Chapter 13: Antimicrobials
Chapters 14/15: Bacterial Pathogenesis

Only parts of the listed chapters will be covered; for specific bacterial diseases, you can see relevant parts of your textbook for support but what you need to know is here.

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Antimicrobials

- Origins of antimicrobial therapy in folk medicine
  - Cinchona tree used to treat malaria (quinine)
  - Moldy bread applied to wounds (?penicillin)
  - Foxglove plant for heart disease (digitalis)
  - Willow bark for pain (aspirin-like compound)

- Start of modern era of antimicrobial therapy is 1930’s
  - Sulfonamides in 1935 could cure deadly systemic bacterial infections: a development of significance incomprehensible in our time
    - Limited effectiveness & problems with toxicity
  - Penicillin: Fleming’s mold, purified by Florey & Chain. Became available to military during WWII (1943)
    - Orson Welles’ movie The Third Man

General properties of antimicrobials: Spectrum of Activity

- Each antibiotic is effective against different types of microbes.

  - Broad spectrum antibiotic:
    - Effective against many types of bacteria (such as both Gram + and Gram –)
    - Use when a patient is seriously ill with infection by unknown organism

  - Narrow spectrum antibiotic:
    - Effective against only a few microbes, or only microbes within a single category
    - Use if the infectious agent is known

Indiscriminate use of broad-spectrum antibiotics creates problems:

1. More antibiotic resistance (by natural selection of larger populations)
2. Undesirable effects on normal microflora (see later slides)

General properties of antimicrobials

- Selective toxicity
  - Drug must harm microbes without causing significant damage to the host
  - Easier to do with prokaryotic & microbial pathogens than multicellular eukaryotes (worms, fungi)

- All drugs are toxic at some level
  - Therapeutic index is an indicator of how toxic
    - Ratio of maximum tolerated dose (per kg body weight) to minimum dose that will treat the disease
      - e.g. Patient can take up to 10 mg/kg without suffering intolerable side effects, less than 5 mg/kg doesn’t work: therapeutic index = 2
      - Bigger is better
Therapeutic index & drug toxicity

• “Tolerated” dose is a relative term
  • Would you “tolerate” nausea, vomiting, severe weight loss, exhaustion, and hair loss from
  – Aspirin taken for a headache?
  – Chemotherapy for cancer?

• Some antimicrobials are “better” than others
  • Often, this is related to mechanism of action
  • Antivirals & anti-parasitic drugs are often “worse”

Drug toxicity (“side effects”):
Toxicity vs Allergy

★ Toxicity: unwanted effects of the drug on the host
  1. Direct consequences of the drug’s mode of action (e.g., inhibition of 70S ribosome affecting host mitochondria)
  2. Indirect effects (damage to kidneys, ears, etc. in a way not obviously related to the drug’s activity)

★ Allergy: an immune reaction to the drug
  • Patient’s immune system responds to “foreign” substance and launches an attack
  • The immune attack itself causes damage to the patient (from skin rash to anaphylactic shock)

Antimicrobial toxicity:
★ Disruption of normal microflora

What is the normal microflora?

Some parts of the body are in contact with the outside world.
These areas are colonized by many microbes which normally do not cause disease.
10x more microbial cells on/in your body than human cells!

Normal microflora: Where & what?
The composition of normal microflora is complex.
• Bacteria but also yeast, fungi, arthropods
• Species present varies from individual to individual (for example, about 1/3 of adults carry S. aureus in their nose: lab!)
• Also varies in the same person over time
• Different species adapted to different body environments
• Normal flora is acquired during or soon after birth, from mother or home environment so prevalence of certain species can be associated with cultural groups

Normal microflora: Dominant bacterial types

★ Skin: Gram + cocci (Staphylococci, Streptococci)
★ Digestive & respiratory tracts: oropharynx; large intestine.
  – Gram negative bacilli (Enterobacteriaceae family including huge numbers of Escherichia coli; Klebsiella; Enterobacter)
★ Genital tract: vaginal Lactobacilli

Urine (inside the bladder), blood, cerebrospinal fluid, deep tissues should be sterile (no permanent normal flora)

Importance of the normal flora

★ Most of the normal microflora are commensals (no direct harm or help to the host; they use the host for food & shelter)

★ Some helpful exceptions:
  ★ • Humans rely on the bacteria of the large intestine to produce Vitamin K, essential for blood clotting
  ★ • Ruminant animals (cows, sheep, etc.) cannot digest cellulose, the main carbohydrate in plants. Bacteria in their guts digest it for them.
Importance of the normal flora

- **Unhelpful exceptions:** Normal bacterial residents of the body can cause disease (opportunistic infection) if:
  - **Host is immunocompromised:** normally, the host’s immune system keeps the microbiota from spreading beyond its usual home, or from multiplying excessively.
  - **Microflora is moved:** injury or surgery can give these bacteria access to areas where they shouldn’t be, resulting in disease
    - Burns, deep wounds, intestinal rupture such as after untreated appendicitis

Exclusion of pathogens (microbial antagonism):

- Bacteria of the normal flora occupy space and compete for nutrients with pathogenic (disease-causing) bacteria.
  - Without the normal flora, pathogens would easily multiply in these places.

Antimicrobial toxicity: Disruption of normal microflora

- Broad spectrum antibiotics can wipe out normal microbiota along with disease-causing bacteria
- A relatively empty environment is created
- **Opportunistic infection** follows
  - Infection by an organism that is NOT normally a pathogen
  - Becomes a problem only when unusual “opportunities” arise
- Such infections following antibiotic treatment of another infection are called **superinfections**
  - **Superinfection:** invasion by replacement microflora

Superinfections

- Follow decimation of normal microbiota as a consequence of using broad-spectrum antibiotic
- Are caused by remaining microbiota which were not affected by the antibiotic
  - They multiply to fill the empty ecological niche

Superinfections: examples

1. **Vaginal yeast infection**
   - *Candida albicans*
   - Yeast are resistant to most antibacterials
   - Extermination of the bacterial flora changes the pH and removes competition for the yeast
     - Yeast overgrowth
   - One treatment is to replenish the normal *Lactobacilli* by ingesting or douching with plain, live-culture yogurt

   (Of course not all vaginal yeast infections are a consequence of antibiotic use.)

2. **“C. dif” colitis (pseudomembranous colitis)**
   - *Clostridium difficile*
   - Sporeforming anaerobe that occasionally colonizes the colon (large intestine)
   - Spores survive antibiotic treatment
     - Often broad spectrum antibiotic was given before abdominal surgery specifically to decrease the microbial content of the colon
   - Germinate after normal microbiota disrupted
   - Vegetative *C. difficile* multiply rapidly & release toxins
   - **Severe:** sometimes fatal inflammation & damage to the colon follow
Antimicrobials: Mechanisms

- **Inhibition of Cell Wall Synthesis**
  - Animal cells do not have cell walls
  - Disruption of cell wall synthesis, esp. in Gram + bacteria, can lead to osmotic lysis (in hypotonic body fluids or water)
  - Penicillins & cephalosporins
    - β-lactam ring prevents crosslinking of peptidoglycan
    - Bacteriostatic
      - Prevents growth of bacteria, doesn't kill them outright

- **Disruption of cell membranes**
  - All cells have cell membranes
  - Those of bacteria & fungi differ enough from animal cells that some selective toxicity is possible
  - These antibiotics act as detergents to make membranes leaky
  - Polymyxins & polyenes
    - amphotericin B, antifungal agent for severe systemic infections nicknamed "amphotemible" for its side effects

- **Inhibition of Nucleic Acid Synthesis**
  - All cells synthesize DNA & RNA but..
  - prokaryotic RNA & DNA polymerases are different from eukaryotic enzymes
  - Rifampin: blocks bacterial RNA transcription

- **Antimetabolites**
  - Interfere with metabolism by:
    - Competitive inhibition of enzymes
    - Being erroneously incorporated into important molecules such as nucleic acids
  - Sulfa drugs (sulfanilamide)
    - nucleotide base analogs
**Sulfa drugs: antimetabolites by competitive inhibition**

- **PABA** (para-aminobenzoic acid) is a crucial intermediate in bacterial production of **folic acid**
- Sulfanilamide binds to active site of enzyme that normally acts on PABA
- **Competitive inhibition of folic acid synthesis**

For animals, folic acid is a required dietary nutrient (no enzymes to make it), so cells are unaffected by this competitive inhibition

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**Antibiotic resistance**

- Microbes may be **sensitive** or **resistant** to an antibiotic
- Resistance may be inherent to the species (for example, the organism does not have the molecular target of the drug), or acquired and thus depends on the particular strain / isolate
  - Kirby-Bauer disk diffusion method (Lab!) can be used to measure antibiotic sensitivity for a particular patient’s infection

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**Mechanisms of antibiotic resistance**

- Antibiotic resistance can be **chromosomal**
  - Most likely due to mutations, followed by natural selection of resistant cells when exposed to antibiotic
- or carried on **R plasmids**
  - Antibiotic resistance genes on plasmids can be widely transferred by conjugation, even across species
  - A single R plasmid can carry several different resistance genes, in a single step creating a huge clinical problem
- Many mechanisms by which bacteria can become resistant

Think about it…

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**Mechanisms of antibiotic resistance**

1. **Keep the antibiotic out of the cell** (prevent entry, or actively pump it out)
2. **Destroy the antibiotic** (**β**-lactamases)
3. **Alter the antibiotic’s target**
   - For example, if target is the ribosome, mutate the structure of the ribosome so the antibiotic can’t bind to it
   - If the antibiotic acts by enzymatic inhibition, change the active site so the antibiotic doesn’t bind
4. **Bypass the affected pathway**
   - For example, resistance to sulfa drugs by “learning” to use ready-made folic acid from the environment

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**Relevant pages from text**

- Ch. 13: p. 353-362; p. 368 (C. difficile)
- Ch. 14: p. 389-390 (normal microflora)