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# Introductory Chapter: The Action Mechanisms of Antibiotics and Antibiotic Resistance

*Sahra Kırmusaoğlu, Nesrin Gareayaghi and Bekir S. Kocazeybek*

## 1. Antibiotics

Pathogenic microorganisms can infect tissues of human by destroying cellular functions. Microorganisms themselves or their toxins can damage host cells. Microbial infections are treated with antimicrobials by either inhibiting the microbial growth or killing the microorganism. Antibiotics are widely being used not only in the treatment of acute and chronic infections, but also in the prophylactic treatment [1]. Targets of antimicrobials are cell membrane, cell wall, protein synthesis, nucleic acid synthesis, and biological metabolic compound synthesis (**Figure 1**) [2]. Over usage of antibiotics, mutations in the genes, carrying resistance genes in chromosomes and plasmids, gaining resistance genes carried by transposons, insertion sequences (IS) and conjugation from the same or other species of microorganisms cause bacteria develop resistance to antimicrobials [3].

## 2. The action mechanisms of antibiotics and antibiotic resistance

### 2.1 Injury to cell membrane

Plasma membrane of microorganism that has selective permeability contributes active transport to gain energy as ATP. Cytoplasmic content and gradient such as micro and macromolecules and ions are controlled by active transportation via integral transporter proteins. When selective membrane permeability is disrupted by antimicrobials, ions are lost and cellular ion gradient is distorted, so, the organism undergoes cellular damage and death [4].

Plasma membranes of bacteria are constructed by fatty acids that can be synthesized in cell or taken from environment as building blocks. Targets of antimicrobials are metabolic steps of fatty acid synthesis and membrane phospholipids. Polymyxin B, that is a bactericidal antibiotic, has been used as one of very few drugs in the treatment of Gram-negative bacteria such as *Pseudomonas* [2]. Polymyxin B that has detergent-like peptides having lipophilic and hydrophilic groups disrupts phosphatidylethanolamine of membrane. Valinomycin, that is an ionophore, disrupts cellular membrane potential that contributes oxidative phosphorylation by forming pores in cellular membrane. Daphtomycin that is widely used in bloodstream, wound, and soft skin infections caused by β-lactam especially vancomycin-resistant *Staphylococcus aureus* disrupts membrane potential by depolarization, that means potassium ions are released from cytoplasm to extracellular matrix [4].
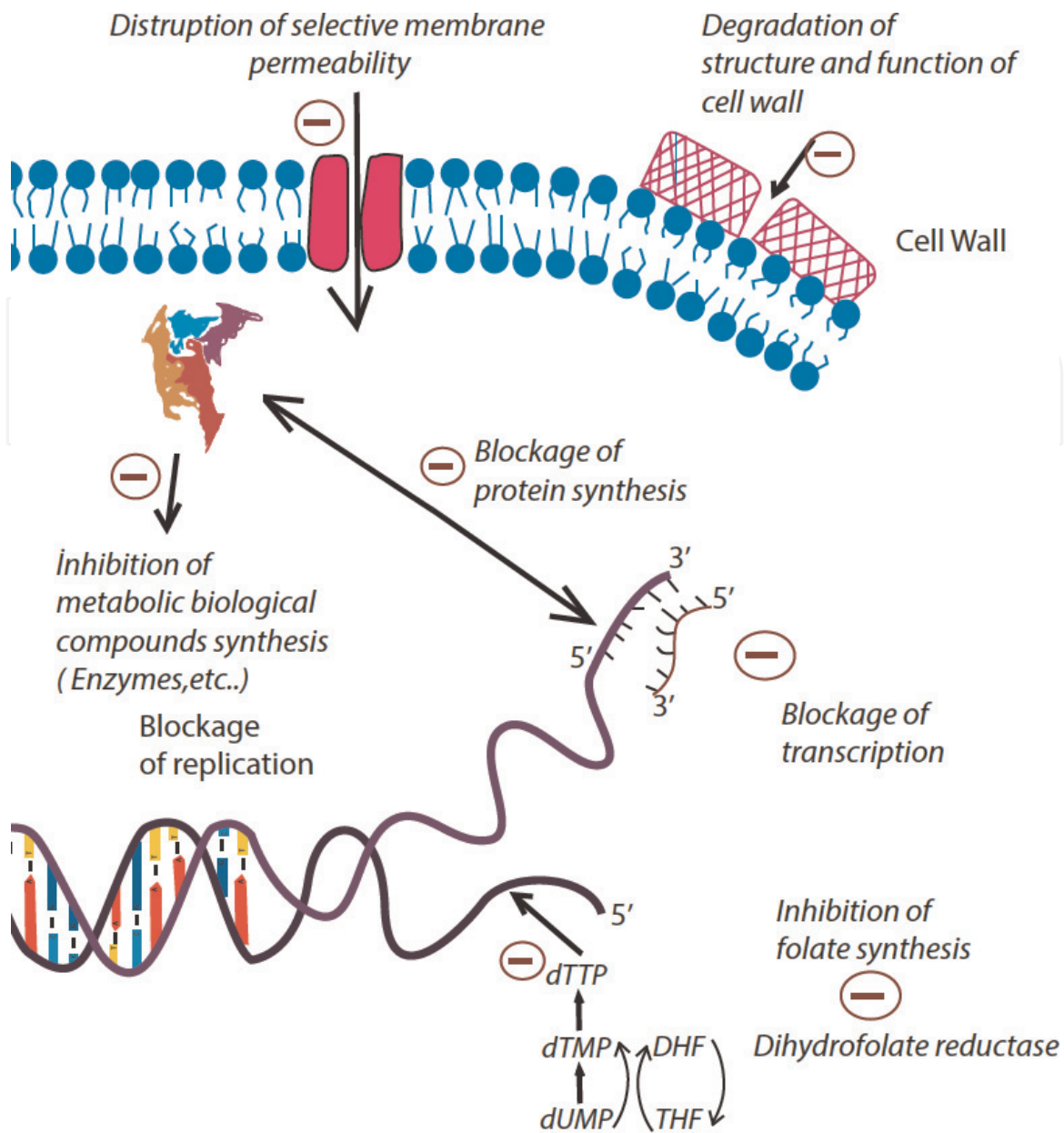
**Figure 1.**
*Targets of antimicrobials.*

Daptomycin, amphotericin B, colistin, imidazoles, and triazoles also act as inhibitors of cell membrane [2, 4].

## 2.2 The effect of antibiotics against cell wall

### 2.2.1 Cell wall synthesis

Cell walls of microorganisms are constructed by peptidoglycan. Glycan polysaccharide strands are linked by crosslink that bind polypeptides bound to N-acetyl muromic acid (NAM) of each polysaccharide strands.

After bactoprenol, which is a membrane-bound acceptor, transfers UDP-NAM-pentapeptide and UDP-NAG from cytoplasm to outer site of cell membrane, transglycosylation, and transpeptidation reactions are catalyzed by penicillin-binding proteins (PBPs) bound to cell membrane as a DD-peptidases to construct peptidoglycan [3].

*2.2.2 Degradation of structure and function of cell wall*

Certain antibiotics such as β-lactam antibiotics react with PBPs having high affinity to β-lactams by binding to PBPs as a substrate. These drugs are structural analogs of acyl-D-alanyl-D-alanine that binds to active site of PBP as a substrate of PBP during transpeptidation reaction. Transpeptidation reaction is blocked by these antibiotics inactivating transpeptidase domain of PBPs. Microorganisms are killed by these cell wall inhibitors that inhibit peptidoglycan biosynthesis [3].

*2.2.3 β-lactamases and the mechanisms of β-lactam resistance*

β-lactamase, that is an enzyme synthesized by many species of Gram-positive and Gram-negative bacteria, inactivates β-lactams degrading amide bond of β-lactam ring of β-lactam antibiotics [4] (bnm MRSA and MSSA). β-lactamases can be mediated by either plasmids or chromosomes, whereas penicillinases of *Staphylococcus aureus* are plasmid mediated, many Gram-negative bacteria are chromosomally mediated. β-lactamases that are plasmid mediated have tendency to be transferred between distinct species of bacteria. Chromosomally mediated β-lactamases can be either produced constructively as in the species of *Bacteroides* and *Acinetobacter* or induced as in the species of *Enterobacter*, *Citrobacter*, and *Pseudomonas*. Extended-spectrum β-lactamases (ESBLs) that are one class of β-lactamases having distinct ability to hydrolyze β-lactam rings of cefotaxime, ceftazidime, and aztreonam can be seen in a few species of Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* [4].

β-lactamase is synthesized by *bla*Z gene and regulated by *bla*I and *bla*RI located in plasmid or transposon. When β-lactam is not used, by binding of BlaI synthesized by *bla*I gene to promoter-operator region of *bla*I-*bla*RI operon, β-lactamase is not transcriped by *bla*Z gene. But in the treatment with β-lactam antibiotics, active site of BlaRI which is a signal transducer integral protein of β-lactam is blocked by β-lactam. After this blockage of active site, intracellular zinc metalloprotease domain of BlaRI releases BlaI bound to *bla*I-*bla*RI operator. By upregulation of *bla*Z gene, β-lactamase enzyme is synthesized, so the microorganism develops resistance to β-lactam used [3].

## 2.3 Inhibition of metabolic biological compounds synthesis

Biological metabolic reactions are catabolized by enzymes that are activated by substrates. Synthesis of metabolic biological compounds can be inhibited by drugs as a competitive inhibition manner. Drugs that are structural analogs of substrates act as substrates for the enzymes used in metabolic reactions.

Para-aminobenzoic acid (PABA) is a substrate for folic acid synthesis that is a coenzyme in the reactions of purines, pyrimidine, and amino acids synthesis [2].

Sulfanilamide and 3,4,5-trimethoxybenzylpyrimidine are the examples of drugs inhibiting synthesis of metabolic biological compounds. Sulfonamides (Sulfa drugs) have been used in many infections such as urinary tract infections. Sulfonamide is widely used in combination with other compounds. Silver sulfadiazine, one of the combined drugs, is used in burn infections [2].

Trimethoprim sulfamethoxazole (TMP-SMZ) is another combined drug used widely because of its synergistic activity. Trimethoprim and sulfamethoxazole block distinct steps of DNA and RNA precursor synthesis, and protein. Sulfamethoxazole that is sulfonamides showing structurally analogy with PABA blocks the reaction
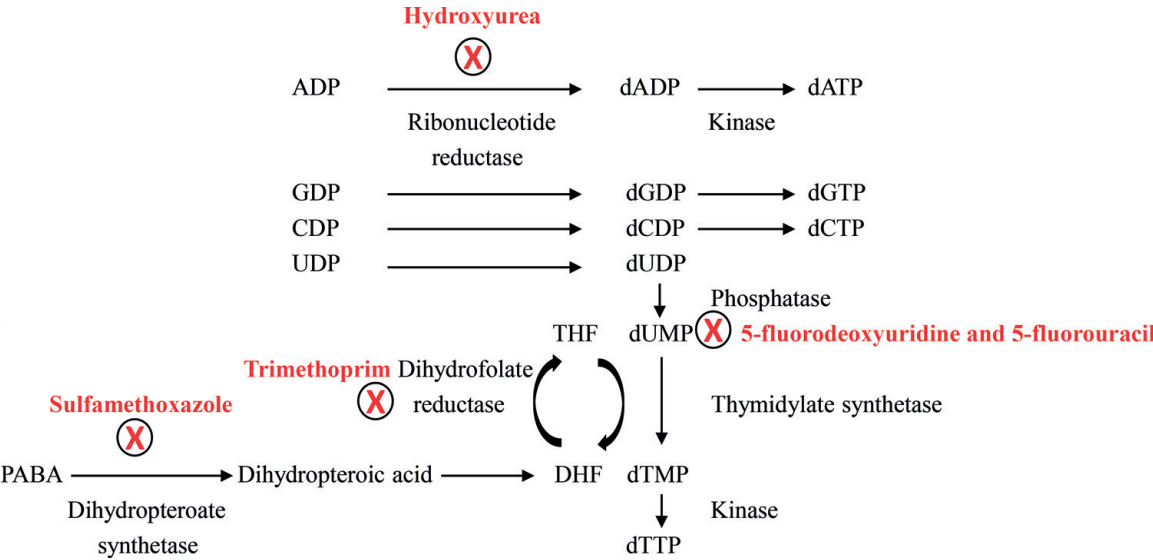
**Figure 2.**
*The synthesis of deoxynucleoside triphosphate precursors (dNTP: dATP, dGTP, dCTP, and dTTP), and the inhibition of tetrahydrofolate (THF) and dNTP synthesis by antibiotics.*

synthesizing dihydrofolic acid (DHF) from PABA, whereas trimethoprim that is sulfonamides showing structurally analogy with DHF blocks the reaction synthesizing tetrahydrofolic acid (THF) from DHF (**Figure 2**) [2].

## 2.4 Inhibition of nucleic acid synthesis

Antibiotics can inhibit replication, transcription, and folate synthesis of microorganisms.

### 2.4.1 Blockage of replication

#### 2.4.1.1 Replication of DNA

Deoxyribonucleotide precursors are synthesized for the polymerization of deoxynucleotides. By kinase enzyme, deoxynucleoside triphosphates (dNTP: dGTP, dCTP, and dATP) are synthesized from deoxynucleoside diphosphates (dNDP: dGDP, dCDP and dADP) that are synthesized from ribonucleosides by ribonucleotide reductase. But deoxythymidine triphosphate (dTTP) is synthesized by different pathway. Deoxyuracil diphosphate (dUDP) that is synthesized from uracil diphosphate (UDP) by ribonucleotide reductase is converted to deoxyuracil monophosphate (dUMP). Thymidylate synthetase catalyzes a reaction that converts dUMP methylated by tetrahydrofolate (THF) to dTMP. In this step, tetrahydrofolate (THF) is synthesized from dihydrofolate (DHF) by dihydrofolate reductase. Finally, dTTP is synthesized from dTMP by kinase (**Figure 2**).

DNA gyrase opens DNA strands for the polymerization of deoxynucleotides by DNA polymerase according to the each circular template strand of chromosome [5].

#### 2.4.1.2 Inhibition of replication and the mechanisms of resistance

Kinolons, such as nalidixic acid and ciprofloxacin that is used in the treatment of infections caused by *Pseudomonas* spp., prevent the formation of replication fork by inhibiting DNA gyrase, as a result of binding to *gyr*A subunit. Novobiocin and coumermycin prevent the formation of replication fork by inhibiting DNA gyrase, as a result of binding to *gyr*B subunit. When *gyr*A and *gyr*B genes of bacteria are

mutated, bacteria develop resistant against these antibiotics [5]. Norfloxacin, gatifloxacin, gemifloxacin, and moxifloxacin are the other groups of floroquinolones having broader spectrum of activity. Floroquinolones are nontoxic. Ciprofloxacin, norfloxacin, gatifloxacin, and gemifloxacin are used to treat urinary tract infection and pneumonia [2].

Synthesis of deoxynucleotide precursors that are used in the replication of DNA can be blocked by trimethoprim, hydroxyurea, 5-fluorodeoxyuridine, and 5-fluorouracil.

Trimethoprim is an inhibitor of folate synthesis. Trimethoprim, that is a structural analog of DHF, prevents the synthesis of THF by inhibiting dihydrofolate reductase. So, dTMP that is a precursor of deoxynucleotide polymer is not synthesized. There are many mechanisms of trimethoprim resistance. If *thyA* gene of microorganism undergoes to mutation that inactivates thymidylate sentetaz dTMP and DHF are not synthesized, as a result of inability in the transfer of methyl group from THF to dUMP. Due to dihydrofolate reductase is not synthesized in the microorganism of which *thyA* gene is mutated, this microorganism is not inhibited by trimethoprim. If the microorganism changes the binding site of the dihydrofolate reductase, the trimethoprim cannot bind to dihydrofolate reductase. So, bacteria develop resistance to trimethoprim. Another mechanism of trimethoprim resistance is carrying gene causing resistance to trimethoprim [5].

Hydroxyurea inhibits ribonucleotide reductase that catalyzes deoxynucleoside diphosphate from ribonucleoside diphosphate (**Figure 2**). If microorganism of gene coding ribonucleotide reductase is mutated, microorganism develops resistance to hydroxyurea [5].

Precursor synthesis can be blocked by 5-fluorodeoxyuridine and 5-fluorouracil with competitive inhibition. Monophosphate forms of 5-fluorodeoxyuridine and 5-fluorouracil are structural analogs of dUMP that is the substrate of thymidylate synthase. They inhibit the synthesis of dTMP. If the microorganism of gene coding thymidylate synthase is mutated, the microorganism develops resistance to 5-fluorodeoxyuridine and 5-fluorouracil [5].

Dideoxynucleotides that are used as drugs are similar with deoxynucleotide precursors, except that the hydroxyl group is absent in their 3′ carbon. Dideoxynucleotides that mimic deoxynucleotide precursors corpore into DNA and then, stop replication, due to it cannot be bound by the next deoxynucleotide.

Mitomycin C blocks replication by binding guanine bases that are located in both template strands of DNA [5].

### 2.4.2 Blockage of transcription

### 2.4.2.1 Transcription

Genetic information is transcribed from DNA to RNA by RNA polymerase that catalyzes a reaction, binds ribonucleotides with phosphodiester bond. RNA polymerase is constructed by 2α, 1β, 1β′, 1ω, and 1σ subunit. Transcription that is initiated by σ subunit that binds to promoter elongates until it is terminated by termination protein P (Rho) that is a RNA-DNA helicase releasing transcript from template DNA by breaking hydrogen bonds produced between template DNA and transcript [5].

### 2.4.2.2 Inhibition of transcription

Rifampin, that is a derivative of rifamycine family of antibiotics, blocks initiation of transcription by binding to β subunit of RNA polymerase. Rifampin is

used in the treatment of infections caused by *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and bacteria for which treatment is hard is not toxic to humans, due to it does not inhibit eukaryotic RNA polymerase. Mutated gene that codes RNA polymerase containing a distinct β subunit structure causes the microorganism to resist against ripampin. Streptolydigin also blocks initiation of transcription by binding with the β subunit of RNA polymerase. Bicyclomycin, the target of which is termination protein P, prevents termination of transcription. Bleomycin produces nicks on DNA. Bleomycin is not suitable in the usage for humans, because they are not specific to bacteria and has high toxic effect against humans and animals; whereas, it is used for the experiments of transcription. Azaserin blocks transcription by inhibition of ribonucleoside triphosphate synthesis [5].

## 2.5 Inhibition of protein synthesis

### 2.5.1 Translation

Protein is translated from mRNA by tRNA in ribosome. Translation is initiated by the binding of formylmethionine tRNA-aminoacyl-tRNA (fMet-tRNA$_f^{Met}$), translation initiation region (TIR) of mRNA and initiation factor 2 (IF-2) to P site of 30S subunit of ribosome and the formation of 70S complex as a result of the release of IF-2. Translation continues with the binding of a new aminoacyl tRNA to A site, transferring the polypeptide from tRNA bond to P site to tRNA bond to A site by peptidyl transferase, and translocating of polypeptidyl tRNA from A to P site by elongation factor-G (EF-G), until translation is terminated by termination protein P (Rho) [5].

### 2.5.2 Inhibitors mimicking tRNA

Puromycin that mimics aminoacyl tRNA enters into ribosome and is added to polypeptide grown, but it is not translocated from A site to P site of ribosome. Polypeptide containing puromycin at the carboxyl terminal is released from ribosome and translation is terminated. Puromycin is toxic to humans and animals, as it inhibits translation of eukaryotes [5].

### 2.5.3 Inhibitors binding to 23S rRNA

#### 2.5.3.1 Chloramphenicol

Chloramphenicol, that is a bacteriostatic agent and the inhibitor of 23S rRNA, inhibits transcription by preventing peptidyl transferase reaction, as a result of preventing the binding of aminoacyl tRNA to A site of ribosome. Due to its ability enter into blood-brain barrier, chloramphenicol is used in the treatment of many central nervous system infections such as bacterial meningitis. If the gene of ribosomal protein is mutated or bacteria has enzyme inactivating chloramphenicol, coded by *cat* gene of Tn9, bacteria resists to chloramphenicol. The product of *cat* gene that acetylates chloramphenicol inactivates chloramphenicol [5].

#### 2.5.3.2 Erythromycin

Erythromycin, that belongs to macrolide class of antibiotics, inhibits translation by binding to 23S rRNA. As a result of the blockage of E site, that is the

exit site for peptidyl-tRNA by erythromycin, premature polypeptide is released in translocation step. Macrolites, such as erythromycin, clarithromycin, azithromycin, and roxithromycin, are used in the treatment of Gram-positive and Gram-negative bacteria such as *Legionella*, *Mycoplasm*, and *Rickettsia*, due to usefulness of antibiotics. Mutational changes in 23S rRNA and efflux pumps, and conformational changes of 23S RNA that is caused by methylation of adenine localized in 23S rRNA by Erm methylase cause bacteria to resist against erythromycin [5].

### 2.5.3.3 Thiostrepton

Thiostrepton and other thiopeptide antibiotics block translation by binding to 23S RNA in the peptidyl transferase reaction and preventing the binding of EF-G that is a translocase translocating polypeptidyl tRNA from A to P site. Thiostrepton is used against Gram-positive bacteria. But the usage of thiostrepton is limited to veterinary and agriculture [5].

### 2.5.4 Inhibitors binding to A site of aminoacyl tRNA

### 2.5.4.1 Tetracyclin

Tetracyclin causes futile cycle to release aminoacyl tRNA from A site of ribosome by binding of release factors mimicking aminoacyl tRNA to A site. Tetracyclin is a broad spectrum antibiotic used to treat infections caused by Gram-positive and Gram-negative bacteria. *tet*M gene transferred from conjugative transposon Tn916 codes not only an enzyme that causes resistance by methylating certain bases of 16S rRNA, but also membrane proteins that pump tetracyclin to out of the cell, consequently resistance is developed. Ribosome protector proteins coded by *tet*O and *tet*Q gene bind to A site of ribosome by mimicking EF-G, consequently tetracyclin is released from A site [5].

### 2.5.5 Inhibitors of translocation

### 2.5.5.1 Aminoglycosides

Aminoglycosides that contain kanamycin, neomycin, gentamycin, streptomycin, amikacin, and tobramycin effect translocation by binding to A site. Aminoglycosides that have broad spectrum of activity cause false reading of mRNA and translation errors. Mutants that are resistant to aminoglycosides are seen rare. Aminoglycoside resistance is caused by the genes that inactivate aminoglycosides by phosphorylation, acetylation, and adenylation of them. *neo* gene that phosphorylates kanamycin and neomycin cause resistance of Gram-negative bacteria against kanamycin and neomycin [5].

### 2.5.5.2 Fusidic acid

Fusidic acid inhibits turnover of EF-G by preventing the release of EF-G from ribosome. Mutations that are occurred in *fus*A gene of *E. coli* cause resistance against fusidic acid. Certain acetyltransferases causing resistance against chloramphenicole can inhibit fusidic acid by binding to fusidic acid [5].

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