I. Antimicrobial chemotherapy (Chapter 13)

A. History

1. **chemotherapeutic agents** = chemical agents used to treat disease
   a. destroy or inhibit growth of pathogens
   b. concentrations low enough not to damage host
   c. include **antibiotics** = microbial products or their derivatives that can kill or inhibit growth of microorganisms

2. Paul Erlich began the modern age of chemotherapy
   a. **magic bullet** = chemical (toxic dye) that would specifically bind to and destroy pathogens
      (1) particularly interested in syphilis and African sleeping sickness
      (2) normal syphilis treatment was ingestion of toxic mercury compounds (often patients died)
      (3) trypanosomiasis treated similarly with arsenic compounds
      (4) deliberately tried preparing organic material containing arsenic which would be less lethal to humans
   b. arsphenamine (Erlich 606) was effective against syphilis (trade name Salversan)

   ![arsphenamine](image)

   c. Gerhard Domagk found that the dye Prontosil Red killed pathogenic staphylococci and streptococci, but didn't harm the animal
      (1) Jacques and Therese Trefouel discovered that Prontosil Red was converted to sulfanilamide, the true active factor
      ![sulfanilamide](image)
      (2) led to development of **sulfa drugs** (sulphonamides)
         (a) specifically designed to stay in gut or be absorbed in bloodstream
         (b) often more active against microbes and less toxic to humans than sulfanilamide
         (c) tremendously effective against pneumonia, puerperal fever (systemic infection due to *Streptococcus pyogenes*)
         (d) general structure similar to PABA

   ![sulfa drugs](image)
i) some microbes require PABA for growth
ii) sulfa drugs are competitive inhibitor; prevent growth and allow body's defense mechanisms to deal with microbes
d. during 40's and 50's, many vitamins discovered and inhibitory analogs made
   (1) worked in test tubes
   (2) too toxic in humans, eliminated too well by kidneys, vitamin concentration in tissues too high, or infecting bacteria did not require vitamin
   (3) not one drug was of practical therapeutic importance
3. penicillin = first antibiotic to be used therapeutically
   a. originally observed by 21-year old French medical student Ernest Duchesne, rediscovered by Alexander Fleming (1928)
   b. Fleming observed a contaminant mold which seemed to be dissolving Staphylococcus colonies
      (1) found that broth from a Penicillium culture could destroy a number of pathogens
      (2) unable to purify the active compound very well
      (3) published some papers and abandoned the area
   c. Howard Florey and Ernst Chain (1939) obtained Penicillium notatum from Fleming
      (1) purified penicillin
      (2) destroyed staph and strep infections in mice
      (3) Fleming, Florey, and Chain shared 1945 Nobel
4. Selman Waksman (1944) discovered streptomycin, an antibiotic produced by the actinomycete Streptomyces griseus (Nobel 1952)
   a. led to increased search for other antibiotic producers
   b. by 1953, microbes producing chloramphenicol, neomycin, terramycin, and tetracycline were isolated
B. Characteristics of antimicrobials
1. selective toxicity = kill or inhibit pathogen without damaging host
   a. therapeutic dose = drug level required for clinical treatment of a particular infection
   b. toxic dose = drug level at which the agent becomes too toxic for host
   c. therapeutic index = ratio of toxic dose to therapeutic dose (larger number = better)
   d. drugs specific for microbial function that doesn't occur in host(e.g., cell wall synthesis) have highest therapeutic index
2. range of effectiveness varies
   a. narrow-spectrum = effective against a limited number
   b. broad-spectrum = effective against many types of pathogens
   c. can be classified based on targeted group
      (1) antibacterial
      (2) antifungal
      (3) antiprotozoan
      (4) antiviral
   d. some agents are effective against more than one group (e.g., sulfa drugs work
against bacteria and some protozoa)

3. chemotherapeutic agents can be **natural**, **synthetic**, or **semi-synthetic**
   - synthetics are
     1. sulfa drugs
     2. trimethoprim
     3. chloramphenicol
     4. isoniazid
     5. dapsone
     6. many antiviral and antiprotozoan drugs
   - semisynthetic are natural antibiotics that have been chemically modified to make them less susceptible to inactivation
     1. ampicillin
     2. carbenicillin
     3. methicillin

4. chemotherapeutic agents can be cidal or static
   - can be concentration dependent
   - effect varies with species
   - static effect relies on host's defense mechanism for elimination of infection

C. General mechanisms of activity

1. pathogen damage can occur through several mechanisms
   - most selective antibiotics interfere with cell wall synthesis
   - high therapeutic index since cell walls not sound in eucaryotes

2. Cell wall synthesis inhibition
   - penicillin, ampicillin, carbenicillin, methicillin, cephalosporins
   - inhibit enzymes for peptidoglycan cross-linking; activate cell wall lytic enzymes
   - bacitracin inhibits CW synthesis by interfering with lipid carrier that transports precursors across the plasma membrane

3. protein synthesis inhibition
   - streptomycin, gentamicin; bind to 30S ribosome subunit and causes misreading of mRNA
   - chloramphenicol binds to 50S ribosomal subunit, inhibits peptidyl transferase, blocking peptide bond formation
   - tetracyclines bind to 30S, interfere with aminoacyl-tRNA binding
   - erythromycin binds to 50S, inhibits peptide chain elongation
   - high therapeutic index because drugs differentiate between procaryotic and eucaryotic ribosomes

4. nucleic acid synthesis inhibition
   - rifampicin
   - inhibits DNA-dependent RNA polymerase, blocking RNA synthesis
   - often toxic to eucaryotic systems also

5. Cell membrane disruption
   - polymyxin B
   - binds to cell membrane, disrupts structure and permeability

6. metabolic antagonism (antimetabolites)
   - sulfa drugs compete with PABA, inhibits folic acid synthesis
b. trimethoprim inhibits dihydrofolate reductase, blocking tetrahydrofolate synthesis  
c. dapsone interferes with folic acid synthesis  
d. isoniazid may disrupt pyridoxal or NAD metabolism and functioning; inhibits synthesis of mycolic acid "cord factor"

7. Several factors determine effectiveness of antimicrobial drugs  
a. drug must reach site of infection, so delivery system important  
   (1) penicillin G unstable in stomach acid  
   (2) gentamicin (aminoglycosides) not well absorbed through gut and must be injected intramuscularly  
   (3) parenteral routes = non-oral administration  
b. concentration must exceed MIC  
   (1) dependent on amount administered,  
   (2) speed of uptake,  
   (3) rate of elimination from body  
   (4) best if drug is absorbed slowly over a long period and excreted slowly  
c. infecting organism  
   (1) dormant bugs less susceptible  
   (2) pathogen must have proper target site  
d. many agents less effective due to resistance mechanisms, spread quickly via plasmids

D. Determining activity levels  
1. dilution susceptibility tests  
   a. a series of broth tubes containing a range of antibiotic concentrations inoculated with test organism  
   b. minimal inhibitory concentration (MIC) = lowest concentration that prevents growth (no growth after 16-20 hr)  
   c. minimal lethal concentration (MLC) = lowest concentration that kills the organism (no growth in subculture)  
   d. cidal drugs usually kills at 2-4x MIC, static drugs kill at much higher concentrations (if at all)  
2. disk diffusion tests  
   a. antibiotic impregnated disks are placed on agar previously inoculated with the test bacterium  
      (1) antibiotic diffuses, forming a gradient  
      (2) resistant organisms grow up to the disk  
      (3) susceptible organisms grow some distance from the disk, displaying a clear zone around the disk  
         (a) wider the clear zone = more susceptible  
         (b) zone width is a function of initial concentration, solubility, diffusion rate, susceptibility of organism  
         (c) zone width cannot be used to compare 2 antibiotics  
   b. Kirby-Bauer most used disk diffusion test  
      (1) Mueller-Hinton agar inoculated with lawn of bacteria  
      (2) disks placed on surface  
      (3) incubation at 35° C for 16-20 hr
(4) diameters of zones measured and compared to tabulated values to determine degree of microbial resistance
  (a) plot MIC vs zone diameters for different strains
  (b) determine from plot if treatment dosage would result in MIC

E. Mechanisms of drug resistance
1. drug cannot enter cell
   a. G- unaffected by penicillin G because it can't penetrate the outer membrane
   b. changes in binding proteins render cells resistant
2. chemical modification
   a. penicillinase hydrolyzes the β-lactam ring
   b. groups can be added which inactivate drugs
3. modification of target
   a. changes in 23S rRNA protects against chloramphenicol or erythromycin
   b. change binding site for sulfanilamide
4. genes for drug resistance can be chromosomal or on plasmids
   a. spontaneous mutations in chromosome are rare
   b. chromosomal changes usually result in changes in drug receptors, preventing binding
   c. R plasmids (resistance plasmids) often code for enzymes that destroy or modify drugs
      (1) implicated in resistance to aminoglycosides, penicillins, cephalosporans, erythromycin, tetracyclines, sulfonamides, chloramphenicol, and others
      (2) plasmids transferred rapidly through populations
      (3) single plasmid can carry resistance to many drugs
5. Overuse of antibiotics has led to many resistant strains
   a. increase drug concentrations to destroy susceptible and spontaneous mutants
   b. use two drugs together
   c. limit use, especially broad-spectrum antibiotics

F. Classes of antibiotics
1. sulfa drugs
   a. structural analog (similar to metabolic intermediate)
   b. similar to PABA, necessary for synthesis of folic acid
2. quinolones
   a. synthetic drug, broad spectrum, bactericidal
   b. inhibits DNA replication and repair, transcription
   c. nalidixic acid, fluoroquinolones (ciprofloxacin, norfoxacin, ofloxacin)
3. penicillins
   a. β-lactam ring is common feature (side chains vary)
   b. penicillinase destroys ring
   c. block peptidoglycan cross-linking, leading to lysis
   d. many people are allergic
4. cephalosporins
   a. originally isolated from *Cephalosporium* (fungus)
   b. β-lactam ring, like penicillins
   c. useful for people allergic to penicillin
d. broad spectrum
5. tetracyclines
   a. naturally produced by *Streptomyces* or semi-synthetic
   b. bind to 30S ribosomal subunit, inhibiting protein synthesis
   c. bacteriostatic
   d. broad spectrum
6. aminoglycoside antibiotics
   a. *Streptomyces* make streptomycin, kanamycin, tobramycin
   b. *Micromonospora purpurea* synthesizes gentamicin
   c. bind to small ribosomal subunit, inhibiting protein synthesis
   d. bactericidal, most effective against gram negatives
   e. quite toxic to humans
   f. widespread resistance
7. erythromycin
   a. macrolide, synthesized by *Streptomyces erythraeus*
   b. broad spectrum, bacteriostatic, most effective against G+
   c. bind to 23S rRNA of 50S ribosomal subunit, inhibiting protein elongation
   d. macrolides have 12- to 22-carbon lactone rings
8. chloramphenicol
   a. synthetic, but originally from *Streptomyces venezuelae*
   b. acts like erythromycin
   c. broad spectrum, bacteriostatic
   d. quite toxic to humans

G. Antifungal drugs
   1. eukaryotic, so drugs often toxic to humans
   2. most fungi have efficient detoxification mechanisms
   3. often target membrane sterols or cell walls

H. Antiviral drugs
   1. most drugs disrupt critical stages in virus life cycle or synthesis of viral-specific nucleic acids
   2. difficult to use drug therapy, since viruses use host's cell machinery