Antimicrobial Drugs

Fading Miracle?
SOME DEFINITIONS

- **Chemotherapy**: The use of drugs to treat a disease
- **Antimicrobial drugs**: Interfere with the growth of microbes within a host
- **Antibiotic**: Substance produced by a microbe that, in small amounts, inhibits another microbe
- **Selective toxicity**: A drug that kills harmful microbes without damaging the host
Chemotherapy

• The use of drugs to treat a disease

• **Selective toxicity**: A drug that kills harmful microbes without damaging the host
Selectively toxic

- Drugs should kill or inhibit microbial cells without simultaneously damaging host tissues.
- As the characteristics of the infectious agent become more similar to the vertebrate host cell, complete selective toxicity becomes more difficult to achieve & more side effects are seen.
Antibiotic/Antimicrobial

• **Antibiotic**: Chemical produced by a microorganism that kills or inhibits the growth of another microorganism

• **Antimicrobial agent**: Chemical that kills or inhibits the growth of microorganisms
Definitions of Antibiotics

• OLD: An antibiotic is a chemical substance produced by various species of microorganisms that is capable in small concentrations of inhibiting the growth of other microorganisms

• NEW: An antibiotic is a product produced by a microorganism or a similar substance produced wholly or partially by chemical synthesis, which in low concentrations, inhibits the growth of other microorganisms
## The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs

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

<table>
<thead>
<tr>
<th>Mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
</tr>
<tr>
<td>Polymyxin</td>
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<tr>
<td>Penicillin</td>
</tr>
<tr>
<td>Streptomycin</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Eukaryotes</th>
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<tbody>
<tr>
<td>Azoles</td>
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<tr>
<td>Niclosamide</td>
</tr>
<tr>
<td>Acyclovir</td>
</tr>
<tr>
<td>Arildone</td>
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<tr>
<td>Ribavirin</td>
</tr>
<tr>
<td>Praziquantel</td>
</tr>
</tbody>
</table>
### Antibiotic Spectrum of Activity

**Table 20.2: The Spectrum of Activity of Antibiotics and Other Antimicrobial Drugs**

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<thead>
<tr>
<th>Prokaryotes</th>
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<tr>
<td><strong>Mycobacteria</strong></td>
<td><strong>Fungi</strong></td>
</tr>
<tr>
<td>Gram-Negative Bacteria</td>
<td>Ketoconazole</td>
</tr>
<tr>
<td>Gram-Positive Bacteria</td>
<td>Mefloquine (malaria)</td>
</tr>
<tr>
<td>Chlamydiads, Rickettsias†</td>
<td>Niclosamide (tapeworms)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Acyclovir</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Praziquantel (flukes)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
</tr>
</tbody>
</table>

*Growth of these bacteria frequently occurs within macrophages or tissue structures.*
†*Obligately intracellular bacteria.*

- No antibiotic is effective against all microbes
### TABLE 12.1

**Characteristics of the Ideal Antimicrobial Drug**

- Selectively toxic to the microbe but nontoxic to host cells
- Microbicidal rather than microbistatic
- Relatively soluble and functions even when highly diluted in body fluids
- Remains potent long enough to act and is not broken down or excreted prematurely
- Not subject to the development of antimicrobial resistance
- Complements or assists the activities of the host’s defenses
- Remains active in tissues and body fluids
- Readily delivered to the site of infection
- Not excessive in cost
- Does not disrupt the host’s health by causing allergies or predisposing the host to other infections
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapeutic drug</td>
<td>Any chemical used in the treatment, relief, or prophylaxis of a disease</td>
</tr>
<tr>
<td>Prophylaxis*</td>
<td>Use of a drug to prevent imminent infection of a person at risk</td>
</tr>
<tr>
<td>Antimicrobial chemotherapy*</td>
<td>The use of chemotherapeutic drugs to control infection</td>
</tr>
<tr>
<td>Antimicrobics</td>
<td>All-inclusive term for any antimicrobial drug, regardless of its origin</td>
</tr>
<tr>
<td>Antibiotics*</td>
<td>Substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganisms</td>
</tr>
<tr>
<td>Semisynthetic drugs</td>
<td>Drugs which are chemically modified in the laboratory after being isolated from natural sources</td>
</tr>
<tr>
<td>Synthetic drugs</td>
<td>The use of chemical reactions to synthesize antimicrobial compounds in the laboratory</td>
</tr>
<tr>
<td>Narrow spectrum (limited spectrum)</td>
<td>Antimicrobics effective against a limited array of microbial types—for example, a drug effective mainly on gram-positive bacteria</td>
</tr>
<tr>
<td>Broad spectrum (extended spectrum)</td>
<td>Antimicrobics effective against a wide variety of microbial types—for example, a drug effective against both gram-positive and gram-negative bacteria</td>
</tr>
</tbody>
</table>
Impact of Modern Healthcare on Life Expectancy

![Graph showing the impact of modern healthcare on life expectancy over time. The graph compares survival rates from 1 AD/1830 to 1985, with notable improvements seen in survival rates as time progresses.]
The Ideal Drug*

1. Selective toxicity: against target pathogen but not against host
2. Bactericidal vs. bacteriostatic
3. Favorable pharmacokinetics: reach target site in body with effective concentration
4. Spectrum of activity: broad vs. narrow
5. Lack of “side effects”
   – Therapeutic index: effective to toxic dose ratio
6. Little resistance development

* There is no perfect drug.
Origins of antimicrobial drugs

• Antibiotics are common metabolic products of aerobic spore-forming bacteria & fungi.
  – bacteria in genera *Streptomyces* & *Bacillus*
  – molds in genera *Penicillium* & *Cephalosporium*

• By inhibiting the other microbes in the same habitat, antibiotic producers have less competition for nutrients & space.
The Action of Antimicrobial Drugs

- Broad-spectrum
- Bactericidal
- Bacteriostatic
Targets of antimicrobial drugs
• Antibiotics fall into one of five classes based on their MECHANISM OF ACTION

• Antibiotic Drugs may (be):
  – Antimetabolites
  – Inhibit cell wall synthesis
  – Inhibit protein synthesis
  – Inhibit nucleic acid synthesis
  – Alter or inhibit cell membrane permeability or transport
• Antibiotics may be classified as
  • Antimetabolites or inhibitors of Metabolic Pathways
  • Cell Wall Active- Inhibit cell wall synthesis
  • Inhibition of Protein Synthesis (Ribosomal)
  • Inhibition of nucleic acid (DNA/RNA) Synthesis, structure or function
  • Cell Membrane Active- Alter or inhibit cell membrane permeability or transport i.e Disruption of cell membrane structure or function
Antibiotic Targets
 Modes of Antimicrobial Action

- Inhibition of cell wall synthesis: Penicillins, cephalosporins, bacitracin, vancomycin
- Inhibition of protein synthesis: Chloramphenicol, erythromycin, tetracyclines, streptomycin

DNA → Replication

Transcription → mRNA → Translation → Protein

- Inhibition of nucleic acid replication and transcription: Quinolones, rifampin
- Enzymatic activity, synthesis of essential metabolites

- Injury to plasma membrane: Polymyxin B
- Inhibition of synthesis of essential metabolites: Sulfanilamide, trimethoprim
Modes of Antimicrobial

Figure 20.4

(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.

(b) In the diagram the black arrows indicate the different points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.
**DNA gyrase**

- Inhibition of cell wall synthesis
  - Penicillins
  - Cephalosporins
  - Vancomycin
  - Bacitracin
  - Isoniazid
  - Ethambutol

- Inhibition of pathogen’s attachment to, or recognition of, host
  - Arildone

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

- Disruption of cytoplasmic membrane
  - Polymyxins
    - Polyenes are antifungal

- Inhibition of general metabolic pathway
  - Sulfonamides
  - Trimethoprim

- Inhibition of protein synthesis
  - Aminoglycosides
  - Tetracyclines
  - Chloramphenicol
  - Macrolides
Antibiotic Mechanisms of Action

Cell wall synthesis
Beta-lactams
Vancomycin
Isoniazid
Ethambutol
Cycloserine
Ethionamide
Bacitracin
Polymyxin

DNA replication
Quinolones
Metronidazole

RNA synthesis
Rifampin
Rifabutin

DNA
mRNA
Ribosomes

50
30
50
30
50
30

Protein synthesis
(50S ribosome)
Chloramphenicol
Macrolides
Clindamycin
Streptogramins

Translation

Antimetabolites
Sulfonamides
Dapsone
Trimethoprim
Para-aminosalicylic acid

Translation

Alteration of Cell Membrane
Polymyxins
Bacitracin
Neomycin
Mechanisms of Antimicrobial Action

- Bacteria have their own enzymes for:
  - Cell wall formation
  - Protein synthesis
  - DNA replication
  - RNA synthesis
  - Synthesis of essential metabolites

- OUR PURPOSE IS TO ATTACK THESE AREAS
Mechanisms of Antimicrobial Action

• Viruses use host enzymes inside host cells
• Fungi and protozoa have own eukaryotic enzymes

• The more similar the pathogen and host enzymes, the more side effects the antimicrobials will have
Antimetabolites Inhibit Metabolic Pathways

- **Bacteriostatic drugs**
  - Sulfonimide
  - Trimethoprim

- **Bactericidal**
  - isoniazid

- **Structural analogs of normal metabolites**

- **Inhibit action of specific enzymes**
Inhibition of Metabolic Pathways

• **Sulfonamides/Trimethoprim** *Bacteriostatic*
  • Inhibit synthesis of tetrahydrofolate
  • Sulfa competes with PABA $\rightarrow$ TH4
  • Trimeth prevents TH4 $\rightarrow$ TH2 $\rightarrow$ TH4 cycle
  • Oral, urine excretion, high levels
  • Low toxicity: nausea
  • **Dangerous to give to Pt. on warfarin!!**

– **Indications**
  • UTI
  • Upper Respiratory tract, otitis media, sinusitis
  • GI v Enterobacteriaceae
  • *Pneumocystis carinii*
**ANTIMETABOLITE Mechanism of Action**

- **Sulfonamides**
  - an analog of PABA, works by competitive inhibition

- **Trimethoprim-sulfamethoxazole**
  - a synergistic combination; useful against UTIs
Mechanism of Action

ANTIMETABOLITE ACTION

(Hydroxymethyl)dihydropteridine

Sulfonamides, Dapsone

Dihydropteroic acid

PABA

PABA

p-aminobenzoic acid

Dihydropteroate synthase

Plus aluminic acid

Dihydrofolate reductase

Trimethoprim

tetrahydrofolic acid
• **Sulfonamides**, which resemble *p*-aminobenzoic acid and dapsone (leprosy and pneumocytis) competitively inhibit dihydropterate synthase.

• **Trimethoprim** inhibits the enzymatic action of dihydrofolate reductase.

• Both of these actions cause interference with the synthesis of folic acid, which is required by bacteria.
(a) Para-aminobenzoic acid (PABA) and its structural analogs, the sulfonamides (sulfa drugs)

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

(c) Inhibition of folic acid synthesis by sulfonamide
Drugs that affect the bacterial cell wall and inhibit cell wall synthesis

Cell Wall Active
Prokaryotic Cell Walls
1 Cell Wall Active Mechanisms of action

• Most bacterial cell walls contain a rigid girdle of peptidoglycan.

• Penicillin and cephalosporin block synthesis of peptidoglycan, causing the cell wall to lyse.

• Penicillins do not penetrate the outer membrane and are less effective against gram-negative bacteria.

• Broad spectrum penicillins and cephalosporins can cross the cell walls of gram-negative bacteria.
Drugs that affect the bacterial cell

- Peptidoglycan
- Exposure to beta-lactam antibiotics
- Weak points lacking peptidoglycan
- Exposure to hypotonic environment
- Membrane bulges out as water diffuses into cell.
- Membrane breaks.
- Cell lyses.

Images (e) and (f) show the effects of these drugs on bacterial cells.
A bacterial cell wall is composed of a macromolecule of peptidoglycan composed 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. Normally, other enzymes link new NAM subunits to old NAM subunits with peptide crosslinks.
Penicillin 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.

The cell bursts from osmotic pressure because the integrity of peptidoglycan is not maintained.
1 Cell Wall Active Mechanisms of action

- **Cell wall synthesis inhibitors**
  - Bactericidal
  - May inhibit transpeptidation of penicillins and cephalosporins
    - $\beta$ lactam drugs
    - May inhibit synthesis of peptidoglycan
      - Cyclocerine, bacitracin, vancomycin
  - May act in cytoplasm, membrane or cell wall
  - May cause bacteria to take on aberrant shapes
EXAMPLES OF CELL WALL SYNTHESIS INHIBITORS

β-Lactam Antibiotics

– Penicillins
– Penicilinase-resistant penicillins
– Extended-spectrum penicillins
– Penicillins + β-lactamase inhibitors
– Cephalosporins
– Carbapenems
– Monobactams
Cell Wall Active

• β Lactams Bactericidal
  – Penicillin:
    • Penicillin G    Penicillin V
  – Aminopenicillin:
    • Amipicillin    Amoxicillin
  – β Lactamase resistant:
    • Methicillin    Oxacillin    Nafcillin
  – Anti-Pseudomonal
    • Carboxypenicillin:     Carbenicillin    Ticarcillin
    • Ureidopenicillin:     Piperacillin    Mezlocillin
• β Lactamase Inhibitors
  • Clavulanic Acid    Sulbactam    Tazobactam
• Cephalosporin:
  • Generations:     1\textsuperscript{st} Narrow    2\textsuperscript{nd} Expanded    3\textsuperscript{rd} Broad    4\textsuperscript{th} Extended
• Carbapenem:
  • Imipenem    Meropenem
• Glycopeptide
  • Vancomycin    Teicoplanin
• Cell wall synthesis inhibitors
  – Penicillins
    • Inhibit transpeptidation enzymes involved in cell wall synthesis
    • More active against gram-positive bacteria
    • React with penicillin binding proteins
    • Have β-lactam ring structure
    • Inactivated by β-lactamase
Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS (cont’d)

β-Lactam ring structure

![β-Lactam ring structure diagram]
(a) Natural (antibiotic) penicillins

- Penicillin G (Requires injection)
- Penicillin V (Can be taken orally)

(b) Semisynthetic penicillins

- Oxacillin (Resistant to penicillinase)
- Ampicillin (Extended spectrum)
Antibiotics that are Inhibitors of Cell Wall Synthesis

• Penicillin (over 50 compounds)
  – Share 4-sided ring (β lactam ring)
• Natural penicillins
  • Narrow range of action
  • Susceptible to penicillinase (β lactamase)
Semisynthetic Penicillins

• **Penicillinase-resistant penicillins**
  • Carbapenems: very broad spectrum
  • Monobactam: Gram negative

• **Extended-spectrum penicillins**

• **Penicillins + β-lactamase inhibitors**
Mechanism of Action
CELL WALL SYNTHESIS INHIBITORS

(cont’d)

Action of β-Lactam antibiotics

1. Bactericidal; **growing cells only**

2. Drug links covalently to regulatory enzymes called PBPs (penicillin-binding proteins)

3. Blocks cross-linkage of peptidoglycan
Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS (cont’d)

Action of β-Lactam antibiotics

For E. coli

$>\text{MIC}$

- wall damage
- autolysins
- spheroplasting
- cell lysis

$<\text{MIC}$

- no septa
- filaments
Non - β-Lactams

- **Vancomycin**
  - active against gram positive cocci, but not gram negative because too large to pass through outer membrane
  - interferes with PG elongation

- **Cycloserine, ethionamide and isoniazid**
  - inhibits enzymes that catalyze cell wall synthesis
  - for *Mycobacterial* infections
<table>
<thead>
<tr>
<th>PATHOGENS</th>
<th>TYPICAL DRUG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td></td>
</tr>
<tr>
<td>Pen-ase (-)</td>
<td>Penicillin G (oral or IM)</td>
</tr>
<tr>
<td>Pen-ase (+)</td>
<td>Methicillin, Nafcillin</td>
</tr>
<tr>
<td>Gram negative</td>
<td></td>
</tr>
<tr>
<td>Enterics, etc.</td>
<td>Ampicillin, gentamicin, etc.</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>Ticarcillin, tobramycin</td>
</tr>
<tr>
<td><em>B. fragilis</em></td>
<td>Clindamycin</td>
</tr>
</tbody>
</table>
### Clinical Uses

<table>
<thead>
<tr>
<th>PATHOGENS</th>
<th>TYPICAL DRUG</th>
</tr>
</thead>
</table>
| Mycobacterium | Streptomycin  
|             | Iso-nicotinic hydrazide (INH)                     |
| Fungi:      | Nystatin  
| Cutaneous   | Amphoterericin B, ketoconazol                     |
| Deep        |                                                   |
| Parasites:  | Chloroquine  
| Plasmodium  | Quinacrine                                       |
| Giardia     |                                                   |
• Other Cell wall synthesis inhibitors
  – Cephalosporins
  • Mechanism of action similar to penicillin
  • Both gram – and gram + bacteria
  • Contain β-lactam ring structure
  • Used for patients allergic to penicillin
General Structure of Cephalosporins
• **Cephalosporin:**
  
  – 1\textsuperscript{st} Narrow
    – cephalothin, cephalexin, cefazolin
      » gram positive, limited gram negative, no anaerobe
  
  – 2\textsuperscript{nd} Expanded
    – cefaclor, cefamandole, cefuroxime, cefotetan, cefoxitin
      » gram negative spectrum increased
  
  – 3\textsuperscript{rd} Broad [note that they all start with “cef” and end with “e” & no “p” like in 4\textsuperscript{th}]
    – cefixime, cefotaxime, ceftriaxone, ceftazidime
      » Pseudomonas activity
  
  – 4\textsuperscript{th} Extended
    – cefepime, cefpirome
    – additional stability v beta lactamases
• Cephalosporins
  – 2nd, 3rd, and 4th generations more effective against gram-negatives

Figure 20.9

[Chemical structures of Cephalosporin and Penicillin nuclei]
Cell Membrane Active

- Polymyxin Bactericidal
  - Polymyxin B  Colistin

  - Active against Gram Negative, including pseudomonas
  - Nephrotoxic and neurotoxic
  - Limited to topical use
Mechanism of Action of CELL WALL SYNTHESIS INHIBITORS

Steps in synthesis:
1. NAM-peptide made in cytoplasm
2. attached to bactoprenol in cell membrane
3. NAG is added
4. whole piece is added to growing cell wall
5. crosslinks added

• the β-Lactams
• the non β-Lactams
Penicillinase (β Lactamase)
Resistance to β-Lactams – Gram pos. (cont’d)

Mechanism of Action
CELL WALL SYNTHESIS INHIBITORS

[Diagram showing the mechanism of action of β-lactams on Gram-positive bacteria]

- Beta-lactam
- Peptidoglycan
  - Fails to bind to altered PBPs
- Drug hydrolyzed by beta-lactamases
  - Cell survives
- Penicillin-binding proteins (PBPs)
- Inhibition of peptidoglycan synthesis
  - Cell killed
Mechanism of Action
CELL WALL SYNTHESIS INHIBITORS

Resistance to β-Lactams – Gram neg (cont’d)
Other Inhibitors of Cell Wall Synthesis

- Polypeptide antibiotics
  - Bacitracin
    - Topical application
    - Against gram-positives
  - Vancomycin
    - Glycopeptide
    - Important "last line" against antibiotic resistant S. aureus
Injury to the Plasma Membrane

- **Polymyxin B (Gram negatives)**
  - Topical
  - Combined with bacitracin and neomycin (broad spectrum) in over-the-counter preparation
Other Inhibitors of Cell Wall Synthesis

- Antibiotics effective against Mycobacteria: interfere with mycolic acid synthesis or incorporation
  - Isoniazid (INH)
  - Ethambutol
• Antimycobacterium antibiotics
  – Isoniazid (INH)
    • Inhibits mycolic acid synthesis
  – Ethambutol
    • Inhibits incorporation of mycolic acid
Drugs that block protein synthesis

• Ribosomes of eucaryotes differ in size and structure from procaryotes, so antimicrobics usually have a selective action against procaryotes.

• Prokaryotes and eukaryotes (80S) have a different structure to their ribosomes so we can use antibiotics for selective toxicity against ribosomes of prokaryotes (70S) ……80S vs. 70 S

• But similar to mitochondrial ribosomes which may account for some toxicity…. they can also damage the eucaryotic mitochondria.
Drugs that block protein synthesis

Figure 12.5
Sites of inhibition on the procaryotic ribosome and major antibiotics that act on these sites. All have the general effect of blocking protein synthesis. Blockage actions are indicated by X.
Mechanism of Action
INHIBITION OF PROTEIN SYNTHESIS:
Steps in synthesis:
1. Initiation
2. Elongation
3. Translocation
4. Termination
Interference with Initiation of Protein Synthesis

30S

1 2 3 GTP

Initiation Factors

50S

GDP + Pi

Aminoglycosides

70S Initiation Complex

Blocks interaction with mRNA

Irreversible binding to 30S
Streptomycin

- **Streptomycin**, a highly basic trisaccharide, interferes with the binding of formylmethionyl-tRNA to ribosomes and thereby prevents the correct initiation of protein synthesis.
Interference with Elongation of Protein Synthesis

- **Tetracycline**
  - Inhibits binding of aminoacyl tRNA

- **Chloramphenicol**
  - Inhibits peptidyl transferase activity

- **Erythromycin**
  - Inhibits release of EFG

- **Fusidic Acid**
  - Inhibits translocation

- **GTP**
  - Ts + GTP → GTP + Ts
  - Tu + GTP → GTP + Tu

- **GDP**
  - Tu + GDP → GDP + Tu
  - Ts + GDP → GDP + Ts

- **Pi**
  - Tu + GDP → GDP + Tu + Pi

- **Inhibits** binding of aminoacyl tRNA
- **Inhibits** peptidyl transferase activity
- **Inhibits** translocation
- **Inhibits** release of EFG
## Antibiotic Inhibitors of Protein Synthesis

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin and other aminoglycosides</td>
<td>Inhibit initiation and cause misreading of mRNA (prokaryotes)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Binds to the 50S subunit and inhibits translocation (prokaryotes)</td>
</tr>
<tr>
<td>Aminoglycosides such as neomycin, kanamycin, and gentamycin</td>
<td>Interfere with the <em>decoding site</em> located near nucleotide 1492 in 16S rRNA of the 30S subunit.</td>
</tr>
</tbody>
</table>
INHIBITORS OF PROTEIN SYNTHESIS

• Aminoglycosides
  – Aminoglycosides (streptomycin, gentamicin) insert on sites on the 30S subunit and cause misreading of mRNA.
  – bind to bacterial ribosome on 30S subunit; and blocks formation of initiation complex. Both actions lead to mis-incorporation of amino acids.
  – Examples:
    - Gentamicin
    - Amikacin
    - Kanamycin
    - Streptomycin
    - Neomycin
    - Tobramycin
    - Spectinomycin
– Tend to be bactericidal and broad spectrum
– **Are actively transported onto the bacterial cell** (this is one reason for selective toxicity - don’t work against animal cells) by a mechanism that involves ox phos.
– Therefore, they have little or no activity against strict anaerobes or those that metabolize only fermentatively (like streptococci)
– **Streptomycin** - hardly used anymore b/c high-level and stable resistant mutants are frequently selected for during therapy
– **Neomycin** - used to reduce the facultative flora of the large intestine before certain types of intestinal surgery. It is poorly absorbed therefore is active in the bowel.
INHIBITORS OF PROTEIN SYNTHESIS (cont’d)

- Aminoglycosides (cont’d)
  - broad spectrum
    - Gram negative rods
    - *P. aeruginosa*
    - Drug-resistant gram negative rods
    - Plague, Tularemia, Gonorrhea
    - Pre-op (bowel)
    - External (skin)
  - toxic at some level to eighth cranial nerve
• **Protein synthesis inhibitors**
  – **Aminoglycosides**
    • **Streptomycin, neomycin, gentamycin**
    • Bactericidal for gram –
    • Bind to 30S ribosomal subunit
    • May irrevocably block initiation of translation and/or cause mRNA misreading
    • Narrow effective concentration range
      – Reanl damage
      – CNVIII toxicity
Inhibition of Protein Synthesis (Ribosomal)

- **Aminoglycosides** Bacteriocidal
  - Gentamicin  Tobramycin  Amikacin
  - Broad Spectrum, highly effective, except v Enterococcus.
  - Used in combination with cell wall active component
  - **High Level Resistance** can occur by altered binding site
  - Narrow window of therapeutic efficacy, use in the hospital, monitor closely
  - Nephrotoxicity, ototoxicity
(a) Incorrect amino acids
Ribosome
50s mRNA
30s Aminoglycosides cause change in 30s shape; mRNA is misread.

(b) Tetracycline blocks docking site of tRNA.
50s 30s

(c) Chloramphenicol binds
Amino acids 50s 30s

(d) Macrolide binds to 50s. mRNA cannot move through ribosome properly. Synthesis stops.
50s 30s
INHIBITORS OF PROTEIN SYNTHESIS

• Most macrolides tend to be bacteriostatic (cidal for some Gm+)

• **Macrolides**: chloramphenicol & erythromycin
  - bind to 50S subunit and blocks the translocation step

- **Chloramphenicol**: broad spectrum
  - Anaerobes
  - Typhoid
  - Meningitis
- **Erythromycin**:
  - Mycoplasma
  - Legionella
  - S. pyogenes
• **Protein synthesis inhibitors**
  – **Chloramphenicol**
    • Bacteriostatic
    • Gram + and –
    • Broad spectrum, NOT anaerobes
      • Meningitis, Rickettsia and Chlamydia
      • Binds to 50S ribosomal subunit
      • Inhibits peptide bond formation
      • Inhibited by chloramphenicol acetyltransferase
      • Last resort because of **aplastic anemia** in 1 in 20,000 recipients
Incorrect amino acids

Ribosome

Aminoglycodies cause change in 30s shape; mRNA is misread.

(a)

Chloramphenicol binds

Amino acids

50s

30s

(c)

Tetracycline blocks docking site of tRNA.

50s

30s

(b)

Macrolide binds to 50s. mRNA cannot move through ribosome properly. Synthesis stops.

50s

30s

(d)
Protein synthesis inhibitors

- **Protein synthesis inhibitors**
  - Macrolides and lincomycins
    - Erythromycin and clindamycin
    - Bacteriostatic
    - Block translocation in 50S subunit
    - Bacteria with mutation in 50S subunit are resistant
      - Prevents binding
• **Clindamycin***---similar mode of action as macrolides
  – binds to 50S subunit and interferes with binding of the amino acid – acyl-tRNA complex and so inhibits peptidyl transferase
  – **works best against**
    • *Staphylococcus*
    • *Bacteroides* & anaerobic gram neg rods
  – **Penicillin allergic people**
    • *Anaerobes* especially B fragilis,
    • PID
    • Pseudomembraneous colitis
Inhibition of Protein Synthesis

• **Macrolides** Bacteriostatic
  • Erythromycin  Azithromycin  Clarithromycin
  • Broad spectrum, Highly effective Gram Positives
  • Legionella, Chlamydia, Mycoplasma
  • Pneumonia, penicillin allergic, Legionnaire’s d.o.c.
  • very safe, GI irritation
• **Protein synthesis inhibitors**
  
  – **Tetracyclines**
  
  • Bacteriostatic
  
  • Bind to 30S ribosomal subunit and prevent binding of aminoacyl tRNA to acceptor site
  
  • May be deposited in teeth and bones
    – Cause structural problem in bones and teeth staining in children
  
  • Not transported into cells with specific resistance factors on the plasmid
• **Tetracyclines**  Bacteriostatic

• Tetracyclines block attachment of tRNA on the A acceptor site and stop further synthesis.

• **Tetracyclines**
  – bind to 30S subunit and interferes with the attachment of the tRNA carrying amino acids to the ribosome

• effective against:
  • *Chlamydia*
  • *Rickettsia*
  • *Mycoplasma*
  • *Brucella*
Tetracycline inhibits the binding of aminoacyl-tRNA into the A site of the bacterial ribosome.
• Tetracycline  Doxycycline  Minocycline
• Doxy: STD, Chlamydia and GC, Walking Pneumonia
• Brucella, Rickettsia
• Discolored teeth, GI upset, phototoxic dermatitis
• Don’t use on children < 8 yo
Protein synthesis inhibitors
- Broad spectrum
- Require bacterial growth

- 30S subunit: aminoglycosides and tetracyclines
- 50S subunit: erythromycin, clindamycin, chloramphenicol
Antibacterial Antibiotics

Inhibitors of Protein Synthesis

• Chloramphenicol
  – Broad spectrum
    • Binds 50S subunit, inhibits peptide bond formation

• Aminoglycosides
  – Streptomycin, neomycin, gentamycin
    • Broad spectrum
      – Changes shape of 30S subunit
Antibacterial Antibiotics
Inhibitors of Protein Synthesis

- **Tetracyclines**
  - Broad spectrum
    - Interferes with tRNA attachment

- **Macrolides**
  - Gram-positives
    - Binds 50S, prevents translocation

- **Erythromycin**
  - Gram-positives
    - Binds 50S, prevents translocation
Inhibitors of Protein Synthesis

• **Broad spectrum, toxicity problems**

• **Examples**

  – Chloramphenicol (bone marrow)
  – Aminoglycosides: Streptomycin, neomycin, gentamycin (hearing, kidneys)
  – Tetracyclines (Rickettsias & Chlamydia; GI tract)
  – Macrolides: Erythromycin (gram +, used in children)
Drugs that inhibit nucleic acid synthesis

- may block synthesis of nucleotides, inhibit replication, or stop transcription
- Sulfonamides and trimethoprim block enzymes required for tetrahydrofolate synthesis needed for DNA & RNA synthesis.
- competitive inhibition – drug competes with normal substrate for enzyme’s active site
- synergistic effect – an additive effect, achieved by multiple drugs working together, requiring a lower dose of each
Drugs that inhibit nucleic acid [DNA/RNA] synthesis

(a) Normal metabolic pathway

(b) Normal folic acid synthesis

(c) Inhibition of folic acid synthesis by sulfa drug
Mechanism of Action

INHIBITION OF DNA/RNA SYNTHESIS

- Rifampin
  - binds to RNA polymerase
  - active against gram positive cocci
  - bactericidal for *Mycobacterium*
  - used for treatment and prevention of meningococcus
• **Rifamycin**
  – Inhibits RNA synthesis
  – Antituberculosis

• **Quinolones and fluoroquinolones**
  – Ciprofloxacin
  – Inhibits DNA gyrase
  – Urinary tract infections
Mechanism of Action
INHIBITION OF DNA/RNA SYNTHESIS

- **Metronidazole**
  - breaks down into intermediate that causes breakage of DNA
  - active against:
    - protozoan infections
    - anaerobic gram negative infections
    - Metronidazole used in treating trichomonas, giardia and amebic infections and some anaerobes, like bacteroides
- Quinolones and fluoroquinolones
  ✓ effect DNA gyrase

• First one was nalidixic acid, but it had limited use because therapeutic levels were only attained in urine and there was high level mutational resistance developed VERY Rapidly.

• Fluorine enhances activity against Gm neg and adds activity against Gm pos.
Mechanism of Action
INHIBITION OF DNA/RNA SYNTHESIS

Nalidixic acid

Norfloxacin

Ciprofloxacin

Inhibits DNA gyrase blocking DNA replication

(cont’d)
Inhibition of DNA/RNA Synthesis

• Fluoroquinolones: Bactericidal
• Ciprofloxacin Ofloxacin Levofloxacin
• *Binds topoisomerases (DNA gyrase)*
• multiple binding sites, low resistance
• Circulate through liver then through intestine
• **Active v. Gram Negative** including
  – P. aeruginosa,
  – Enterobacteriaceae,
  – Intracellular orgs Legionella,
  – Brucella,
  – Salmonella,
  – Mycobacterium
• **Inactive v. anaerobes, gram positives** except *S. aureus*, *B. anthracis*
• Low toxicity, but GI upset, cartilage damage, CNS
Drugs that disrupt cell membrane function
Drugs that disrupt cell membrane function

- A cell with a damaged membrane dies from disruption in metabolism or lysis.
- These drugs have specificity for a particular microbial group, based on differences in types of lipids in their cell membranes.
- Polymyxins interact with phospholipids and cause leakage, particularly in gram-negative bacteria.
- Amphotericin B and nystatin form complexes with sterols on fungal membranes which causes leakage.
Mechanism of Action

ALTERATION OF CELL MEMBRANES

- **Polymyxins and colistin**
  - destroys membranes
  - active against gram negative bacilli
  - serious side effects
  - used mostly for skin & eye infections
Mechanism of Action
ALTERATION OF CELL MEMBRANES
(cont’d)
Antifungal Drugs

- Fungi are eukaryotes
- Have unique sterols in their cell walls
- Pathogenic fungi are often outside the body
ANTIFUNGAL DRUGS

- Protein synthesis inhibitors
  - Griseofulvin
    - Fungistatic
    - Inhibits protein assembly
    - Interferes with cell division by blocking microtubule assembly
Mechanisms of action

• Mycolic acid synthesis inhibitor
  – Bactericidal
  – Inhibits mycobacterial mycolic acid synthesis
Mechanisms of action

• Cytopasmic membrane inhibitor
  – *Alters osmotic properties of membrane*
    • polymyxin
  – *Inhibit membrane lipid synthesis*
    • Miconazole and ketoconazole
  – *Some gram –*
  – *Sterol containing mycoplasma*
  – Fungal
  – Toxic to host
  – Topical or severe
Antifungal drugs

- **Macrolide polyene**
  - Amphotericin B – mimic lipids, most versatile & effective, topical & systemic treatments
  - Nystatin – topical treatment
- **Griseofulvin** – stubborn cases of dermatophyte infections, nephrotoxic
- **Synthetic azoles** – broad-spectrum; ketoconazole, clotrimazole, miconazole
- **Flucytosine** – analog of cytosine; cutaneous mycoses or in combination with amphotericin B for systemic mycoses
Antihelminthic Drugs

- Prevent ATP generation (Tapeworms)
- Alters membrane permeability (Flatworms)
- Neuromuscular block (Intestinal roundworms)
- Inhibits nutrient absorption (Intestinal roundworms)
- Paralyzes worm (Intestinal roundworms)
Antiparasitic drugs

- **Antimalarial drugs** – quinine, chloroquinine, primaquine, mefloquine
- **Antiprotozoan drugs** - Metronidazole (Flagyl), quinicrine, sulfonamides, tetracyclines
- **Antihelminthic drugs** – immobilize, disintegrate, or inhibit metabolism
  - mebendazole, thiabendazole- broad-spectrum – inhibit function of microtubules, interferes with glucose utilization & disables them
  - pyrantel, piperazine- paralyze muscles
  - niclosamide – destroys scolex
Antiviral Drugs

• Viruses are composed of nucleic acid, protein capsid, and host membrane containing virus proteins

• Viruses live inside host cells and use many host enzymes

• Some viruses have unique enzymes for DNA/RNA synthesis or protein cutting in virus assembly
Antiviral drugs

- **Block penetration into host cell**
- **Block transcription or translation**
  - Nucleotide analogs
    - Acyclovir – herpesviruses
    - Ribavirin – a guanine analog – RSV, hemorrhagic fevers
    - AZT – thymine analog - HIV
- **Prevent maturation of viral particles**
  - Protease inhibitors – HIV
- **Interferon - HCV**
Antiviral Drugs

Nucleoside and Nucleotide Analogs

(a) Structural resemblance between acyclovir and guanine-containing nucleoside
Analogs Block DNA Synthesis

Figure 20.16b, c
Antiviral Drugs
Enzyme Inhibitors

• Inhibit assembly
  – Indinavir (HIV)
• Inhibit attachment
  – Zanamivir (Influenza)
• Inhibit uncoating
  – Amantadine (Influenza)
Antiviral Drugs
Enzyme Inhibitors

• Interferons prevent spread of viruses to new cells (Viral hepatitis)
• Natural products of the immune system in viral infections
Side effects of antimicrobial drugs

1. Toxicity to organs
2. Allergic responses
3. Suppression and alteration of microflora
Considerations in selecting an antimicrobial drug

1. nature of microbe causing infection
2. degree of microbe’s sensitivity to various drugs
3. overall medical condition of patient
• **Minimum inhibitory concentration (MIC)**- smallest concentration of drug that visibly inhibits growth

• **Therapeutic index** – the ratio of the dose of the drug that is toxic to humans as compared to its minimum effective dose
## Major Classes of Antibiotics

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism of action</th>
<th>Major resistance mechanisms</th>
</tr>
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</table>
| Beta-lactams  | Inactivate PBPs (peptidoglycan synthesis)                | • Beta-lactamases  
               |                                            | • Low affinity PBPs  
               |                                            | • Decreased transport                       |
| Glycopeptides | Bind to precursor of peptidoglycan                      | • Modification of precursor                       |
| Aminoglycosides | Inhibit protein synthesis (bind to 30S subunit) | • Modifying enzymes  
               |                                            | (add adenyl, PO$_4$, or acetyl group)     |
| Macrolides    | Inhibit protein synthesis (bind to 50S subunit)          | • Methylation of rRNA  
               |                                            | • Efflux pumps                            |
| Quinolones    | Inhibit topoisomerases (DNA synthesis)                  | • Altered target enzyme  
               |                                            | • Efflux pumps                            |
### Major Classes of Antibiotics

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</table>
Genetic Basis of Resistance

- Spontaneous mutations in endogenous genes
  - Structural genes: expanded spectrum of enzymatic activity, target site modification, transport defect
  - Regulatory genes: increased expression
- Acquisition of exogenous sequences
  - Usually genes that encode inactivating enzymes or modified targets, regulatory genes
  - Mechanisms of DNA transfer: conjugation (cell-cell contact); transformation (uptake of DNA in solution); transduction (transfer of DNA in bacteriophages)
- Expression of resistance genes
  - Reversible induction/repression systems can affect resistance phenotypes
1. Given sufficient time and drug use, antibiotic resistance will emerge.

2. Resistance is progressive, evolving from low levels through intermediate to high levels.

3. Organisms resistant to one antibiotic are likely to become resistant to other antibiotics.

4. Once resistance appears, it is likely to decline slowly, if at all.

5. The use of antibiotics by any one person affects others in the extended as well as the immediate environment.
I. Inactivation by bacterial enzymes

A. Destroys the drug  
B. Chemically alters the drug
II. Decreased drug accumulation

A. Prevents Drug Penetration

B. Pumps out the drug
III. Alteration of the drug target

A. Prevents Drug Attachment

B. Affects the metabolic pathway
## ANTIBIOTIC RESISTANCE MECHANISMS

<table>
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<th>Altered target</th>
<th>Reduced accumulation</th>
<th>Bypass</th>
</tr>
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<td>Tetracyclines</td>
<td>Trimethoprim Sulphonamides</td>
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<tr>
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<td>Streptomycin</td>
<td>β-lactams</td>
<td></td>
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<tr>
<td></td>
<td>Rifampicin</td>
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</tbody>
</table>

IMPACT OF ANTIBIOTIC RESISTANCE

• Acquired rather than intrinsic resistance poses the greatest threat to antibiotic therapy

• Many valuable antibiotic treatments are no longer available or under threat
  – Sulphonamides for meningitis
  – Ampicillin for *H.influenzae* infections
  – “Low dose” penicillin for gonorrhoea
  – Ampicillin for hospital-acquired coliform infections

• Many bacteria are resistant to almost all clinically useful antibiotics
  – MRSA - Methicillin-resistant *Staphylococcus aureus*

• Vancomycin is often the only useful antibiotic left
  – Vancomycin resistance has now been reported in enterococci
STRATEGIES FOR COUNTERACTING RESISTANCE

- Develop new antibiotics
  - Assisted by genome sequencing projects?

- Enzyme inhibitors
  - Clavulanic acid is a beta-lactamase inhibitor (irreversible)
    - Amoxycillin = Augmentin
    - Ticarcillin = Timentin

- Limit antibiotic use
  - Save potent antibiotics for when they are needed (who pays the pharmaceutical companies?)
  - Antibiotic rotation encourages loss of resistance

- Prevent cross-infection between hospital patients
  - Requires epidemiological investigation of hospital-acquired infections
STRAIN OF 1907

YOU ARE THE NEXT CLASS OF DRUG-RESISTANT BACTERIA. AS HUMANS CONTINUE TO ABUSE AND OVERUSE ANTIBIOTICS, YOUR RANKS WILL SWELL. SO, GO OUT THERE AND MUTATE! AND REMEMBER: THAT WHICH DOES NOT KILL US MAKES US STRONGER!!
“Hey, I got news for you, sweetheart! ... I am the lowest form of life on earth!”
“Don’t forget to take a handful of our complimentary antibiotics on your way out.”