Control or the destruction of microorganisms that reside within the bodies of humans & other animals is of tremendous importance. This unit introduces the principles of chemotherapy & discusses the ideal characteristics for successful chemotherapeutic agents (including the concept of selectively damaging the target microorganism while minimizing damage to the host). The course unit also presents characteristics of some commonly used antibacterial, antifungal, and antiviral drugs.

Chapter Objectives: After reading this course unit, you should be able to:

- discuss the various ways in which antimicrobial agents can damage pathogens while causing minimal damage to the host
- discuss the various factors that influence the effectiveness of a chemotherapeutic agent
- discuss the increasingly serious problem of drug-resistant pathogens
- discuss the increasing demand for and availability of antifungal and antiviral agents

Study Outline
A. Introduction
   1. Chemotherapeutic agents are chemical agents used to treat disease
   2. Antibiotics are microbial products or their derivatives that kill or inhibit susceptible microorganisms
   3. Synthetics are drugs that are not synthesized by microbes

B. The Development of Chemotherapy
   1. Paul Ehrlich (1904-1909)- aniline dyes and arsenic compounds
   2. Gerhard Domagk, and Jacques and Therese Trefouel (1939)- sulfanilamide
   3. Ernest Duchesne (1896) discovered penicillin; however, this discovery was not followed up and was lost for 50 years
   4. Alexander Fleming (1928) accidentally discovered the antimicrobial activity of penicillin on a contaminated plate; however, follow-up studies convinced him that penicillin would not remain active in the body long enough to be effective
   5. Howard Florey and Ernst Chain (1939) aided by the biochemist, Norman Heatley, worked from Fleming’s published observations, obtained a culture from him, and demonstrated the effectiveness of penicillin
   6. Selman Waksman (1944) -streptomycin; this success led to a worldwide search for additional antibiotics, and the field has progressed rapidly since then

C. General Characteristics of Antimicrobial Drugs
   1. Selective toxicity-ability to kill or inhibit microbial pathogen with minimal side effects in the host
i. Therapeutic dose—the drug level required for clinical treatment of a particular infection
ii. Toxic dose—the drug level at which the agent becomes too toxic for the host (produces undesirable side effects)
iii. Therapeutic index—the ratio of toxic dose to therapeutic dose: the larger the better
2. Drugs with narrow spectrum activity are effective against a limited variety of pathogens; drugs with broad-spectrum activity are effective against a wide variety of pathogens
3. Chemotherapeutic agents can occur naturally, be synthetic, or semisynthetic (chemical modifications of naturally occurring antibiotics)
4. Drug can be cidal (able to kill) or static (able to reversibly inhibit growth)
5. Minimal inhibitory concentration (MIC) is the lowest concentration of the drug that prevents growth of a pathogen; minimal lethal concentration (MLC) is the lowest drug concentration that kills the pathogen

D. Determining the Level of Antimicrobial Activity
1. Dilution susceptibility tests—a set of broth-containing tubes are prepared; each tube in the set has a specific antibiotic concentration; a standard number of test organisms is added to each
   i. The lowest concentration of the antibiotic resulting in no microbial growth is the MIC
   ii. Tubes showing no growth are sub-cultured into tubes of fresh medium that do not contain antibiotic to determine the lowest concentration of the drug from which the organism does not recover; this is the MLC
2. Disk diffusion tests—disks impregnated with specific drugs are placed on agar plates inoculated with the test organism; clear zones (no growth) will be observed if the organism is sensitive to the drug; the size of the clear zone is used to determine the relative sensitivity according to tables prepared for the various available drugs; zone width is a function of initial concentration, solubility, and diffusion rate of the antibiotic
3. Measurement of drug concentrations in the blood can be done using microbiological, chemical, immunological, enzymatic, and/or chromatographic assays

E. Mechanisms of Action of Antimicrobial Agents
1. Inhibition of cell wall synthesis
2. Inhibition of protein synthesis
3. Inhibition of nucleic acid synthesis
4. Disruption of cell membranes
5. Inhibition of metabolic activities (antimetabolites)

F. Factors Influencing the Effectiveness of Antimicrobial Drugs
1. Drug’s ability to reach the site of infection—this is greatly influenced by the mode of administration (e.g., oral, topical, parenteral), but can also be influenced by exclusion from the site of infections (e.g., blood clots or necrotic tissue protects bacterium)
2. Susceptibility of pathogen— influenced by growth rate and by inherent properties (e.g., whether or not pathogen has target of the drug)
3. Factors influencing drug concentration in the body—must exceed the pathogen's MIC for the drug to be effective; this will depend on
   i. Amount of drug administered
   ii. Route of administration
   iii. Speed of uptake
   iv. Rate of clearance (elimination) from the body
   v. Drug resistance has become an increasing problem.

G. Antibacterial Drugs
   1. Sulfonamides or sulfa drugs—structural analogues of metabolic intermediates; they inhibit folic acid synthesis in bacteria (humans don't synthesize folic acid, so are not affected); resistance is increasing and many patients are allergic to these drugs
   2. Quinolones—inhibit bacterial DNA gyrase, thereby disrupting replication, repair, and other processes involving DNA
   3. Penicillins—inhibit cell wall synthesis; many types have been identified or synthesized; they differ in spectrum of activity and administration route; resistance is an increasing problem; some patients are allergic to these antibiotics
   4. Cephalosporins—inhibit cell wall synthesis, broad spectrum of activity; they can be given to some patients with penicillin allergies
   5. Tetracyclines—inhibit protein synthesis; broad spectrum
   6. Aminoglycosides—inhibit protein synthesis; quite toxic to patients
   7. Erythromycin and other macrolides—inhibit protein synthesis; broad spectrum
   8. Vancomycin and teicoplanin—glycopeptide antibiotics that block peptidoglycan synthesis
   9. Chloramphenicol—inhibits protein synthesis; it has a broad spectrum but is toxic

H. Drug Resistance
   1. Mechanisms of drug resistance
      i. Prevent entrance of drug (e.g., alter drug transport into cell)
      ii. Pump the drug out of the cell once it has entered
      iii. Enzymatic inactivation of the drug—chemical modification of the drug by cellular enzymes can render it inactive before it has a chance to damage the cell
      iv. Alteration of target enzyme or organelle—modification of the target so that it is no longer susceptible to the action of the drug
      v. Use of alternative pathways and increased production of the target metabolite have been used by some organisms to minimize the effects of the drug
   2. The origin and transmission of drug resistance
      vi. Spontaneous mutations in chromosomal genes; these are then inherited by progeny of the resistant mutant
      vii. Transfer of R plasmids
      viii. Super-infection-growth of drug-resistant pathogens as the result of extensive drug treatment
ix. Several strategies can be used to discourage emergence of drug resistance (e.g., administration of high doses, simultaneous treatment with two different drugs, limited use of broad-spectrum antibiotics

x. Drug resistance has become an increasing problem; new drugs are constantly being developed and new treatment methods (e.g., phage treatment of bacterial infections) are being explored

I. Antifungal Drugs

1. Fungal infections are more difficult to treat than bacterial infections, because the greater similarity between fungi and host limits the ability of a drug to have a selective point of attack; furthermore, many fungi have detoxification systems that inactivate drugs

2. Superficial mycoses are infections of superficial tissues and can often be treated by topical application of antifungal drugs such as Miconazole, Nystatin, and griseofulvin, thereby minimizing systemic side effects

3. Systemic mycoses are more difficult to treat and can be fatal; however, amphotericin B and flucytosine have been used with limited success; amphotericin B is highly toxic and must be used with care; flucytosine must be converted by the fungus to an active form, and animal cells are incapable of this; some selectivity is possible, but severe side effects have been observed with both drugs

4. Drug resistant fungal strains are also beginning to emerge

J. Antiviral Drugs

1. Selectivity has been a problem because viruses use the metabolic machinery of the host

2. Antiviral drugs target specific steps of life cycle, especially enzymes that function in the life cycle (e.g., amantadine, vidarabine, acyclovir, and azidothymidine)

3. Human interferon is used to treat some viral infections
## Definition of Basic Terms in Antimicrobial Chemotherapy

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Chemotherapy</td>
<td>The use of drugs to treat a disease</td>
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<tr>
<td>Antibiotic</td>
<td>✓ A compound naturally produced by certain molds and bacteria that inhibits the growth or kills other microorganisms.</td>
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<td>✓ A Substance produced by a microbe that, in small amounts, inhibits another microbe</td>
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<tr>
<td>Antimicrobial drugs (antimicrobials)</td>
<td>Chemical substances that Interfere with the growth of microbes within a host. This term includes antibiotics and chemically synthesized drugs.</td>
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<tr>
<td>Antibacterial drug</td>
<td>An antimicrobial used to treat disease caused by bacteria</td>
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<tr>
<td>Antiviral drug</td>
<td>A drug that interferes with the replication of viruses. All antiviral drugs are chemically synthesized. None are antibiotics</td>
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<tr>
<td>Selective toxicity</td>
<td>A drug that kills harmful microbes without damaging the host</td>
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<tr>
<td>Therapeutic index</td>
<td>A measure expressing the relative toxicity of a chemotherapeutic drug. It is the lowest dose toxic to the patient divided to the dose used for therapy.</td>
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<tr>
<td>Antibiotic spectrum</td>
<td>Range of activity of an antimicrobial. A broad-spectrum antibacterial drug can inhibit a wide variety of gram-positive and gram-negative bacteria, whereas a narrow-spectrum drug is active only against a limited variety of bacteria.</td>
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<tr>
<td>Bacteriostatic activity</td>
<td>The level of antimicrobial activity that inhibits the growth of an organism. This is determined in vitro by testing a standardized concentration of organisms against a series of antimicrobial dilutions. The lowest concentration that inhibits the growth of the organism is referred to as the minimum inhibitory concentration (MIC).</td>
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<tr>
<td>Bactericidal activity</td>
<td>The level of antimicrobial activity that kills the test organism. This is determined in vitro by exposing a standardized concentration of organisms to a series of antimicrobial dilutions. The lowest concentration that kills 99.9% of the population is referred to as the minimum bactericidal concentration (MBC).</td>
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<tr>
<td>Antibiotic combination</td>
<td>Combinations of antibiotics that may be used:</td>
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<td>✓ To broaden the antibacterial spectrum for empiric therapy or the treatment of polymicrobial infections,</td>
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<tr>
<td></td>
<td>✓ to prevent the emergence of resistant organisms during therapy, and</td>
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<td></td>
<td>✓ To achieve a synergistic killing effect.</td>
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<tr>
<td>Antibiotic Synergism</td>
<td>Combinations of two antibiotics that have enhanced bactericidal activity when tested together compared with the activity of each antibiotic.</td>
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<tr>
<td>Antibiotic antagonism</td>
<td>Combination of antibiotics in which the activity of one antibiotic interferes With the activity of the other (e.g., the sum of the activity is less than the activity of the individual drugs).</td>
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<tr>
<td>Beta-lactamase</td>
<td>An enzyme that hydrolyzes the beta-lactam ring in the beta-lactam class of antibiotics, thus inactivating the antibiotic. The enzymes specific for penicillins and cephalosporins are the penicillinases and cephalosporinases, respectively</td>
</tr>
<tr>
<td>Allergic Reactions</td>
<td>Some people develop hypersensitivities to antimicrobials</td>
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<tr>
<td>Intrinsic Resistance</td>
<td>Resistance due to an inherent characteristic of a microorganism</td>
</tr>
<tr>
<td>Suppression of normal flora</td>
<td>When normal flora killed, other pathogens may be able to grow to high numbers</td>
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</table>
The Criteria (Principles) of the Ideal Antibiotic

1. Selectively toxic to microbe but nontoxic to host.
2. Soluble in body-tissue distribution
3. Remains in body long enough to be effective - resists excretion and breakdown.
4. Long Shelf life
5. Does not lead to resistance.
6. Cost not excessive (affordable prices).
8. Microbicidal rather than microbiostatic.
9. Concerns suppression of normal flora - antibiotic associated colitis with *Clostridium difficile* and its toxins or *Candida albicans*.

Mechanism of Action of Antimicrobial Agents

A. Inhibition of Cell Wall Synthesis: β-lactam Drugs
   1. Irreversibly inhibit enzymes involved in the final steps of cell wall synthesis. These enzymes mediate formation of peptide bridges between adjacent stands of peptidoglycan.
   2. Drug binds to enzyme, competitively inhibit enzymatic activity.
   3. Some bacteria produce β-lactamase- the enzyme that breaks the critical β-lactam ring.
   4. β-lactam drugs include: penicillins and cephalosporins.
   5. **Penicillins (Benzylpenicillin)**
      a. Effective against Gram positive bacteria (phenoxymethylpenicillin).
      b. Broad spectrum penicillins: amoxicillin and ampicillin are more hydrophilic and therefore, are active against gram-negative bacteria.
      c. Penicillinase-resistant penicillins – Flucloxacillin
         ✓ Indicated in infections caused by penicillinase-producing penicillin-resistant staphylococci.
         ✓ Less effective than Benzylpenicillin, So, should be used only for penicillin-resistant infections.
         ✓ Staphylococci aureas-resistant strains to Flucloxacillin and MRSA (methicillin-resistant Staph aureas) – this is an increasing problem!!
6. **Broad-Spectrum Penicillins**
   a. Ampicillin and amoxicillin – very active against non-β-lactamase-producing gram+ bacteria.
   b. Because they diffuse readily into Gram- bacteria, also very active against many strains of E. coli, H. influenzae, and Salmonella typhimurium.
   c. Orally, amoxicillin is better because absorption is better.
   d. Ineffective against penicillinase-producing bacteria (e.g., S. aureus, 50% of E. coli strains, and up to 15% of H. influenzae strains.
   e. Many bacterial β-lactamases are inhibited by clavulanic acid ± amoxicillin (co-amoxiclav) antibiotic is effective against penicillinase-producing organisms.
   f. Co-amoxiclav indicated in respiratory and UT infections, which are confirmed to be resistant to amoxicillin.

7. **Cephalosporins**
   a. Used for treatment of meningitis, pneumonia, and septicemia.
   b. Same mechanism and pharmacology as that of penicillins.
   c. May stimulate allergic reaction and cross-reactivity to penicillin.
   d. Similar to penicillins in broad-spectrum antibacterial activity.
   e. Cedadroxil (for UTI) in case of antibacterial resistance.
   f. Cefuroxime (prophylactic in surgery) – Resistant to inactivation by β-lactamases and used in severe infections (others ineffective).
   g. Ceftazidine – wide range of activity against gram- including Pseudomonas aeruginosa), but is less active than cefurozime against gram+ bacteria (S aureus).
   h. Used in meningitis (CNS-accessible) caused by gram negative bacteria.

8. **Vancomycin**
   a. Inhibits peptidoglycan formation.
   b. Active against most gram + (positive) organisms.
   c. Intra-Venous (I.V) treatment for septicemia or endocarditis caused by MRSA.
   d. Used for pseudomembranous colitis (superinfection of the bowel by Clostridium difficile – produces a toxin that damages the colon mucosa)

B. **Antibacterial Medications that Inhibit Protein Synthesis**
   1. Target ribosomes of bacteria
   2. Aminoglycosides: bind to 30S subunit causing it to distort and malfunction; blocks initiation of translation
      a. Against many gram negative and some gram positive bacteria.
      b. Narrow Therapeutic Index – very potentially toxic.
      c. Most important adverse side-effect: cranial nerve toxicity and kidney damage.
      d. Resistance – several mechanisms: inactivation of the drug by acetylation, phosphorylation or adenylation to prevent drug access, and the binding site of the 30S subunit (streptomycin only).
      e. Examples include
✓ Gentamicin – used for acute, life-threatening gram negative infections. Has synergism with penicillin and Vancomycin and combination.
✓ Amikacin – used for bacteria that are gentamicin resistant.
✓ Netilmicin – less toxic than gentamicin.
✓ Neomycin – too toxic for parenteral use. Used for topically for skin infections and orally for sterilizing bowel before surgery.
✓ Streptomycin – active against Mycobacterium tuberculosis. But because of its ototoxicity, rifampicin replaces.
✓ Rifampicin – resistance develops quickly alone; so, with TB, combine with isoniazid, ethambutol, and pyrazinamide or with rifampicin and isoniazid.

3. **Tetracyclines: Bind to 30S subunit blocking attachment of tRNA.**
   a. Broad-spectrum.
   b. Penetrate microorganisms well.
   c. Sensitive organisms accumulate it through partly passive diffusion and partly through active transport.
   d. Resistant organisms develop an efflux pump and do not accumulate the drug.
   e. Genes for tetracycline resistance are transmitted by plasmids.
   f. Closely associated with those for other drugs to which the organisms will also be resistant (e.g., sulphonamides, aminoglycosides, chloramphenicol).
   g. Tetracyclines bind to Calcium in growing bones and teeth (can discolor teeth). So, should be avoided in children less than 8 years old.

4. **Macrolides: Bind 50S subunit and prevents protein synthesis from continuing.**
   a. Very safe drugs, usually given orally.
   b. Erythromycin and clarithromycin
   c. Effective against gram negative bacteria and can be used as an alternative to penicillin-sensitive patients, especially in infections caused by streptococci, staphylococci, pneumococci, and clostridia.
   d. Ineffective against meningitis.
   e. Resistance occurs because of plasmid-controlled change of their receptor on the 50S subunit.
   f. Erythromycin – in high doses, may cause nausea and vomiting.
   g. Azithromycin – very long half-life (~40-60 hr.) and a single dose is as effective in treating chlamydial non-specific urethritis as tetracycline administration over 7 days.

5. **Chloramphenicol**
   a. Broad-spectrum.
   b. Serious side-effects: bone marrow aplasia, suppression of RBCs, WBCs, encephalopathy, optic neuritis.
   c. Inhibits the actions of other drugs and may increase the actions of phenytoin, sulphonlureas, and warfarin.
   d. Neonates cannot metabolize the drug rapidly; its accumulation causes the ‘grey baby’ syndrome (pallor, abdominal distension, vomiting, and collapse).