Controlling Microbial Growth in the Body: Antimicrobial Drugs
The History of Antimicrobial Agents

• Drugs
  • Chemicals that affect physiology in any manner

• Chemotherapeutic agents
  • Drugs that act against diseases

• Antimicrobial agents (antimicrobials)
  • Drugs that treat infections
The History of Antimicrobial Agents

- Paul Ehrlich
  - “Magic bullets”
    - Arsenic compounds that killed microbes
- Alexander Fleming
  - Penicillin released from *Penicillium*
- Gerhard Domagk
  - Discovered *sulfanilamide*
- Selman Waksman
  - Antibiotics
    - Antimicrobial agents produced naturally by organisms
Figure 10.1 Antibiotic effect of the mold *Penicillium chrysogenum*. 

*Penicillium chrysogenum* (fungus) 

*Staphylococcus aureus* (bacterium) 

Zone where bacterial growth is inhibited
The History of Antimicrobial Agents

• **Semisynthetics**
  • Chemically altered antibiotics that are more effective, longer lasting, or easier to administer than naturally occurring ones

• **Synthetics**
  • Antimicrobials that are completely synthesized in a lab
Table 10.1  Sources of Some Common Antibiotics and Semisynthetics

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Antimicrobial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>Penicillin G</td>
</tr>
<tr>
<td><em>Penicillium griseofulvum</em></td>
<td>Griseofulvin</td>
</tr>
<tr>
<td><em>Acremonium</em> a spp. b</td>
<td>Cephalothin</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Amycolatopsis orientalis</em></td>
<td>Vancomycin</td>
</tr>
<tr>
<td><em>Amycolatopsis rifamycinica</em></td>
<td>Rifampin</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>Bacitracin</td>
</tr>
<tr>
<td><em>Bacillus polymyxa</em></td>
<td>Polymyxin</td>
</tr>
<tr>
<td><em>Micromonospora purpurea</em></td>
<td>Gentamicin</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>Mupirocin</td>
</tr>
<tr>
<td><em>Saccharopolyspora erythraea</em></td>
<td>Erythromycin</td>
</tr>
<tr>
<td><em>Streptomyces griseus</em></td>
<td>Streptomycin</td>
</tr>
<tr>
<td><em>Streptomyces fradiae</em></td>
<td>Neomycin</td>
</tr>
<tr>
<td><em>Streptomyces aureofaciens</em></td>
<td>Tetracycline</td>
</tr>
<tr>
<td><em>Streptomyces venezuelae</em></td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td><em>Streptomyces nodosus</em></td>
<td>Amphotericin B</td>
</tr>
<tr>
<td><em>Streptomyces avermitilis</em></td>
<td>Ivermectin</td>
</tr>
</tbody>
</table>

a This genus was formerly called Cephalosporium.  
b spp. is the abbreviation for multiple species of a genus.
The History of Antimicrobial Agents

• **Tell Me Why**
  • Why aren’t antibiotics effective against the common cold?
Mechanisms of Antimicrobial Action

- Successful chemotherapy requires **selective toxicity**
- Antibacterial drugs constitute largest number and diversity of antimicrobial agents
- Fewer drugs to treat eukaryotic infections
- Antiviral drugs limited
Figure 10.2  Mechanisms of action of microbial drugs.

- **Inhibition of cell wall synthesis**
  - Penicillins
  - Carbapenems
  - Cephalosporins
  - Lipoglycopeptides
  - Bacitracin
  - Isoniazid
  - Ethambutol
  - Echinocandins (antifungal)

- **Inhibition of protein synthesis**
  - Aminoglycosides
  - Tetracyclines
  - Chloramphenicol
  - Macrolides
  - Antisense nucleic acids

- **Inhibition of DNA or RNA synthesis**
  - Actinomycin
  - Nucleotide analogs
  - Quinolones
  - Rifampin

- **Disruption of cytoplasmic membrane**
  - Polymyxins
  - Polyenes (antifungal)

- **Inhibition of general metabolic pathway**
  - Sulfonamides
  - Trimethoprim
  - Dapsone

- **Inhibition of pathogen's attachment or entry into host cell**
  - Arildone
  - Pleconaril
  - Enfuvirtide
Chemotherapeutic Agents: Modes of Action

- Inhibition of DNA & RNA Synthesis
- Inhibition of Protein Synthesis
- Inhibition of Metabolic Pathway
- Inhibition of Cell Wall Synthesis
- Disruption of Plasma Membrane
Mechanisms of Antimicrobial Action

• **Inhibition of Cell Wall Synthesis**
  
  • Inhibition of synthesis of bacterial walls
    
    • Most common agents prevent cross-linkage of NAM subunits
    
    • **Beta-lactams** are most prominent in this group
      
      • Functional groups are beta-lactam rings
      
      • Beta-lactams bind to enzymes that cross-link NAM subunits
    
    • Bacteria have weakened cell walls and eventually lyse
Figure 10.3a-b  Bacterial cell wall synthesis and the inhibitory effects of beta-lactams on it.

A bacterial cell wall is made of peptidoglycan, which is made of NAG-NAM chains that are cross-linked by peptide bridges between the NAM subunits.

New NAG and NAM subunits are inserted into the wall by enzymes, allowing the cell to grow. Other enzymes link new NAM subunits to old NAM subunits with peptide cross-links.
Figure 10.3c-e  Bacterial cell wall synthesis and the inhibitory effects of beta-lactams on it.

(c)  
- Penicillin G (natural) 
- Methicillin (semisynthetic) 

(d)  
- New cross-links inhibited by beta-lactam 
- Previously formed crossbridge 

Beta-lactam interferes with the linking enzymes, and NAM subunits remain unattached to their neighbors. However, the cell continues to grow as it adds more NAG and NAM subunits.

(e)  
- The cell bursts from osmotic pressure because the integrity of peptidoglycan is not maintained.
Mechanisms of Antimicrobial Action

• Inhibition of Cell Wall Synthesis
  • Inhibition of synthesis of bacterial walls
    • Semisynthetic derivatives of beta-lactams
      • More stable in acidic environments
      • More readily absorbed
      • Less susceptible to deactivation
      • More active against more types of bacteria
Mechanisms of Antimicrobial Action

• **Inhibition of Cell Wall Synthesis**
  • Inhibition of synthesis of bacterial walls
    • Vancomycin and cycloserine
      • Interfere with particular bridges that link NAM subunits in many Gram-positive bacteria
    • Bacitracin
      • Blocks transport of NAG and NAM from cytoplasm
  • Isoniazid and ethambutol
    • Disrupt *mycolic acid* formation in mycobacterial species
Mechanisms of Antimicrobial Action

• **Inhibition of Cell Wall Synthesis**
  • Inhibition of synthesis of bacterial walls
    • Prevent bacteria from increasing amount of peptidoglycan
    • Have no effect on existing peptidoglycan layer
    • Effective only for growing cells
Mechanisms of Antimicrobial Action

• **Inhibition of Cell Wall Synthesis**
  • Inhibition of synthesis of fungal walls
    • Fungal cells composed of various polysaccharides not found in mammalian cells
    • **Echinocandins** inhibit the enzyme that synthesizes glucan
Mechanisms of Antimicrobial Action

• **Inhibition of Protein Synthesis**
  • Interference with prokaryotic ribosomes
    • Prokaryotic ribosomes are 70S (30S and 50S)
    • Eukaryotic ribosomes are 80S (40S and 60S)
  • Drugs can selectively target translation
  • Mitochondria of animals and humans contain 70S ribosomes
    • Can be harmful
Figure 10.4 Some mechanisms by which antimicrobials target prokaryotic ribosomes to inhibit protein synthesis.

- **(a)** Incorrect amino acids can interfere with protein synthesis. For example, streptomycin can cause a change in the 30S ribosomal subunit, leading to misreading of the mRNA.

- **(b)** Tetracycline and some aminoglycosides can block the docking site of tRNA, preventing the correct alignment of amino acids.

- **(c)** Chloramphenicol can block peptide bond formation, stopping protein synthesis.

- **(d)** Lincosamides or macrolides bind to the 50S subunit, blocking proper mRNA movement through the ribosome, thus preventing synthesis.

- **(e)** Antisense nucleic acid can hybridize with the mRNA, preventing translation.

- **(f)** Oxazolidinone can bind to the 30S ribosomal subunit, inhibiting protein synthesis.

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Mechanisms of Antimicrobial Action

• Inhibition of Protein Synthesis
  • Interference with charging of transfer RNA molecules
    • *Aminoacyl-tRNA synthetases* load amino acids onto tRNA molecules
  • **Mupirocin** selectively binds to isoleucyl-tRNA synthetase
    • Prevents loading of isoleucine only in Gram-positive bacteria
Mechanisms of Antimicrobial Action

• **Disruption of Cytoplasmic Membranes**
  • Some drugs form channel through cytoplasmic membrane and damage its integrity
  • *Nystatin* and *amphotericin B* attach to *ergosterol* in fungal membranes
    • Humans somewhat susceptible because cholesterol similar to ergosterol
    • Bacteria lack sterols; not susceptible
Figure 10.5 Disruption of the cytoplasmic membrane by the antifungal amphotericin B.
Mechanisms of Antimicrobial Action

- **Disruption of Cytoplasmic Membranes**
  - **Azoles** and **allylamines** inhibit ergosterol synthesis
  - **Polymyxin** disrupts cytoplasmic membranes of Gram-negative bacteria
    - Toxic to human kidneys
  - Some parasitic drugs act against cytoplasmic membranes
Mechanisms of Antimicrobial Action

• Inhibition of Metabolic Pathways
  • *Antimetabolic agents* can be effective when pathogen and host metabolic processes differ
    • *Atovaquone* interferes with electron transport in protozoa and fungi
    • Heavy metals inactivate enzymes
    • Agents that disrupt tubulin polymerization and glucose uptake by many protozoa and parasitic worms
    • Drugs that block activation of viruses
    • Metabolic antagonists
Figure 10.6 The antimetabolic action of sulfonamides in inhibiting nucleic acid synthesis.

(a) Para-aminobenzoic acid (PABA) and some of its structural analogs, the sulfonamides

(b) Role of PABA in folic acid synthesis in bacteria and protozoa

(c) Inhibition of folic acid synthesis by sulfonamide
Mechanisms of Antimicrobial Action

• **Inhibition of Metabolic Pathways**
  • Trimethoprim also interferes with nucleotide synthesis.
  • Antiviral agents can target unique aspects of viral metabolism.
    • *Amantadine, rimantadine*, and weak organic bases prevent viral uncoating.
  • *Protease inhibitors* interfere with an enzyme that HIV needs in its replication cycle.
Mechanisms of Antimicrobial Action

• **Inhibition of Nucleic Acid Synthesis**
  • Several drugs block DNA replication or RNA transcription
  • Drugs often affect both eukaryotic and prokaryotic cells
  • Not normally used to treat infections
  • Used in research and perhaps to slow cancer cell replication
Mechanisms of Antimicrobial Action

- **Inhibition of Nucleic Acid Synthesis**
  - Quinolones and fluoroquinolones
    - Act against prokaryotic *DNA gyrase*
  - Nucleotide or nucleoside analogs
    - Interfere with function of nucleic acids
    - Distort shapes of nucleic acid molecules and prevent further replication, transcription, or translation
    - Most often used against viruses
    - Effective against rapidly dividing cancer cells
Figure 10.7 Nucleosides and some of their antimicrobial analogs.
Mechanisms of Antimicrobial Action

- **Inhibition of Nucleic Acid Synthesis**
  - Inhibitors of RNA polymerase
  - Reverse transcriptase inhibitors
    - Act against an enzyme HIV uses in its replication cycle
    - Do not harm people because humans lack reverse transcriptase
Mechanisms of Antimicrobial Action

• Prevention of Virus Attachment, Entry, or Uncoating
  • *Attachment antagonists* block viral attachment or receptor proteins
  • New area of antimicrobial drug development
  • *Pleconaril* blocks viral attachment
  • *Arildone* prevents viral uncoating
Tell Me Why

Some antimicrobial drugs are harmful to humans. Why can physicians safely prescribe such drugs despite the potential danger?
Clinical Considerations in Prescribing Antimicrobial Drugs

- **Ideal Antimicrobial Agent**
  - Readily available
  - Inexpensive
  - Chemically stable
  - Easily administered
  - Nontoxic and nonallergenic
  - Selectively toxic against wide range of pathogens
Clinical Considerations in Prescribing Antimicrobial Drugs

• **Spectrum of Action**
  • Number of different pathogens a drug acts against
    • **Narrow-spectrum** effective against few organisms
    • **Broad-spectrum** effective against many organisms
      • May allow for secondary or superinfections to develop
      • Killing of normal flora reduces microbial antagonism
Figure 10.8  Spectrum of action for selected antimicrobial agents.

<table>
<thead>
<tr>
<th>The Spectrum of Activity of Selected Antimicrobial Drugs</th>
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</thead>
<tbody>
<tr>
<td><strong>Prokaryotes</strong></td>
</tr>
<tr>
<td>Mycobacteria</td>
</tr>
<tr>
<td>Isoniazid</td>
</tr>
<tr>
<td>Streptomycin</td>
</tr>
<tr>
<td>Gram-negative bacterium</td>
</tr>
<tr>
<td>Polymyxin</td>
</tr>
<tr>
<td>Penicillin</td>
</tr>
<tr>
<td>Gram-positive bacterium</td>
</tr>
<tr>
<td>Eukaryotes</td>
</tr>
<tr>
<td>Chlamydia, rickettsia</td>
</tr>
<tr>
<td>Penicillin</td>
</tr>
<tr>
<td>Erythromycin</td>
</tr>
<tr>
<td>Tetracycline</td>
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<tr>
<td>Sulfonamides</td>
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<tr>
<td>Viruses</td>
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<tr>
<td>Protozoa</td>
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<tr>
<td>Fungi</td>
</tr>
<tr>
<td>Helminths</td>
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<tr>
<td>Niclosamide</td>
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<tr>
<td>Arildone</td>
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<tr>
<td>Ribavirin</td>
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<tr>
<td>Praziquantel</td>
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<tr>
<td>Acyclovir</td>
</tr>
</tbody>
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Clinical Considerations in Prescribing Antimicrobial Drugs

• **Effectiveness**
  • Ascertained by
    • Diffusion susceptibility test
    • Minimum inhibitory concentration test
    • Minimum bactericidal concentration test
Figure 10.9 Zones of inhibition in a diffusion susceptibility (Kirby-Bauer) test.
Figure 10.10  Minimum inhibitory concentration (MIC) test in wells.

Turbid wells  Clear wells

Increasing concentration of drug
Figure 10.11 An Etest, which combines aspects of Kirby-Bauer and MIC tests.
Figure 10.12  A minimum bactericidal concentration (MBC) test.

Concentration of antibacterial drug (µg/ml)

- Clear MIC tube
- 8 µg/ml
- 16 µg/ml
- 25 µg/ml

Drug-free media

Bacterial colonies
No colonies
No colonies
Clinical Considerations in Prescribing Antimicrobial Drugs

• **Routes of Administration**
  • *Topical* application of drug for external infections
  • *Oral* route requires no needles and is self-administered
  • *Intramuscular* administration delivers drug via needle into muscle
  • *Intravenous* administration delivers drug directly to bloodstream
  • Must know how antimicrobial agent will be distributed to infected tissues
Figure 10.13 The effect of route of administration on blood levels of a chemotherapeutic agent.
Clinical Considerations in Prescribing Antimicrobial Drugs

• Safety and Side Effects
  • Toxicity
    • Cause of many adverse reactions poorly understood
    • Drugs may be toxic to kidneys, liver, or nerves
    • Consideration needed when prescribing drugs to pregnant women
  • Therapeutic index is the ratio of the dose of a drug that can be tolerated to the drug's effective dose
Figure 10.14 Some side effects resulting from toxicity of antimicrobial agents.
Clinical Considerations in Prescribing Antimicrobial Drugs

• **Safety and Side Effects**
  
  • **Allergies**
    
    • Allergic reactions are rare but may be life threatening
    
    • *Anaphylactic shock*
  
  • **Disruption of normal microbiota**
    
    • May result in secondary infections
    
    • Overgrowth of normal flora causing superinfections
    
    • Of greatest concern for hospitalized patients
Clinical Considerations in Prescribing Antimicrobial Drugs

• **Tell Me Why**
  • Why don’t physicians invariably prescribe the antimicrobial with the largest zone of inhibition?
Resistance to Antimicrobial Drugs

• The Development of Resistance in Populations
  • Some pathogens are naturally resistant
  • Resistance by bacteria acquired in two ways:
    • New mutations of chromosomal genes
    • Acquisition of **R plasmids** via transformation, transduction, and conjugation
Figure 10.15  The development of a resistant strain of bacteria.
Antibiotic Resistance: Origins of Resistance

Transducing phage (bacterial DNA) into a resistant cell.
Resistance to Antimicrobial Drugs

- **Mechanisms of Resistance**
  - At least seven mechanisms of microbial resistance
    - Produce enzyme that destroys or deactivates drug
    - Slow or prevent entry of drug into the cell
    - Alter target of drug so it binds less effectively
    - Alter their own metabolic chemistry
    - Pump antimicrobial drug out of the cell before it can act
    - Bacteria in biofilms can resist antimicrobials
    - *Mycobacterium tuberculosis* produces MfpA protein
      - Binds DNA gyrase, preventing the binding of fluoroquinolone drugs
Figure 10.16 How beta-lactamase (penicillinase) renders penicillin inactive.
• In the Interactive Microbiology tutorial in Chapter 10, we learn about antibiotic resistance.
  • Rebecca enters the hospital to have an appendectomy but becomes sick with pneumonia.
  • Her pneumonia is caused by *Klebsiella pneumoniae*.
  • Antimicrobial drugs bind to and disrupt specific bacterial components.
  • Bacteria such as *K. pneumoniae* can resist antimicrobials through several mechanisms.
Resistance to Antimicrobial Drugs

- **Multiple Resistance and Cross Resistance**
  - Pathogen can acquire resistance to more than one drug
  - Common when R plasmids exchanged
  - Develop in hospitals and nursing homes
    - Constant use of drugs eliminates sensitive cells
  - **Multiple-drug-resistant pathogens** are resistant to at least three antimicrobial agents
  - **Cross resistance** can occur when drugs are similar in structure
Resistance to Antimicrobial Drugs

• **Retarding Resistance**
  • Maintain high concentration of drug in patient for sufficient time
    • Inhibit the pathogen so immune system can eliminate
  • Use antimicrobial agents in combination
    • **Synergism** occurs when one drug enhances the effect of a second drug
    • **Antagonism** occurs when drugs interfere with each other
Figure 10.17  An example of synergism between two antimicrobial agents.

Disk with semisynthetic amoxicillin-clavulanic acid

Disk with semisynthetic aztreonam
Resistance to Antimicrobial Drugs

• **Retarding Resistance**
  
  • Use antimicrobials only when necessary
  
  • Develop new variations of existing drugs
    • *Second-generation drugs*
    • *Third-generation drugs*
  
  • Search for new antibiotics, semisynthetics, and synthetics
    • *Bacteriocins*
    • Design drugs complementary to the shape of microbial proteins to inhibit them
Resistance to Antimicrobial Drugs

• **Tell Me Why**
  
  • Why is it incorrect to say that an individual bacterium develops resistance in response to an antibiotic?