• Antimicrobial drugs act by inhibiting or interfering with the growth of microbes.
• Antimicrobial drugs act within the host without damaging the host.
• Antibiotics are one of the most important discoveries of modern medicine.
• Resistance to antibiotics poses a "major global threat" to public health according to WHO.

Pseudomonas aeruginosa resistant to many antibiotics

The History of Chemotherapy

Learning Objectives

Identify the contributions of Paul Ehrlich and Alexander Fleming to chemotherapy.

Name the microbes that produce most antibiotics.

Selective toxicity: selectively finding and destroying pathogens without damaging the host

Chemotherapy: the use of chemicals to treat a disease

Antibiotic: a substance produced by a microbe that, in small amounts, inhibits another microbe

Antimicrobial drugs: synthetic substances that interfere with the growth of microbes
The History of Chemotherapy

• Birth of modern chemotherapy is credited to the efforts of Paul Ehrlich, Germany
• He speculated about a “magic bullet” that would selectively target and destroy pathogens but not harm the host.
• Father of chemotherapy
• Made the first magic bullet: Salvarsan to treat syphilis
• Nobel prize in 1908

The History of Chemotherapy

• 1928: Fleming discovered penicillin, produced by Penicillium
• 1932: Prontosil red dye used for streptococcal infections
• 1940: First clinical trials of penicillin
• Today there is a growing problem of antibiotic resistance

“One sometimes finds what one is not looking for.”
Alexander Fleming

Microbes from natural environments (soil) will show bacterial inhibition by antibiotics produced by bacteria

Most commonly Streptomyces species

<table>
<thead>
<tr>
<th>TABLE 20.1 Representative Sources of Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microorganism</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>GRAM-POSITIVE RODS</strong></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Flavobaccillus polymyx</td>
</tr>
<tr>
<td><strong>ACTINOMYCETES</strong></td>
</tr>
<tr>
<td>Streptomyces nodosus</td>
</tr>
<tr>
<td>Streptomyces venezuelae</td>
</tr>
<tr>
<td>Streptomyces aureofaciens</td>
</tr>
<tr>
<td>Saccharopolyspora erythraea</td>
</tr>
<tr>
<td>Streptomyces fraiae</td>
</tr>
<tr>
<td>Streptomyces griseus</td>
</tr>
<tr>
<td>Micromonospora purpurea</td>
</tr>
<tr>
<td><strong>FUNGI</strong></td>
</tr>
<tr>
<td>Cephalosporium spp.</td>
</tr>
<tr>
<td>Penicillium griseofaci</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
</tr>
</tbody>
</table>
Spectrum of Antimicrobial Activity

Learning Objectives

Describe the problems of chemotherapy for viral, fungal, protozoan, and helminthic infections.

Define the following terms: spectrum of activity, broad-spectrum antibiotic, superinfection.

The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs

- **Narrow spectrum of microbial activity**: drugs that affect a narrow range of microbial types
- **Broad-spectrum antibiotics**: affect a broad range of gram-positive or gram-negative bacteria

<table>
<thead>
<tr>
<th>Prokaryotes</th>
<th>Eukaryotes</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacteria</td>
<td>Gram-negative bacteria</td>
<td>Good resistance</td>
</tr>
<tr>
<td></td>
<td>Gram-positive bacteria</td>
<td>Poor resistance</td>
</tr>
<tr>
<td></td>
<td>Chlamydia, Rickettsia</td>
<td>Poor resistance</td>
</tr>
<tr>
<td></td>
<td>Protozoa</td>
<td>Good resistance</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Poor resistance</td>
</tr>
<tr>
<td></td>
<td>Helminths</td>
<td>Poor resistance</td>
</tr>
</tbody>
</table>

Antibiotics target the normal flora as well as the pathogen.

Survivors may flourish and become opportunistc pathogens due to overgrowth.

**Superinfection**: overgrowth of normal microbiota that is resistant to antibiotics.
The Action of Antimicrobial Drugs

Learning Objective
Identify five modes of action of antimicrobial drugs.

Five Major Targets of Antibacterial Drugs
Antimicrobial drugs target essential functions of the microbe.

1. Inhibition of cell wall synthesis: penicillins, cephalosporins, bacitracin, vancomycin
2. Inhibition of protein synthesis: chloramphenicol, erythromycin, tetracyclines, aminoglycosides
3. Inhibition of nucleic acid replication and transcription: quinolones, rifampin
4. Injury to plasma membrane: polymyxin B
5. Inhibition of synthesis of essential metabolites: sulfanilamide, trimethoprim

The Action of Antimicrobial Drugs
• Bactericidal
  – Kill microbes directly
• Bacteriostatic
  – Prevent microbes from growing
  – Host's immune system usually kills the pathogen

• Inhibiting cell wall synthesis
  – Peptidoglycan is found only in bacterial cell walls.
  – Penicillins prevent the synthesis of peptidoglycan
  – Cell wall is weakened and undergoes lysis.

Gram-Positive Bacterial Cell Wall
Gram-Negative Bacterial Cell Wall
The Action of Antimicrobial Drugs

- Inhibiting protein synthesis
  - Target bacterial 70S ribosomes
  - Eukaryotes have 80S ribosomes
  - Difference in ribosome structure accounts for selective toxicity
  - However, mitochondria have 70S ribosomes so some toxicity may occur
  - Chloramphenicol, erythromycin, streptomycin, tetracyclines

Inhibition of bacterial cell wall synthesis by penicillin

Protein synthesis

Inhibition of protein synthesis by antibiotics.
The Action of Antimicrobial Drugs

• Injuring the plasma membrane
  – Polypeptide antibiotics change membrane permeability
  – Antifungal drugs combine with membrane sterols

• Injury to the plasma membrane of a yeast cell caused by an antifungal drug.

• Cell releases its cytoplasmic contents as the plasma membrane is disrupted by antifungal drugs.

The Action of Antimicrobial Drugs

• Inhibiting nucleic acid synthesis
  – Interfere with DNA replication and transcription
  – Some interfere with mammalian DNA and RNA as well.

• Inhibiting the synthesis of essential metabolites
  – Antimetabolites compete with normal substrates for an enzyme
    • Sulfanilamide competes with \textit{para}-aminobenzoic acid (PABA)
    • Stops synthesis of folic acid, a vitamin that functions as a coenzyme in the synthesis of nucleic acids

Competitive Inhibition of an Enzyme

PABA - substrate for an enzymatic reaction in the synthesis of folic acid, a vitamin that functions as a coenzyme in the synthesis of nucleic acids

\[
\text{Sulfanilamide} \quad \text{PABA}
\]

Antifungal, Antiviral, Antiprotozoan Drugs

Learning Objectives

Explain modes of action of current antifungal drugs.
Explain modes of action of current antiviral drugs.
Explain modes of action of current antiprotozoan and antihelminthic drugs.
Mode of Action for Antifungal Drugs

- **Disrupting membrane permeability**
  - Interrupt the synthesis of a fungal sterol, ergosterol, making the membrane excessively permeable
  - Ergosterol - principle sterol in the fungal cell wall
- **Inhibiting cell wall synthesis**
  - Inhibit the synthesis of $\beta$-glucan
- **Inhibiting nucleic acids synthesis**
  - Cytosine analog interferes with RNA synthesis

Fungal Cell Wall

Mode of Action for Antiviral Drugs

- **Entry and fusion inhibitors**
  - Block the receptors on the host cell that bind to the virus
  - Block fusion of the virus and cell
- **Uncoating, genome integration, and nucleic acid synthesis inhibitors**
  - Prevent viral uncoating
  - Inhibit viral DNA integration into the host genome
  - Nucleoside analogs inhibit RNA or DNA synthesis
- **Interference with assembly and release of viral particles**
  - Protease inhibitors block cleavage of protein precursors
- **Viral exit inhibitors**
  - Inhibit neuraminidase, an enzyme required for some viruses to bud from the host cell
Replication of a Virus

Guanine nucleotide

Host thymidine kinase

DNA polymerase

Phosphate

Nucleoside

DNA polymerase blocked by false nucleotide. Assembly of DNA stops.

False nucleotide (acyclovir triphosphate)

Viral-encoded thymidine kinase

Acyclovir (resembles nucleoside)

• Acyclovir has no effect on a cell not infected by a virus.
• In a virally infected cell, the thymidine kinase is altered and converts the acyclovir to a false nucleotide, which blocks DNA synthesis by DNA polymerase.

Mode of Action for Antiprotozoan and Antihelminthic Drugs

Many antiprotozoan and antihelminthic drugs are available now but are considered still experimental.

• One malarial drug targets the asexual stages of *Plasmodium*, which causes malaria.
• Another protozoan drug interferes with anaerobic respiration, which is similar to that in bacteria, and works on obligate anaerobic bacteria as well.
Tests to Guide Chemotherapy

Learning Objective
Describe two tests for microbial susceptibility to chemotherapeutic agents.

Susceptibility to Antimicrobials

- Different in different species and strains
- Changes with time, even during therapy
- Several tests are used to indicate an effective therapeutic agent
  - Broth dilution tests
  - Disk-Diffusion Method
  - E-test

The Diffusion Methods

- **Disk-diffusion method** (Kirby-Bauer test)
  - Tests the effectiveness of chemotherapeutic agents
  - Paper disks with a chemotherapeutic agent are placed on agar containing the test organism
  - The chemotherapeutic agent diffuses into the agar
  - The further the agent diffuses the lower the concentration
  - If the agent is effective, a zone of no growth around the disk occurs
  - **Zone of inhibition** around the disk determines the sensitivity of the organism to the antibiotic
  - The zone diameter is measured and the larger the zone of inhibition the more sensitive the microbe is to the agent
Disk-diffusion method for determining the activity of antimicrobials.

- Disks containing antimicrobials are placed on a lawn of bacterial growth
- Plates are incubated for a standardized time
- Zone of inhibition is compared to a standard table that reports the sensitivity as
  - Sensitive
  - Intermediate
  - Resistant

The Diffusion Methods

- E test
  - Determines the minimal inhibitory concentration (MIC)
    - Lowest antibiotic concentration that prevents visible bacterial growth
    - A plastic coated strip contains a gradient of antibiotic concentrations
    - The MIC is read from a scale printed on the strip

E test (for epsilometer), a gradient diffusion method that determines antibiotic sensitivity and estimates minimal inhibitory concentration (MIC)

Broth Dilution Tests

- Determine the MIC and minimal bactericidal concentration (MBC) of an antimicrobial drug
- Test organism is placed into the wells of a tray containing decreasing concentrations of a drug
- Growth is determined
- The wells that do not show growth may be cultured in drug-free medium to determine if the drug is bactericidal or bacteriostatic.

Antibiograms
  - Reports that record the susceptibility of organisms encountered clinically
Resistance to Antimicrobial Drugs

Learning Objective
Describe the mechanisms of drug resistance.

- **Persister cells**: subpopulation of microbes with genetic characteristics that allow their survival when exposed to an antibiotic without undergoing genetic change; type of dormant cell
- **Superbugs**: bacteria that are resistant to large numbers of antibiotics
- Resistance genes are often spread horizontally among bacteria on plasmids or transposons via conjugation or transduction

Antibiotic resistance tests
**Superbugs** - resistant to most antibiotic chemical classes - Left most image
Mechanisms of Resistance

1. Destruction or inactivation of the drug by enzymes
   • Beta-lactamases breakdown penicillin-like drugs
2. Prevention of penetration to the target site within the microbe
   • Gram-negative porins are altered
3. Alteration of the drug’s target site
   • mutations affect drug interaction but not function of the target
4. Rapid efflux (ejection) of the antibiotic
5. Variations of mechanisms of resistance

Example mechanisms of antibiotic resistance

Rapid development of an antibiotic-resistant mutant during antibiotic therapy

"The microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out. . . . In such cases, the thoughtless person playing with penicillin is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted."

— Alexander Fleming
Antibiotic Misuse

• Misuse of antibiotics has selected for resistance mutants leading to antibiotic resistance in bacterial strains

• Misuse includes:
  – Using outdated or weakened antibiotics
  – Using antibiotics for the common cold and other inappropriate conditions
  – Using antibiotics in animal feed
  – Failing to complete the prescribed regimen
  – Using someone else’s leftover prescription

• Antibiotics have been sold without prescriptions for many decades in much of the world.
• Rural Bangladesh - 8% of antibiotics are prescribed by a physician

CDC estimates for unnecessary antibiotic prescriptions in the United States

<table>
<thead>
<tr>
<th>Section of Use</th>
<th>Percentage unnecessary</th>
<th>Prescriptions per year (in millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Infection</td>
<td>30%</td>
<td>23m</td>
</tr>
<tr>
<td>Common Cold</td>
<td>100%</td>
<td>18m</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>80%</td>
<td>16m</td>
</tr>
<tr>
<td>Sore Throat</td>
<td>50%</td>
<td>13m</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>50%</td>
<td>13m</td>
</tr>
</tbody>
</table>

The frequency with which doctors prescribe antibiotics varies greatly from state to state. The reasons for this variation are being studied and might suggest areas where improvements in antibiotic prescribing (fewer unnecessary prescriptions) would be most helpful.
At least 70% of antibiotics produced in the US are NOT used to treat disease but are used in animal feeds to promote growth.

- In 2006, European Union banned the use of antibiotics as a growth enhancer
- In 2012, FDA banned use of one class of antibiotics in feed
- In 2013, FDA promoted a voluntary plan for industry to phase out use of some antibiotics
- October 10, 2015, the first US state to ban use of antibiotics as growth enhancers

California governor signs bill to require a veterinarian’s prescription for therapeutic antibiotic uses in livestock and ban other uses.

- *Campylobacter jejuni* causes over 2.5 million foodborne illness annually in the US.
- Fluoroquinolone (FQ)-resistance in *C. jejuni* emerged in 1990s.
- Emergence corresponded with presence of FQ-resistant *C. jejuni* in grocery store-purchased chicken.
- However, patients infected with the FQ-resistant *C. jejuni* had not taken the antibiotic prior to their illness and had not traveled out of the United States.
- In 2005, the use of FQ in chicken feed was banned in hopes of reducing FQ resistance.

Clinical Focus: Antibiotics in Animal Feed Linked to Human Disease


Antibiotic Cost and Prevention

- Developing new drugs is a costly process
- May result in drugs that are too costly for many to afford
- Strategies to prevent antibiotic resistance:
  - Finish the full antibiotic prescription
  - Never use leftover antibiotics to treat new illnesses
  - Avoid unnecessary prescriptions
  - Ensure the choice and dose are appropriate
  - Avoid broad-spectrum antibiotics if possible
Antibiotic Safety

- Therapeutic index: risk versus benefit
- Reactions of antibiotics with other drugs
- Damage to organs
- Risk to the fetus
- Allergies to antibiotics

Effects of Combinations of Drugs

Learning Objective

Compare and contrast synergism and antagonism.
**Effects of Combinations of Drugs**

- **Synergism**: the effect of two drugs together is greater than the effect of either alone
  
  Damage to cell walls by penicillin makes it easier for streptomycin to enter the cell

- **Antagonism**: the effect of two drugs together is less than the effect of either alone

  By stopping the growth of the bacteria with the bacteriostatic tetracycline, it prevents the action of penicillin, which require bacterial growth

**Future of Chemotherapeutic Agents**

**Learning Objective**

Name three areas of research on new chemotherapeutic agents.

**Future of Chemotherapeutic Agents**

- Target virulence factors
- Sequester iron, which feeds pathogens
- Antimicrobial peptides produced by various organisms
- Phage therapy
- Bacteriocins: antimicrobial peptides produced by bacteria
- Natural products
New methods for discovery