Chapter 14: Antimicrobial Drugs

1. Fundamentals of Antimicrobial Drugs
2. Antibacterial Drugs
3. Drugs That Target Eukaryotic Pathogens
4. Antiviral Drugs
1. Fundamentals of Antimicrobial Drugs
History of Antimicrobial Drug Discovery

Paul Ehrlich (early 1900s):
• developed anti-syphilis drug “Salvarsan” through systematic chemical approach still in use today

Alexander Fleming (1928):
• discovered penicillin

Selman Waksman (1940s):
• discovered multiple antibiotics produced by Actinomycetes in the soil (e.g., *Streptomyces*)
An antibiotic is a substance naturally produced by a microorganism to inhibit or kill other microorganisms:

- e.g., the mold *Penicillium chrysogenum* produces penicillin which is very effective against Gram+ bacteria

An antimicrobial drug ("antimicrobial") may be synthetic, semi-synthetic, or a naturally produced antibiotic.
Selective Toxicity

For an antimicrobial drug to be effective, it must have selective toxicity, i.e., it must cause significantly greater harm to the pathogen than to the host being treated:

Chemotherapeutic index = \frac{\text{Toxic dose}}{\text{Therapeutic dose}}

The higher the chemotherapeutic index the safer the drug.
Spectrum of Action

The range of pathogens targeted by a specific antimicrobial drug is referred to as its spectrum, which can be “broad” or “narrow”:

Broad-spectrum drugs

• usually refers to drugs effective against more than one general category of pathogens

Narrow-spectrum drugs

• usually refers to drugs effective against only one general category of pathogens
### Examples of Spectrum of Action

<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>Gram-positive bacteria</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Polymyxin</td>
<td>Penicillin</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Erythromycin</td>
<td>Tetracycline</td>
</tr>
</tbody>
</table>

- e.g., isoniazid & polymyxin are “narrow spectrum”, tetracycline & erythromycin are “broad spectrum”

**Broad spectrum drugs are not necessarily preferable due to their effects on the normal microbiota.**
Antimicrobial Drug Resistance

The introduction of an antimicrobial is a selective factor that over time can select for resistant pathogens. The main ways to minimize this problem are:

1. Take antimicrobial drugs *only* when prescribed & necessary.
   - overuse is a huge problem

2. Administer the drug at the prescribed dose for the prescribed duration.
   - premature stoppage allows the more resistant pathogens to survive

3. Use of antimicrobial drugs in combination.
   - much lower odds of resistance to multiple drugs
How is Resistance Obtained?

By the acquisition of antimicrobial drug resistance genes via transformation, conjugation, transduction or mutation.

Resistance genes can be of several types:

- **enzymes** that degrade or modify the drug
- **efflux pumps** that rapidly expel the drug
- changes in membrane proteins that **limit entry** of antibiotic
- **target modification** which prevents binding of antibiotic
- **target mimicry** via molecules similar to target that will bind drug
- **target overproduction** to exceed the capacity of the drug
Types of Antimicrobial Drug Resistance

- **Efflux pump**
  - fluoroquinolones
  - aminoglycosides
  - tetracyclines
  - β-lactams
  - macrolides

- **Blocked penetration**
  - β-lactams
  - tetracyclines
  - fluoroquinolones

- **Inactivation of enzymes**
  - β-lactams
  - aminoglycosides
  - macrolides
  - rifamycins

- **Target modification**
  - fluoroquinolones
  - rifamycins
  - vancomycin
  - β-lactams
  - macrolides
  - aminoglycosides
Superinfection

Another problem that can result from antimicrobial drug use is **superinfection** – expansion of opportunistic pathogens due to the loss of normal microbiota species.

- e.g., colitis caused by *Clostridium difficile* or “C diff”

1. Normal microbiota keeps opportunistic pathogens in check.
2. Broad-spectrum antibiotics kill nonresistant cells.
3. Drug-resistant pathogens proliferate and can cause a superinfection.
Antimicrobial Testing

**Kirby-Bauer test**
- antimicrobial drug discs are placed on lawn of bacteria on special agar
- zone of inhibition size indicates susceptibility or resistance

**Etest**
- strip with antibiotic gradient reveals minimum inhibitory concentration (MIC)
Minimum Inhibitory Concentration

The MIC can also be determined by culturing cells in media containing various levels or dilutions of the drug.
Minimum Bacteriocidal Concentration

Concentration of antibacterial drug (µg/ml)

- Clear MIC tube
- 8 µg/ml: Bacterial colonies
- 16 µg/ml: No colonies
- 32 µg/ml: No colonies

MBC test

Drug-free media

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Antimicrobial Targets

A variety of unique microbial features can be targeted by antimicrobial drugs w/o harming host cells:

1) cell walls of bacteria (peptidoglycan), fungi (chitin)
2) protein synthesis (prokaryotic ribosomes)
3) unique features of bacterial, fungal membranes
4) nucleic acid synthesis (microbial polymerases)
5) metabolic pathways not found in host
6) attachment to host cells (viruses)
2. Antibacterial Drugs
Antibacterial Targets

**Cell wall**
- β-lactams
- penicillins
- cephalosporins
- monobactams
- carbapenems
- Glycopeptides
- vancomycin
- Bacitracin

**DNA synthesis**
- Fluoroquinolones
  - ciprofloxacin
  - levofloxacin
  - moxifloxacin

**RNA synthesis**
- Rifamycins
  - rifampin

**Plasma membrane**
- Polymyxins
  - polymyxin B
  - colistin
- Lipopeptide
  - daptomycin

**Ribosomes**
- 30S subunit
  - aminoglycosides
  - tetracyclines
- 50S subunit
  - macrolides
  - lincosamides
  - chloramphenicol
  - oxazolidinones

**Metabolic pathways**
- Folic acid synthesis
- sulfonamides
- sulfones
- trimethoprim
- Mycolic acid synthesis
- izoniazid
Drugs that Target the Cell Wall – β-lactams

The β-lactams all have the same core β-lactam ring structure:

- some are natural, some are semisynthetic (i.e., have man-made “R” groups)
- binds and inhibits the bacterial transpeptidase enzyme
- prevents final cross-linking step in peptidoglycan synthesis

Penicillin Derivatives

<table>
<thead>
<tr>
<th>R group</th>
<th>Drug name</th>
<th>Spectrum of activity</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>-CH₂-O-</td>
<td>penicillin G</td>
<td>G+ and a few G−</td>
<td>parenteral</td>
</tr>
<tr>
<td>-CH₂-NH₂</td>
<td>penicillin V</td>
<td>similar to penicillin G</td>
<td>oral</td>
</tr>
<tr>
<td>-CH₂-OH</td>
<td>ampicillin</td>
<td>G+ and more G− than penicillin</td>
<td>parenteral and oral</td>
</tr>
<tr>
<td>CH₃O</td>
<td>amoxicillin</td>
<td>similar to ampicillin</td>
<td>oral (better than ampicillin)</td>
</tr>
<tr>
<td>CH₃O</td>
<td>methicillin</td>
<td>G+ only, including β-lactamase producers</td>
<td>parenteral</td>
</tr>
</tbody>
</table>
Effect of Transpeptidase Inhibition

Inhibition of peptidoglycan cross-linking makes bacterial cells vulnerable to lysis due to osmotic pressure.
Microbial Defenses to $\beta$-lactams

- some bacteria can avoid the effects of penicillin by the production of $\beta$-lactamases (e.g., penicillinase), enzymes that break the $\beta$-lactam ring
- many semisynthetic derivatives are resistant to such enzymes
Vancomycin

- a glycopeptide antibiotic that binds to and blocks cell wall precursor molecules preventing their addition

Bacitracin

- a polypeptide that prevents movement of cell wall precursor molecules to the outside of the cell
- can be toxic taken internally, usually topical
Drugs That Inhibit Protein Synthesis

Major classes of protein synthesis–inhibiting antibacterials

**Chloramphenicol, macrolides, and lincosamides**
- Bind to the 50S ribosomal subunit
- Prevent peptide bond formation
- Stop protein synthesis

**Aminoglycosides**
- Bind to the 30S ribosomal subunit
- Impair proofreading, resulting in production of faulty proteins

**Tetracyclines**
- Bind to the 30S ribosomal subunit
- Block the binding of tRNAs, thereby inhibiting protein synthesis
Inhibitors of Nucleic Acid Synthesis

Rifamycins (e.g., rifampicin)
- inhibit bacterial transcription (mRNA synthesis)
- penetrates host cells well, effective against intracellular bacteria such as Mycobacteria

Fluoroquinolones (e.g., ciprofloxacin)
- inhibit the bacterial DNA gyrase, thus DNA replication

Metronidazole
- inhibits dNTP synthesis, thus DNA replication, in anaerobes
Drugs that target Membranes

Polymyxins

- disrupt plasma membrane, outer membrane
- especially effective against Gram- bacteria
- usually in topical ointments since it is poorly absorbed and rather toxic internally

polymyxin B
Metabolic Inhibitors – Sulfonamides

AKA, “sulfa drugs”.

Inhibit folic acid synthesis from PABA (para-aminobenzoic acid).

- folic acid synthesis in animals does not involve PABA
- has a bacteriostatic effect
- synthetic compounds first used in 1930s
Antimycobacterial Drugs

Mycobacteria are difficult targets due to their unique “acid-fast” outer membrane containing mycolic acids, and being intracellular pathogens.

The most effective antibiotics for mycobacterial infections are:

Rifamycins
- penetrate mycolic acid rich cell wall, inhibit transcription

Isoniazid
- directly inhibits mycolic acid synthesis
- a narrow spectrum metabolic inhibitor
3. Drugs That Target Eukaryotic Pathogens
Challenges of Eukaryotic Pathogens

Unlike bacteria, eukaryotic pathogens have less features that differ from host cells and thus less targets to work with:

- e.g., ribosomes and other metabolic processes are basically the same as ours

For this reason there are fewer drugs to turn to in order to treat eukaryotic infections, however, there are some unique features with which to target many eukaryotic pathogens...
Fungal cells differ from animal cells like ours in the following ways thus providing targets for drug treatment:

- **cell walls with chitin** (e.g., echinocandins)
- **ergosterol in fungal membranes**
  - **polyenes**: a class of drugs that are somewhat toxic
  - **azoles**: a class of drugs used topically in many antifungal creams

![Chemical structures of cholesterol, ergosterol, and amphotericin B](image)
Targets of Antifungal Drugs

- Inhibit DNA and RNA synthesis: flucytosine
- Disrupt microtubule function: griseofulvin
- Inhibit mitochondria function: naphthoquinone
- Disrupt membrane: polyenes
- Inhibit ergosterol synthesis: imidazole and allylamine
- Inhibit chitin synthesis: polyoxins and nikkomycins
- Inhibit synthesis of β(1→3) glucans: echinocandins
Drugs That Target Protozoans

**Quinolones** (e.g., chloroquine)
- used to treat malaria, amoebiasis

**Metronidazole** (Flagyl)
- used for *Trichomonas vaginalis*, *Giardia intestinalis*
- inhibits DNA replication in anaerobes

**Pentamidine**
- used for trypanosome based illness (e.g., sleeping sickness)
- disrupts DNA structures unique to mitochondria of trypanosomes and several related protozoans
Drugs That Target Helminths

Benzimidazoles

• prevent microtubule formation

Ivermectin

• paralyzes and kills many nematodes

Niclosamide

• not absorbed well into body, inhibits ATP production in tapeworms

Praziquantel

• paralyzes flukes and tapeworms
4. Antiviral Drugs
Limits of Antiviral Drugs

Viruses are especially difficult to target with drugs for several reasons:

1) viruses tend to use host cell metabolic processes
   • it’s difficult to target viral processes w/o harming host

2) viruses are intracellular parasites
   • the drug must effectively enter host cells

3) viruses can be latent
   • there’s very little a drug can do to combat a provirus

4) viruses can mutate very rapidly
   • thus their molecular targets can change rapidly
Antiviral drugs generally target the following:

**Virus-specific enzymes**
- e.g., reverse transcriptase, integrase (inserts viral DNA into host DNA), proteases (process viral proteins)

**Viral DNA replication, transcription**
- drugs inhibiting these processes may kill host cell also

**Fusion of enveloped viruses**
- most enveloped viruses gain entry via fusion with the host membrane which can also be blocked
Nucleoside, Nucleotide Analogs

Many antiviral drugs are analogs of nucleosides (sugar + base) or nucleotides (sugar + base + phosphates):

- incorporated into DNA or RNA
- function as “chain terminators”, preventing elongation

Acyclovir is a common antiviral drug that is an analogue of the nucleoside guanosine.
Acyclovir: Mechanism of Action

- Viral thymidine kinase will add phosphate to acyclovir, human thymidine kinase will not

Drug is thus activated only in virally infected cells.
Enzyme Inhibitors

Some antiviral drugs bind directly to viral enzymes and inhibit their activity:

**Reverse transcriptase inhibitors**
- blocks conversion of RNA to DNA

**Integrase inhibitors**
- Prevents insertion of viral DNA into host chromosome

**Protease inhibitors**
- inhibits viral assembly
Key Terms for Chapter 14

• antimicrobial vs antibiotic
• selective toxicity, chemotherapeutic index
• narrow vs broad spectrum, superinfection
• Minimum Inhibitory vs Bactericidal Concentration (MIC vs MBC)
• ergosterol, chitin
• nucleoside & nucleotide analogs
• thymidine kinase, chain terminators