Antimicrobial Drugs

Chemotherapy is the use of chemicals for treatment of disease. Chemotherapeutic agents are chemicals used within the body for therapeutic purposes. Chemicals used against some kind of microorganism are commonly referred to as antimicrobials. An antibiotic is a naturally made chemical produced by one organism used to inhibit another organism. Antibiotics are used as a chemotherapeutic agent to control bacteria growth in animals.

There are three basic types of antimicrobials, classified according to their development: antibiotics (drugs produced by bacteria and fungi, like penicillin), semi-synthetics (naturally produced antibiotics which are modified in the laboratory to give the drug more action), and synthetics (drugs produced entirely in the laboratory, like sulfonamides).

Antimicrobials can be classified according to their activity: antibacterials, which work on bacteria; antivirals, which work on viruses; antifungals, which work on fungi; antiprotozoals, which work on parasitic protozoa; antimalarials, which work on malaria; and antihelminths, which work on parasitic worm infections.

Some antimicrobials do not actually kill the agent causing disease. The suffix "-statis" means to inhibit. Bacteriostatic means to inhibit the growth of bacteria; thereby, lowering their numbers within the host. The host's own defenses (i.e., non-specific host defenses and adaptive immune system) must help to destroy the pathogen. Antimicrobials that kill the agent are differentiated by the use of the suffix "-cidal" meaning to kill. Bactericidal for example would mean to kill bacteria directly.

A major host problem associated with the use of antibiotics is the loss of normal microorganisms from the urogenital and gastrointestinal tracts. That loss may occur because an antibiotic is not selective in its action or because the physician may have chosen a broad-spectrum drug to bring an infection under control. A broad-spectrum antibiotic is one that is able to control a wide range of microbes. An example of this would be tetracycline, which can be used in the treatment of infections caused by Gram-positive and Gram-negative bacteria, aerobic and anaerobic bacteria, spirochetes, rickettsias, mycoplasmas, chlamydia, and some protozoa. Narrow-spectrum antibiotics are those that are able to control a narrow range of microorganisms. An example would be polymyxin B, which is effective against certain Gram-negative bacteria. Extended-spectrum antibiotics are those that act on either Gram-positive or Gram-negative bacteria but have a limited effect on the other group. When nonspecific, broad-spectrum drugs are given orally, they may "accidentally" kill E. coli and other beneficial bacteria that normally live in the body. If they are lost, the normal microbiota becomes imbalanced and secondary infections such as antibiotic associated diarrhea caused by Clostridium difficile or yeast infections caused by the loss of Lactobacillus sp. in the vagina.
Six Characteristics of an Ideal Antimicrobial Drug:

When research is conducted on a new antibiotic, investigators set their standards as high as possible. They look for the optimum antibiotic that will be most effective for the longest period. The ideal antibiotic:

- **Must demonstrate selective toxicity.** This means it must be toxic to the pathogen (i.e., destroy it) without harming the body tissues of the host.
- **Should be microcidal.** This means it should be capable of killing the targeted microbe.
- **Should be stable.** This means it should be soluble in body fluids, but it should not be broken down too rapidly in the body or eliminated too quickly from body (i.e., it should have a long half-life). Drug resistance is an ever-increasing problem. Antimicrobials should be structurally stable enough that it is difficult for the microbe to develop resistance to the agent.
- **Must be complementary to host defenses.** This means the antimicrobial should stimulate or enhance the immune response of the body. It should not suppress immunity in any way. It should not stimulate hypersensitivity reactions in most patients.
- **Should have extensive tissue distribution.** This means that depending on how the antimicrobial is administered to the patient (oral, intramuscular, intravenous, etc.) that the drug should be able to become well distributed throughout the body tissues, including entry across the blood-brain barrier as necessary.
- **Should remain active in the presence of organic compounds.** This means the antimicrobial must not become inactive due to the presence of organic compounds such as proteins or serum. If the antimicrobial is going to become bound to proteins, the active site on the agent must remain available for binding to the microbe. (If the active site were hidden, the antimicrobial would not be able to bind with the microbe, and would be ineffective.)

These are ideal characteristic that researchers strive for, with the understanding that they may never find or produce all of them in a single antibiotic.
Mechanisms of Action of Antibacterial Agents

Fig. 11.1 Cycle of Events in the Life of a Bacterial Cell image by A. Swarthout

This diagram illustrates the basic events in the life of a bacterial cell. The DNA controls transcription, which controls translation. The proteins created during translation are often enzymes, which in turn control the metabolism of the cell. The cell makes energy during catabolism, and makes other cell structures such as cell membrane, cell wall, nucleic acids and proteins as a result of anabolism.

In order for the bacterial cell to live and reproduce, it must carry out all of the functions in the diagram above. But, if that bacterial cell is living inside of a human and making that human sick, we don't want the cell to be able to continue the cycle. So, we need to find points in the cycle where we can "screw up" the bacterial cell without harming the human host in the process. The diagram below indicates points in the cycle that we target with antibiotics.

Fig. 11.2 Potential Targets for Disruption of the Cell Cycle image by A. Swarthout

Table 11.1 Kendall Hunt Image [http://webcom.grtxle.com/customization/uploads/TABLE05-04.PDF]

1. **Interfere with nucleic acid synthesis.** Depending on the drug and the microbe, this can be interfering with RNA synthesis or DNA synthesis.
Fluoroquinolones and quinolones are drugs that bind to gyrase, an enzyme that prevents DNA from being damaged by over-winding during DNA replication. With the drugs bound to gyrase, the enzyme cannot bind to the DNA and protect from over-winding. The DNA strands then tangle and break and the cell cannot complete DNA replication.

Some drugs work by binding to RNA polymerase, which you should remember, is the enzyme that carries out transcription. Without transcription, there is no translation, and without translation no new proteins are made in the cell. That means that old, worn out proteins are not replaced and different metabolic pathways cannot be turned on when necessary. Rifampin is one drug that works in this way.

2. Interfere with protein synthesis. Most drugs that work by inhibiting protein synthesis work by binding to the ribosome. They may bind to either the 30S (small) subunit or the 50S (large) subunit. When tetracycline or aminoglycoside drugs bind to the ribosome, their presence prevents the proper binding of mRNA and tRNA to the ribosome, blocking translation.

Most drugs that interfere with protein synthesis maintain selective toxicity by taking advantage of the differences between eukaryotic and prokaryotic ribosomes. Our ribosomes are 80S ribosomes and consist of a 60S and 40S subunit, while the 30S and 50S subunit of the bacterial ribosomes make up 70S ribosomes.

Endosymbiotic Theory is a theory that human mitochondria are actually descended from bacterial cells! According to the theory, an ancient eukaryote endocytosed an ancient bacterium and they established a symbiotic relationship that evolved into our current cell structure.

In fact the ribosomes found inside of mitochondria are 70S, like those of bacteria, and drugs that affect bacterial ribosomes will have an adverse effect on mitochondrial ribosomes as well.

Table 11.2 Kendall Hunt Image Table 5.5 Inhibitors of Protein Synthesis (Microbiology Line Art, Growth & Control of Growth)

3. Interfere with metabolic pathways by interfering with enzymes involved in these metabolic pathways. Sulfonamides are drugs that are also competitive inhibitors. They bind to the first enzyme in the folic acid synthesis pathway, stopping the conversion of PABA to dihydrofolic acid, and thereby stopping the production of folic acid. Folic acid is necessary to make DNA and RNA.

Trimethoprim is another anti-metabolic agent, and it binds to the second enzyme in the folic acid synthesis pathway and prevents the conversion of dihydrofolate into tetrahydrofolate, again stopping the production of folic acid.
4. **Disrupt the integrity of the cell membrane.** There aren't many antibacterial drugs that work this way, in large part because the bacterial cell membrane is similar enough to ours that selective toxicity becomes an issue. Polymyxin antibiotics work this way, but are toxic to human kidneys, and are therefore used as topical agents.

5. **Interfere with cell wall synthesis.** As far as selective toxicity goes, this is a great way to target bacterial cells because we do not have peptidoglycan in our cells.

Penicillins and cephalosporins are both beta-lactam drugs. They both have a chemical structure, the **beta-lactam ring**, which is similar to the substrate that forms the pentaglycine crossbridges of peptidoglycan. They bind competitively to the enzyme that builds the crossbridges and prevent crossbridge formation. This makes the peptidoglycan weak and the cell unable to resist osmotic pressure, ultimately causing lysis of the cell.
Fig. 11.4 Different types of Penicillins

**Penicillins**

The side chains on penicillins influence the spectrum of activity for the antibiotics. Penicillins are divided into the following groups according to their modification and activity:

- Natural penicillins - such as penicillin G & penicillin V
- Penicillinase-resistant penicillins (PRPs) - such as methicillin, cloxacillin, nafcillin, oxacillin and dicloxacillin
- Broad-Spectrum penicillins - such as ampicillin, amoxicillin, ampicillin + sulbactam in combination, and amoxicillin + clavulanate potassium in combination
- Extended-Spectrum penicillins - such as mezlocillin, ticarcillin and piperacillin

**Cephalosporins**

First, second, and third generations of cephalosporins have been created by modifying the side groups of the beta lactam ring. These changes influence the spectrum of activity for the
antibiotics. Cephalosporins are divided into the following groups according to their modification and activity:

- First generation cephalosporins - such as cephalexin, cefadroxil, cefazolin, cephalothin, cepahaprin, and cephradine
- Second generation cephalosporins - such as cefaclor, cefamandole, cefmetazole, cefonicid, cefoteten, cefoxitin, cefprozil, cefuroxime, and loracarbef
- Third generation cephalosporins - such as cefdinir, cefepime, cefixime, Cefoperazone, cefotaxime, cefpodoxime, ceftazidime, cefibutin, ceftizoxime, ceftriazone, and moxalactam

Vancomycin also disrupts peptidoglycan synthesis, but it is not a beta-lactam drug as it does not have a beta lactam ring, but it inhibits cross-bridge formation by another mechanism.

Bacitracin prevents the transport of the NAG and NAM subunits from out of the cell, which halts peptidoglycan synthesis.

**Synergism**

*Sometimes one antibiotic is not enough to successfully treat a patient. With the rise in antibiotic resistant bacterial strains physicians may result to prescribe a combination of antibiotics. When multiple antibiotics are prescribed we are relying on the phenomenon called synergism. Synergism* is when two drugs are used in combination to increase the effectiveness of the treatment while decreasing the concentrations of the drugs needed. By using smaller concentrations of the drugs, there is less risk of harmful side effects to the patient. Also, the overall effectiveness of the treatment is increased!

An example of synergy is the combination of trimethoprim with sulfamethoxazole, a sulfa drug. Because both drugs act upon the same pathway, the pathway receives two "hits." This ensures the pathway is shut down, especially if the bacterium were to develop resistance to one of the drugs.

**A Special Case: Treatment of infections caused by Mycobacterium tuberculosis**

*Mycobacterium tuberculosis* is a Gram-positive bacillus that causes the disease tuberculosis. This organism is referred to as an acid fast bacterium and has several characteristics that make it intrinsically resistant to antibacterial drugs:

1. Mycolic acids in the cell wall make it waxy, enabling it repel drugs, disinfectants and antiseptics
2. It has a very long generation time
3. It mutates
Because of these factors, treatment of TB usually takes at least 6 months to treat and requires the patient to take a combination of two drugs at a time. TB drugs are divided into 1st line and 2nd line drugs. The 1st line drugs are the more effective, less toxic drugs. Examples include rifampin, isoniazid, ethambutol and pyrazinamide. If the microbe develops resistance to the first line drugs, the less effective, more toxic 2nd line drugs must be used. Many TB patients are required to undergo **directly observed therapy**, whereby they must be observed taking their treatment by a qualified professional. This is due in part to the length of time antibiotic therapy takes. Many people stop taking their antibiotic before the infection is cleared from the system, leaving the more resistant microbes alive in the body to continue to reproduce.

**Drug Resistance, Superinfections, and Drug Side Effects**

Antibiotics act as agents of natural selection. The bacterial cells that have the genetic ability to withstand the effects of the drug will survive and become the “grandparents” of a new population of drug-resistant bacteria. Since one essential life characteristic is genetic change, the prevention of drug resistance is almost impossible. Worldwide, the incidence of antibiotic drug resistance is increasing at an alarming rate. Unchecked, some pathogenic bacteria will ultimately become uncontrollable by antibiotics.

Bacteria can develop drug resistance through mutation or through gene transfer. Remember that mutations are random events that are statistically more likely to be harmful than helpful. However, because bacteria replicate and mutate so quickly, it is likely that a few beneficial mutations will pop in the population. These spontaneous mutations "just happen"; *the bacteria are not mutating on purpose in order to become resistant*. In most cases, the new, mutated bacteria would not fare any better or worse than the rest of their population. But when pressure in the form of antibiotics is applied to the population, and some cells have developed resistance, the non-mutated cells are killed off leaving more space and nutrients for the resistant bacteria to grow. As these new bacteria grow, they can accumulate more mutations until they become fully resistant to an antibiotic, resistant to multiple antibiotics or both.
Bacteria can also share antibiotic resistance genes via transduction, transformation and conjugation. Plasmids that code for antibiotic resistance are termed **R** (resistance) **plasmids**. Gene transfer does not have to occur between bacteria of the same type, it can occur between two different genera of bacteria.

If the normal microbiota is wiped out (due to such actions as use of antibiotics) the pH rises, which allows the yeast to become dominant. This overgrowth of yeast is known as a **superinfection**. A superinfection occurs when opportunistic microbiota are allowed to increase in numbers uncontrollably, leading to an infection. There are individuals who do experience harm when taking antibiotics to control an infection. This kind of harm is referred to as a side effect and may include anemia, kidney toxicity, allergic reactions, and tooth discoloration.

The antigenic properties of antibiotics are also important. These drugs should not be able to stimulate the immune system of the host. If that should occur, the patient could: (a) show an allergic response to the presence of the drug by producing antibodies against the antibiotic; or (b) produce antibodies that would destroy the drug.

**Mechanisms of Drug Resistance**

Resistance to antibiotics is due to the added capabilities of the resistant microbes. In order to resist the effects of antibiotics, bacteria can:

- produce enzymes that actively degrade antibiotics. Beta-lactamase degrades beta-lactam drugs. Penicillinase is beta-lactamase that specifically degrades penicillin. The production of beta-lactamase occurs in many penicillin and methicillin resistant strains of *Staphylococcus*.
- develop efflux pumps that specifically bind to the antibiotic once it has entered the cell and then pump it out
- alter the target of drug, such as changing the shape of the ribosome slightly, causing protein-synthesis inhibitors to stop working
- altering their metabolic pathways, for example, some bacteria will stop producing their own folic acid when exposed to sulfa drugs, they will absorb it from the environment instead
- more tightly regulate what comes into the cell via porins in the cell wall
- form biofilms to protect themselves from coming in contact with the antibiotic
Multiple drug resistance (MDR) occurs when a microbe is resistant to 2 or more drugs. Cross-resistance occurs when resistance to one drug results in resistance to other, similar drugs. For example, bacteria that are resistant to penicillin may also be resistant to cephalosporin because they both have the same mode of action.

![Graph showing the emergence of antibiotic resistance over time](image)

**Fig. 11.6 Drug Resistant Microbes**

**Slowing the Emergence of Antibiotic Resistance**

In the fight against bacteria and antibiotic resistance, it often seems as if we simply can't win. But there are things that we can and should do to help prevent the emergence of further antibiotic resistance:

- always finish all antibacterial drugs as prescribed
- don't take antibacterial drugs for viral infections (cold & flu)
- don't over-use antimicrobial products such as hand-soaps and other household cleaners unnecessarily
- limit the use of antibiotics in cattle, chicken, pigs and other animals sold for human consumption. This is a major source of antibiotic resistance.
- keep patients with infections caused by antibiotic resistant microbes from coming in contact with other patients with other resistant microbes and use proper PPE, Standard and Universal Precautions when dealing with these patients
• Educate ourselves and others about the dangers of the over-use of antibiotics and antimicrobial products!

Mechanisms of Action of Antiviral Drugs

Unfortunately, the treatment choice for viral infections is much more limited than for bacterial infections, because viruses have fewer targets than bacteria. A major complication is the fact that they don't carry out any metabolism on their own, and most drugs work by targeting and interfering with some type of metabolic process. A virus that is not active has virtually no targets, and one that is active is taking advantage of our cellular metabolism, meaning we have to essentially target ourselves, and we must be very careful in how we do this.

Table 11.3 Kendall Hunt Image: http://webcom.grtxle.com/customization/uploads/TABLE05-06.PDF

A few viral targets:

1. **Prevent viral uncoating**: think back to learning viral replication. The virus must "uncoat" or separate the nucleic acid from the capsid so the nucleic acid has access to our cell's organelles. If the virus stays in the coat, it can't access our cellular machinery to take over. A few drugs, rimantidine and amantadine (used to treat Influenza A) work this way.

2. **Interfere with nucleic acid synthesis**: nucleoside and nucleotide analogs are substances that "look like" the nucleic acid bases adenine, guanine, cytosine, thymine and uracil, but are fakes. They look enough like the real thing that the enzymes involved DNA replication and transcription use them in place of the real thing, but the DNA and RNA created using these analogs are non-functional. These are selectively toxic because viruses do not "proofread" their genetic material as well as eukaryotic cells, which would catch these fakes and remove them.

The HIV treatment AZT and the herpes treatments acyclovir and valcyclovir are examples of nucleoside drugs.

3. **Inhibit the action of Protease**: Viruses often make long, non-functional polypeptides during translation that are later cut or cleaved into the smaller, functional proteins by the enzyme protease. Interfering with the action of protease stops the production of functional viral proteins. Protease inhibitors are used to treat HIV.

Antifungal Drugs

Because fungi are eukaryotes, many of their cell structures and metabolic pathways are similar enough to ours to cause problems with selective toxicity. Most antifungal drugs work by interfering with the integrity of the fungal cell membrane. Fungal cell membranes contain
ergosterol, a sterol that is similar to cholesterol in structure and function. Allylamines and azoles inhibit ergosterol synthesis which disrupts cell membrane integrity. Because humans do not make ergosterol allylamines and azoles are harmless to humans. Polyenes and Amphitericin B work by binding to the ergosterol in the membrane in order to disrupt membrane integrity. Due to ergosterol’s similarity to cholesterol these drugs can bind to cholesterol (found in our cell membranes) and can be harmful to us in high concentrations.

Antiprototazoal and Antihelminthic Drugs

Antiprototazoal and Antihelminthic drugs are very limited categories of antimicrobial drugs. Treatment of protozoal and helminth infections is complicated by the fact that these organisms are also eukaryotes, and many of them have complex life cycles with different targets at different stages. A further issue is the fact that most of these types of infections occur in under-developed parts of the world where people do not have money to pay for the drugs or the development of drugs.

Testing the Efficacy of Antibacterial Drugs

One way to test whether an antibacterial drug will be effective against a certain of bacterium is to conduct a Kirby-Bauer Test. Disks impregnated with different antibiotics are placed on a Petri plate which has been inoculated with the suspected bacterium. After incubation, the size of clearing (the zone of inhibition) around the disk indicates the effectiveness of the antibacterial. If there is a large zone, this would indicate sensitivity. If there is no zone (i.e., the bacterium was able to grow right up to the edge of the disk), this would indicate resistance. Evaluation tables have been published which standardize this process of determining susceptibility or resistance. This method is used to determine the types and concentrations of antibiotics that could be selected for treatment of a disease.

A similar test that provides drug concentration information is the E test. Rather than disks, a strip containing the antibiotic is used, and the concentration along the strip gradually increases from zero. This test shows not only that the antibiotic works, but the minimum inhibitory concentration as well.

The minimum inhibitory concentration (MIC) of a drug is the lowest dose that inhibits microbial growth. It can be found by creating a series of serial dilutions of drug, then inoculating them with bacteria. The lowest concentration that inhibits growth is the MIC.

The minimum bactericidal concentration (MBC) is the lowest dose that actually kills the microbes and is found by taking bacteria from the MIC tube and using them inoculate new media, and finding the lowest concentration at which the bacteria are killed. This allows the
physician to more accurately prescribe the smallest concentration of an antibiotic known to kill or inhibit the infecting bacteria.

Fig. 11.8 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Tests image by A. Swarthout