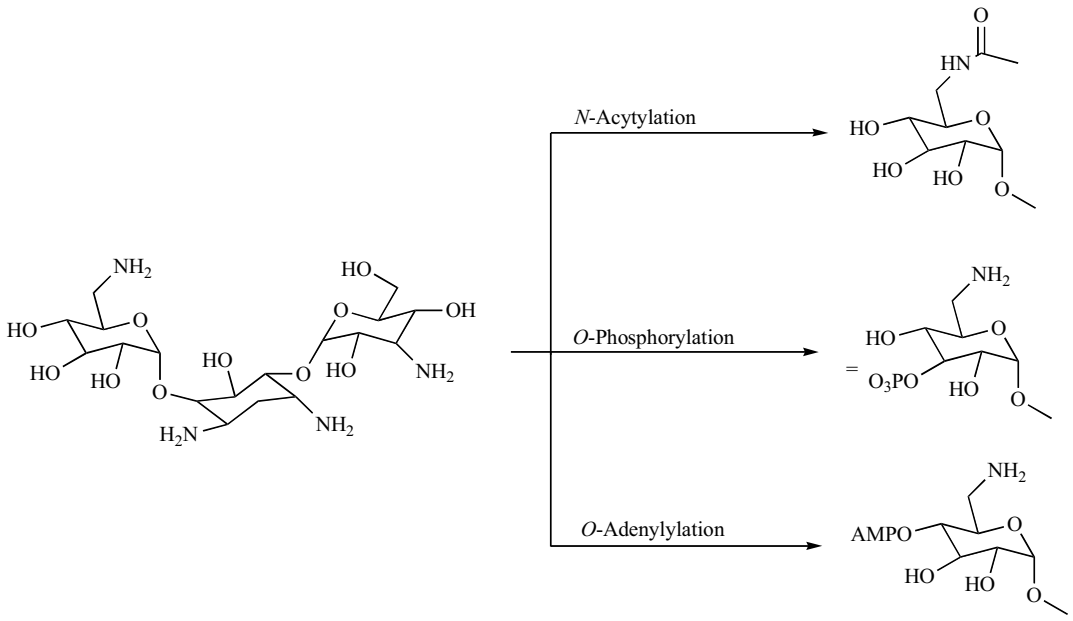



**Fig. (10).** The aminoglycoside antibiotic Kanamycin modified at three sites by three kinds of enzymatic processing.

binding proteins to lower-affinity forms as well as by expression of new PBPs with lower affinity for the antibiotic. The acquisition by *S. aureus* of the mecA gene that encodes a PBP protein with low affinity for all β-lactam antibiotics provides the molecular basis for the MRSA (Methicillin Resistant *S. aureus*) phenotype that is now widely disseminated [31-33].

## NOVEL STRATEGY TO TARGET AND KILL ANTIBIOTIC RESISTANT BACTERIA

Antibiotic resistance propagates in bacteria by moving DNA strands containing the resistance genes to neighboring cells. Putting bacteria on birth control could stop the spread of drug resistant microbes, and researchers at the University of North Carolina at Chapel Hill have found a way to do just that. The team discovered a key weakness in the enzyme that helps fertile bacteria swap genes for drug resistance. Interfering with the enzyme has the added effect of annihilating antibiotic resistant bacteria in laboratory cultures. Animal studies of the drugs are now underway.

Every time someone takes an antibiotic, the drug kills the weakest bacteria in the bloodstream. Any bug that has a protective mutation against the antibiotic survives. These drug resistant microbes quickly accumulate useful mutations and share them with other bacteria through conjugation – the microbe equivalent of mating. Conjugation starts when two bacteria fuse their membranes together and then each opens a

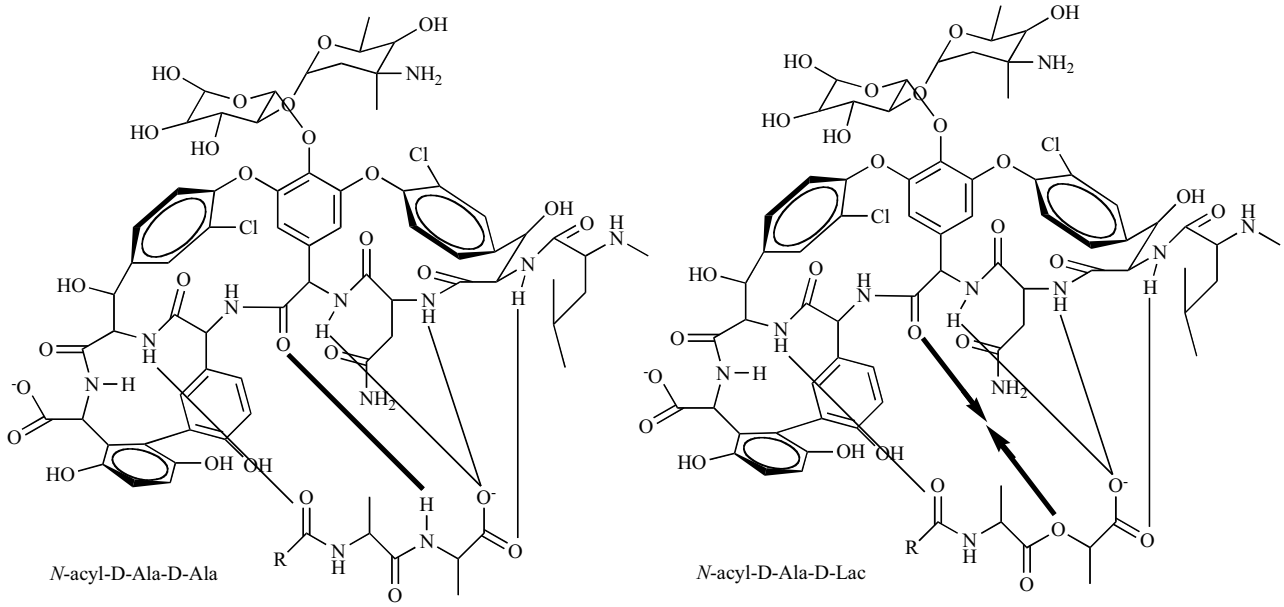



**Fig. (11).** The target structure in the bacteria reprogrammed to have a low affinity for antibiotic recognition.

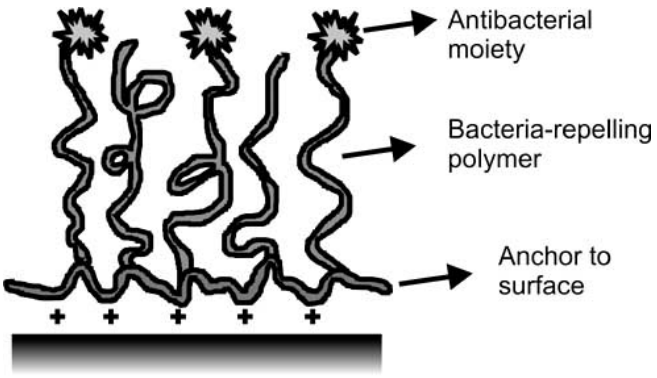



**Fig. (12).** Schematic illustration of a dual-function antibacterial polymer coating.

hole in their membrane and one squeezes out a single strand of DNA to the other. Then the two go on their merry way, one with new genes for traits such as drug resistance. Many highly drug resistant bacteria rely on an enzyme, called DNA relaxase, to obtain and pass on their resistance genes. The researchers analyzed relaxase because it plays a crucial role in conjugation. The enzyme starts and stops the movement of DNA between bacteria. From the three-dimensional picture of the relaxase protein it is predicted that the enzyme's weak link is the spot where it handles DNA. Relaxase must juggle two phosphate-rich DNA strands at the same time. Chemical decoy – a phosphate ion – could plug this dual DNA binding site. There are several bisphosphonates in the market like clodronate and etidronate, which steal the DNA binding site, preventing relaxase from handling DNA. This wreaks havoc inside *E. coli* bacteria that are preparing to transfer their genes. Exactly how bisphosphonates destroy each bacterium is still unknown, but the drugs are potent, wiping out any *E. coli* carrying relaxase. By targeting these bacteria, the drugs act like birth control pills and prevent antibiotic resistance from spreading [34].

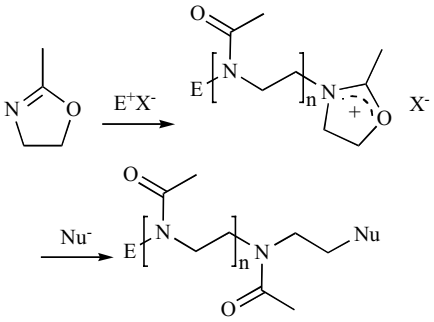



**Fig. (13).** Polymerization of 2-methyl-2-oxazoline. Initiation by an electrophile (E+), e.g. trifluoromethylsulfonate, termination by a nuclephile, e.g. potassium hydroxide.

### Novel Antibacterial Surface Coatings: 'Click' Chemistry

Infections of implanted devices are usually very difficult to treat by systemic administration of antibiotics since the bacteria reside in biofilms where they are up to 1500 times more resistant to antimicrobial agents than their planktonic counterparts.

It is therefore logical, to combat bacterial infection by coating the implant surface with antibacterial polymers [35].

A poly(L-Lysine) cationic backbone serves as an anchor to the surface *via* multiple electrostatic interactions as depicted in Fig. (**12**). Graft copolymers with this architecture have been shown to readily self assemble onto negatively charged metal oxide surfaces such as $SiO_2$, $TiO_2$ and $Nb_2O_5$. This allows an almost arbitrary number of surfaces of any shape to be coated in a simple dip-and-rinse procedure once the polymer is synthesized and characterized in bulk [36].

Bacteria-repellent polymer side-chains act against the first step in bacterial infection, that is, bacterial adhesion onto the surface (biopassive function). Typically, the polymer of choice to prevent protein and bacteria adhesion onto a surface is PEG (polyethylene glycol). However, it has been shown that PEG degrades under *in vivo* conditions forming hazardous (hydro) peroxide byproducts [37]. Alternative polymer, PMOXA (poly 2-methyl-2-oxazoline) can be used and has been shown to be equally capable to reduce bacteria adhesion as PEG ('PLL-g-PEG'). Furthermore, the living character of the cationic ring-opening polymerization of 2-methyl-2-oxazoline allows introducing functional groups at any position and in any quantity in the polymer chain as depicted in Fig. (**13**). Graft copolymers using PMOXA can be constructed and introduced with acetylene functional groups known as acetylene-functional PLL-g-PMOXA.

An antibacterial moiety that can actively kill bacteria (bioactive function) can be coupled to the polymers using 'click' chemistry. 'Click' chemistry is a newly established bioconjugation method that allows attaching azide-functionalized molecules (a component of the antibacterial moiety) under mild conditions (aqueous solutions, room temperature) to an acetylene-functionalized substrate (like the acetylene-functional PLL-g-PMOXA). This reaction tolerates almost any other functional groups thus avoiding the need for protecting groups, especially natural [38-40].

Since bacterial colonization is prevented in two ways, these coatings should be advantageous as compared to conventional antibacterial surfaces. The amount of antibacterial agent needed would be reduced and adverse effects such as toxicity and the development of bacterial resistance would be minimized. In addition, the biopassive layer would also act against the adsorption of bacterial lysate thus preventing a blockade of the antibacterial surface functionality.

## CONCLUSION

In the last decade, almost every type of bacteria has become more resistant to antibiotic treatment. These bugs cause deadly infections that are difficult to treat and expensive to cure. A thorough knowledge of the resistance mechanisms would go a long way in the rational design of existing antibiotic molecules by bringing about alterations, which are difficult for the bug to understand or in the design of new molecules to combat the resistance mechanisms.

## REFERENCE

[1] Balows, A. *Manual of Clinical Microbiology,* American Society for Microbiology: Washington, **1991**.

[2] Tomasz, A. The mechanism of the irreversible antimicrobial effect of penicillins. *Ann. Rev. Microbiol.,* **1979**, *33,* 113-37.

[3] Willmott, C. J. R.; Critchlow, S. E.; Eperon, C.; Maxwell, A. The complex of DNA gyrase and quinolone drugs with DNA forms a barrier to transcription by RNA polymerase. *J. Mol. Biol.,* **1994**, *242,* 351-63.

[4] Edwards, D. I. Nitroimidazole drugs - action and resistance mechanisms I. Mechanism of action. *J. Antimicrob. Chemother.,* **1993**, *31,* 9-20.

[5] Taber, H.W.; Mueller, J. P.; Arrow, A. S. Bacterial uptake of aminoglycoside antibiotics. *Microbiol. Rev.,* **1987**, *51,* 439-57.

[6] Aumercier, M.; Le, G. F. In *Macrolides - Chemistry, Pharmacology and Clinical Uses,* Bryskier, A.J.; Butzler, J.P.; Neu, H.C.; Tulkens, P.M., Ed.; Arnette: Paris, **1993**, pp. 115-23.

[7] Sykes, R. B.; Mathew, M. The β-lactamases of gram-negative bacteria & their role in resistance to β-lactam antibiotics. *J. Antimicrob. Chemother.,* **1976**, *2,* 115.

[8] Bush, K.; Jacoby, G. A.; Medeiros, A. A. A functional classification scheme for β-lactamses and its correlation with molecular structure. *Antimicrob. Agents Chemother.,* **1995**, *39,* 1211.

[9] Ambler, R. P.; Phil T. R. The structure of β-lactamases. *R. Soc. Lond.,* **1980**, 289-21.

[10] Segelman, A. B.; Farnsworth, N. R. Penicillin stability to alcohols. *J. Pharm. Sci.,* **1970**, *59,* 725.

[11] Foye W. O.; Lemke T. L.; Williams, D. A. *Foye` Principles of Medicinal Chemistry,* Lippincott Williams and Wilkins: Philadelphia, **2008**.

[12] Knowles, J. R. Penicillin resistance: the chemistry of β-lactamase inhibition. *Acc. Chem. Res*., **1985**, *18,* 97.

[13] Henquell, C.; Chanal, C.; Sirot, D.; Labia, R.; Sirot, J. Molecular characterization of nine different types of mutants among 107 inhibitors-resistant TEM β-lactamases from clinical isolates of *Escherichia coli. Antimicrob. Agents Chemother.,* **1995**, *39,* 427-30.

[14] Philippon, A.; Paul, G.; Nevot, P. β-lactamases : incidence et interet clinique. *Reanim. Soins. Intens. Med. Urg.,* **1987**, *3,* 230-37.

[15] Block, J. H.; Beale, J. M. Jr. *Wilson and Gisvold`s Textbook of Organic Medicinal and Pharmaceutical Chemistry,* Lippincott Williams and Wilkins: Philadelphia, **2004**.

[16] Brözel, V.S.; Cloete, T.E. Resistance of *Pseudomonas aeruginosa* to sodium dimethyldithiocarbamate by adaptation. *Curr. Microiol.,* **1993**, *26,* 275-80.

[17] Brözel, V. S., Cloete, T. E. Resistance of *Pseudomonas aeruginosa* to isothiazolone. *J. Appl. Bacteriol.,* **1994**, *76,* 576-82.

[18] Brinkman, F. S.; Brian, M.; Hancock, R.E. The amino terminus of *Pseudomonas aeruginosa* outer membrane protein OprF forms channels in lipid bilayer membranes: correlation with a three-dimensional model. *J. Bacteriol*., **2001**, *182,* 5251-55.

[19] Nikaido, H.; Vaara, M. Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev.,* **1985**, *49,* 1-32.

[20] Gilleland, L. B.; Gilleland, H. E.; Gibson, J. A.; Champlin, F. R. Adaptive resistance to aminoglycoside antibiotics in *Pseudomonas aeruginosa*. *J. Med. Microbiol*., **1989**, *29,* 41-51.

[21] Lambert, P. A. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *J. R. Soc. Med.,* **2002**, *95,* 22-23.

[22] Li, X. Z.; Livermore, D. M.; Nikaido, H. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenichol and norfloxacin. *Antimicrob. Agents Chemother.,* **1994**, *38,* 1732-41.

[23] Nikaido, H. Multiple antibiotic resistance and efflux. *Curr. Opin. Microbiol*., **1998**, *1,* 516.

[24] Sum, P. E.; Sum, F. W.; Projan, S. J. Recent developments in tetracycline antibiotics. *Curr. Pharm. Des*., **1998**, *4,* 119.

[25] Someya, Y.; Yamaguchi, A.; Sawai, T. A novel glycylcycline, 9-(N,N-dimethyl glycylamido)-6-demethyl-6-deoxytetracycline, is neither transported nor recognized by the transposon Tn 10-encoded metal- tetracycline 1H+ antiporter. *Antimicrob. Agents Chemother.*, **1995**, *39,* 247.

[26] Nikaido, H.; Basina, M.; Nguyen, V.; Rosenberg, E.Y. Multiple antibiotic resistance and efflux. *J. Bacteriol.,* **1998**, *180,* 4686.

[27] Nikaido, H. Prevention of drug access to bacterial targets: permeability barrier and active efflux. *Science,* **1994**, *264,* 382-88.

[28] Lomovskaya, O.; Watkins, W. J. Efflux pumps: their role in antibacterial drug discovery. *Curr. Chem.*, **2001**, *8,* 1705.

[29] Walsh, C.; Fisher, S. L.; Park, I. S.; Prahalad, M.; Wu, Z. Bacterial resistance to vancomycin: five genes and one missing hydrogen bond tell the story. *Chem. Biol.,* **1996**, *3,* 21-28.

[30] Bugg, T. D. H. Molecular basis for vancomycin resistance in E*nterococcus faecium*, BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry,* **1991**, *30,* 10408-15.

[31] Song, M. D.; Wachi, M.; Doi, M.; Ishino, F.; Matsuhashi, M. Evolution of an inducible penicillin target protein in MRSA by gene fusion. *FEBS Lett.,* **1987**, *221,* 167.

[32] Spratt, B. G. Resistant to antibiotics mediated by target alteration. *Science,* **1994**, *264,* 388-93.

[33] Chu, D. W.; Plattner, J. J.; Katz, L. New directions in antibacterial research. *J. Med. Chem.,* **1996**, *39,* 3853-74.

[34] New way to target and kill antibiotic-resistant bacteria found, www.physorg.com/news103220423. html, **2007**.

[35] Costerton, J. W. Introduction to biofilm. *Inter. J. Antimicrob. Agents,* **1999**, *11,* 217-21.

[36] Pasche, S.; De Paul, S. M.; Voeroes, J.; Spencer, N. D.; Textor, M. Poly (L-lysine)- graft-poly(ethylene glycol) assembled monolayers on niobium oxide surface: a quantitative study of the influence of polymer interfacial architecture on resistance to protein adsorption by Tof-SIMS and in situ OWLS. *Langmuir,* **2003**, *19,* 9216-25.

[37] Shen, M.; Martinson, L.; Wagner, M. S.; Castner, D. G.; Ratner, B. D.; Horbett, T. A. PEO-like plasma polymerized tetracylyme surface interactions with leukocytes & proteins: in vitro and in vivo studies. *J. Biom. Sci.,* Polymer Edition, **2002**, *13,* 367-90.

[38] Kolb, H. C.; Sharpless, K. B. The growing impact of click chemistry on drug discovery. *Drug Discov. Today,* **2003**, *8,* 1128-37.

[39] Bock, V. D.; Hiemstra, H.; Van Maarseveen, J. H. Cul-catalyzed alkyne-azide "click". cycloadditions from a mechanistic & synthetic perspective. *Eur. J. Org. Chem.,* **2005**, *1,* 51-68.

[40] Luxenhofer, R.; Jordan, R. Click chemistry with poly(2-oxazoline)s. *Macromolecules*, **2006**, *39, 10,* 3509-16.

**Received: 12 August, 2008 Revised: 30 October, 2008 Accepted: 09 September, 2008**