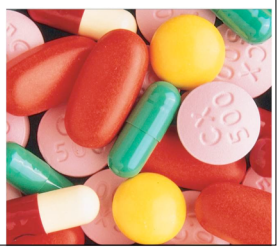
Chapter 35

Antimicrobial Chemotherapy

Antibiotics

- ✓ Substances produced by microorganisms
- ✓ Active in small quantities
- ✓ act on the metabolism of cells

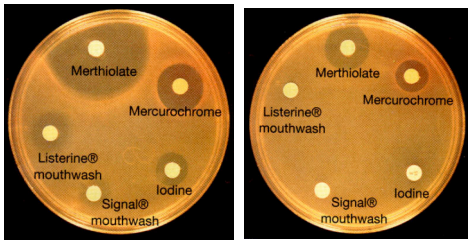


Measuring Antimicrobial Activity

- ✓ Tube dilution method
- ✓ Disk-plate method

Disk-plate method

Staphylococcus aureus *Escherichia coli*



Chemotherapeutic agents

- ✓ chemical agents used to treat disease
- ✓ destroy pathogenic microbes or inhibit their growth within host
- ✓ most are antibiotics
  - ✒ microbial products or their derivatives that kill susceptible microbes or inhibit their growth

The Development of Chemotherapy

- ✓ Paul Ehrlich (1904)
  - ✒ developed concept of selective toxicity
  - ✒ identified dyes that effectively treated African sleeping sickness
- ✓ Sahachiro Hato (1910)
  - ✒ working with Ehrlich, identified arsenic compounds that effectively treated syphilis (salvarsan)
- ✓ Gerhard Domagk, and Jacques and Therese Trefouel (1935)
  - ✒ discovered sulfonamides and sulfa drugs (Prontosil Red)

Penicillin

- ✓ first discovered by Ernest Duchesne (1896), but discovery was forgotten
- ✓ accidentally discovered by Alexander Fleming (1928)
  - ✒ observed penicillin activity on contaminated plate
  - ✒ did not think could be developed further
- ✓ effectiveness demonstrated by Florey, Chain, and Heatley (1939)

Later discoveries

- ✓ streptomycin discovered by Selman Waksman (1944)
- ✓ by 1953 chloramphenicol, terramycin, neomycin, and tetracycline isolated

General Characterisitics of Antimicrobial Drugs

- ✓ selective toxicity
  - ✒ ability of drug to kill or inhibit pathogen while damaging host as little as possible
- ✓ therapeutic dose
  - ✒ drug level required for clinical treatment
- ✓ toxic dose
  - ✒ drug level at which drug becomes too toxic for patient (i.e., produces side effects)
- ✓ therapeutic index
  - ✒ ratio of toxic dose to therapeutic dose

cidal - kills **static** – inhibits growth

Table 35.1 Properties of Some Common Antibacterial Drugs

Drug	Primary Effect	Spectrum	Side Effects*
Ampicillin	Cidal	Broad (gram +, some –)	Allergic responses (diarrhea, anemia)
Bacitracin	Cidal	Narrow (gram +)	Renal injury if injected
Carbenicillin	Cidal	Broad (gram +, many –)	Allergic responses (nausea, anemia)
Cephalosporins	Cidal	Broad (gram +, some –)	(Allergic responses, thrombophlebitis, renal injury)
Chloramphenicol	Static	Broad (gram +, –; rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions
Ciprofloxacin	Cidal	Broad (gram +, –)	Gastrointestinal upset, allergic responses
Clindamycin	Static	Narrow (gram +, anaerobes)	Diarrhea
Dapsone	Static	Narrow (mycobacteria)	(Anemia, allergic responses)
Erythromycin	Static	Narrow (gram +, mycoplasma)	(Gastrointestinal upset, hepatic injury)
Gentamicin	Cidal	Narrow (gram –)	(Allergic responses, nausea, loss of hearing, renal damage)
Isoniazid	Static or cidal	Narrow (mycobacteria)	(Allergic reactions, gastrointestinal upset, hepatic injury)
Methicillin	Cidal	Narrow (gram +)	Allergic responses (renal toxicity, anemia)
Penicillin	Cidal	Narrow (gram +)	Allergic responses (nausea, anemia)
Polymyxin B	Cidal	Narrow (gram –)	(Renal damage, neurotoxic reactions)
Rifampin	Static	Broad (gram +, mycobacteria)	(Hepatic injury, nausea, allergic responses)
Streptomycin	Cidal	Broad (gram +, –; mycobacteria)	(Allergic responses, nausea, loss of hearing, renal damage)
Sulfonamides	Static	Broad (gram +, –)	Allergic responses (renal and hepatic injury, anemia)
Tetracyclines	Static	Broad (gram +, –; rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration (renal and hepatic injury)
Trimethoprim	Cidal	Broad (gram +, –)	(Allergic responses, rash, nausea, leukopenia)
Vancomycin	Cidal	Narrow (gram +)	Hypotension, neutropenia, kidney damage, allergic reactions

*Occasional side effects are in parentheses. Other side effects not listed may also arise.

broad-spectrum drugs – attack many different pathogens

narrow-spectrum drugs – attack only a few different pathogens

Table 35.2 Microbial Sources of Some Antibiotics

Microorganism	Antibiotic
Bacteria	
Streptomyces spp.	Amphotericin B
	Chloramphenicol (also synthetic)
	Erythromycin
	Kanamycin
	Neomycin
	Nystatin
	Rifampin
	Streptomycin
	Tetracyclines
	Vancomycin
Micromonospora spp.	Gentamicin
Bacillus spp.	Bacitracin
	Polymyxins
Fungi	
Penicillium spp.	Griseofulvin
	Penicillin
Cephalosporium spp.	Cephalosporins

Determining the Level of Antimicrobial Activity

- ✓ effectiveness expressed in two ways
  - ✒ minimal inhibitory concentration (MIC)
    - ⇨ lowest concentration of drug that inhibits growth of pathogen
  - ✒ minimal lethal concentration (MLC)
    - ⇨ lowest concentration of drug that kills pathogen
- ✓ two techniques are routinely used to determine MIC and MLC

Dilution Susceptibility Tests

- ✓ involves inoculating media containing different concentrations of drug
  - ✒ broth or agar with lowest concentration showing no growth is MIC
  - ✒ if broth used, tubes showing no growth can be subcultured into drug-free medium
    - ☞ broth from which microbe can't be recovered is MLC

Disk Diffusion Tests

- ✓ disks impregnated with specific drugs are placed on agar plates inoculated with test microbe
- ✓ drug diffuses from disk into agar, establishing concentration gradient
- ✓ observe clear zones (no growth) around disks

Kirby-Bauer method

- ✓ standardized method for carrying out disk diffusion test
- ✓ sensitivity and resistance determined using tables that relate zone diameter to degree of microbial resistance
- ✓ table values plotted and used to determine if concentration of drug reached in body will be effective

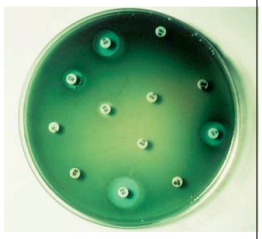
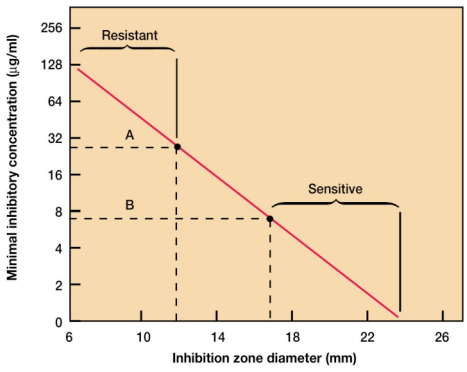


Table 35.3 Inhibition Zone Diameter of Selected Chemotherapeutic Drugs

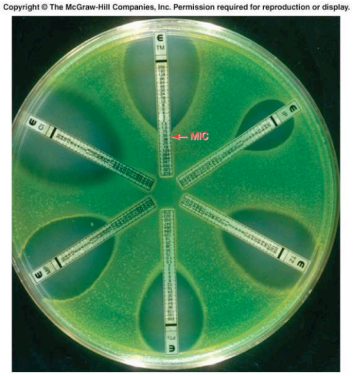
		Zone Diameter (Nearest mm)		
Chemotherapeutic Drug	**Disk Content**	**Resistant**	**Intermediate**	**Susceptible**
Carbenicillin (with *Proteus* spp. and *E. coli*)	100 µg	≤17	18–22	≥23
Carbenicillin (with *Pseudomonas aeruginosa*)	100 µg	≤13	14–16	≥17
Ceftriaxone	30 µg	≤13	14–20	≥21
Chloramphenicol	30 µg	≤12	13–17	≥18
Erythromycin	15 µg	≤13	14–17	≥18
Penicillin G (with staphylococci)	10 U[a]	≤20	21–28	≥29
Penicillin G (with other microorganisms)	10 U	≤11	12–21	≥22
Streptomycin	10 µg	≤11	12–14	≥15
Sulfonamides	250 or 300 µg	≤12	13–16	≥17
Tetracycline	30 µg	≤14	15–18	≥19

[a]One milligram of penicillin G sodium = 1,600 units.



The E test

- ✓ convenient for use with anaerobic pathogens
- ✓ similar to disk diffusion method, but uses strip rather than disk
- ✓ E-test strips contain a gradient of an antibiotic
- ✓ intersection of elliptical zone of inhibition with strip indicates MIC



Measurement of Drug Concentrations in the Blood

- ✓ concentration of drug at infection site must be > MIC to be effective
- ✓ microbiological, chemical, immunological, enzymatic, or chromatographic assays can be used to determine concentration of drug in blood

Mechanism of Action of Antimicrobial Agents

- ✓ can impact pathogen by targeting some function necessary for its reproduction or survival
- ✓ targeted function is very specific to pathogen → higher therapeutic index

have high therapeutic index

Table 35.4 Mechanisms of Antibacterial Drug Action

Drug	Mechanism of Action
Cell Wall Synthesis Inhibition	
Penicillin Ampicillin Carbenicillin Methicillin Cephalosporins	Inhibit transpeptidation enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall peptidoglycan. Activate cell wall lytic enzymes.
Vancomycin	Binds directly to the D-Ala-D-Ala terminus and inhibits transpeptidation.
Bacitracin	Inhibits cell wall synthesis by interfering with action of the lipid carrier that transports wall precursors across the plasma membrane.
Protein Synthesis Inhibition	
Streptomycin Gentamicin	Binds with the 30S subunit of the bacterial ribosome to inhibit protein synthesis and causes misreading of mRNA.
Chloramphenicol	Binds to the 50S ribosomal subunit and blocks peptide bond formation through inhibition of peptidyl transferase.
Tetracyclines	Bind to the 30S ribosomal subunit and interfere with aminoacyl-tRNA binding.
Erythromycin and clindamycin	Bind to the 50S ribosomal subunit and inhibit peptide chain elongation.
Fusidic acid	Binds to EF-G and blocks translocation.
Nucleic Acid Synthesis Inhibition	
Ciprofloxacin and other quinolones	Inhibit bacterial DNA gyrase and thus interfere with DNA replication, transcription, and other activities involving DNA.
Rifampin	Blocks RNA synthesis by binding to and inhibiting the DNA-dependent RNA polymerase.
Cell Membrane Disruption	
Polymyxin B	Binds to the plasma membrane and disrupts its structure and permeability properties.
Metabolic Antagonism	
Sulfonamides	Inhibit folic acid synthesis by competition with *p*-aminobenzoic acid.
Trimethoprim	Blocks tetrahydrofolate synthesis through inhibition of the enzyme dihydrofolate reductase.
Dapsone	Interferes with folic acid synthesis.
Isoniazid	May disrupt pyridoxal or NAD metabolism and functioning. Inhibits the synthesis of the mycolic acid "cord factor."

antimetabolites

Factors Influencing the Effectiveness of Antimicrobial Drugs

- ✓ ability of drug to reach site of infection
- ✓ susceptibility of pathogen to drug
- ✓ ability of drug to reach concentrations in body that exceed MIC of pathogen

Ability of drug to reach site of infection

✓ depends in part on mode of administration
- ✒oral
  - ☞some drugs destroyed by stomach acid
- ✒topical
- ✒parenteral routes
  - ☞Non-oral routes of administration

✓ drug can be excluded by blood clots or necrotic tissue

Factors influencing ability of drug to reach concentrations exceeding MIC

✓ amount administered
✓ route of administration
✓ speed of uptake
✓ rate of clearance (elimination) from body

Antibacterial Drugs

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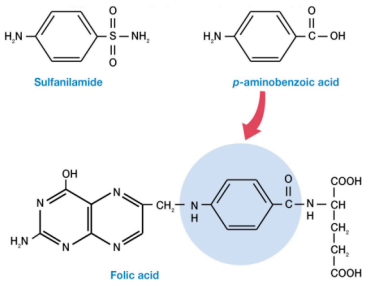
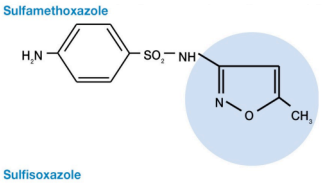
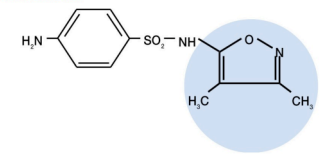
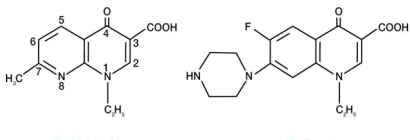
Sulfonamides or Sulfa Drugs

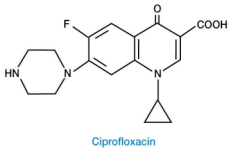




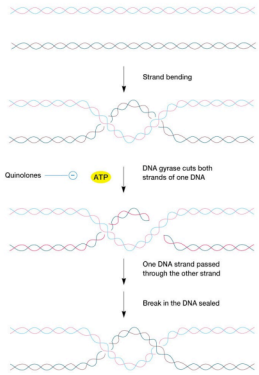


Quinolones



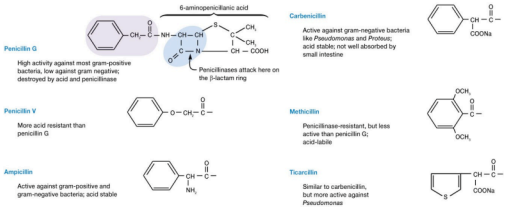


inhibit DNA gyrase activity



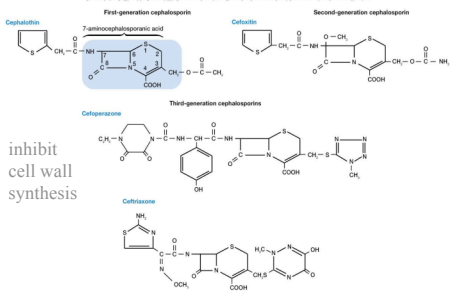
Penicillins

inhibit peptidoglycan synthesis



penicillinase-enzyme produced by penicillin-resistant bacteria

Cephalosporins

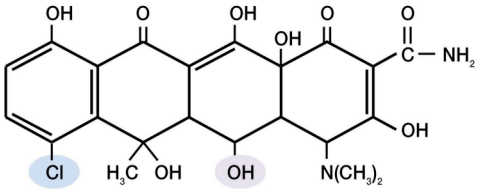


inhibit cell wall synthesis

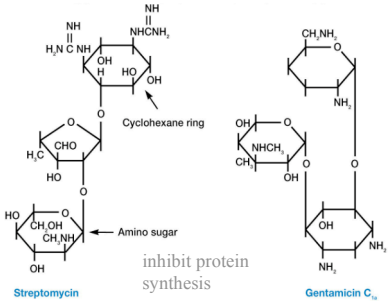
The Tetracyclines

inhibit protein synthesis

Tetracycline (chlortetracycline, doxycycline)



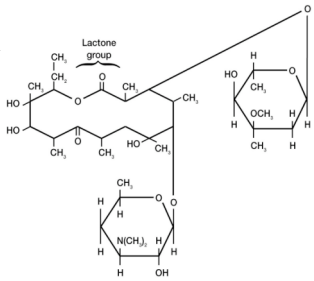
Aminoglycoside Antibiotics



inhibit protein synthesis

Erythromycin and Other Macrolides

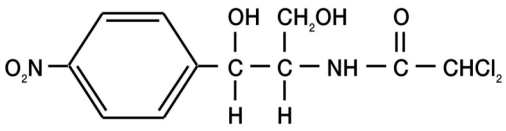
inhibit protein synthesis



Vancomycin and Teicoplanin

- ✓ glycopeptide antibiotics
- ✓ inhibit cell wall synthesis
- ✓ vancomycin has been important for treatment of antibiotic resistant staphylococcal and enterococcal infections
  - ✒ vancomycin resistance on rise

Chloramphenicol



inhibits protein synthesis

Drug Resistance

- ✓ an increasing problem
- ✓ once resistance originates in a population it can be transmitted to other bacteria

Mechanisms of Drug Resistance

- ✓ exclusion of drug
  - ✒ drug can't bind to or penetrate pathogen
- ✓ pump drug out
- ✓ inactivation of drug
  - ✒ chemical modification of drug by pathogen
- ✓ alteration of target enzyme or organelle
- ✓ use of alternative pathways or increased production of target metabolite

The Origin and Transmission of Drug Resistance

- ✓ resistance genes can be chromosomal or on plasmids
  - ✒ small DNA molecules that can exist separate from chromosome or integrated into it

Origin and spread of resistance genes

- ✓ chromosomal genes
  - ✒ mutations, usually of drug target
- ✓ R plasmids
  - ✒ resistance plasmids
  - ✒ can be transferred to other cells by conjugation, transduction, and transformation
  - ✒ can carry multiple resistance genes

Origin and spread…

- ✓ transposons
  - ✒ integron-genetic element with a site into which genes can be inserted
- ✓ gene cassettes
  - ✒ sets of resistance genes
  - ✒ can exist as separate genetic elements
  - ✒ can be part of transposon, integron or chromosome

Superinfection

- ✓ development and spread of drug-resistant pathogens
  - ✒ caused by drug treatment, which destroys drug sensitive strains
- ✓ e.g., pseudomembranous enterocolitis
  - ✒ caused when treatment with certain antibiotics kills intestinal flora, leaving *Clostridium difficile* to flourish and produce a toxin

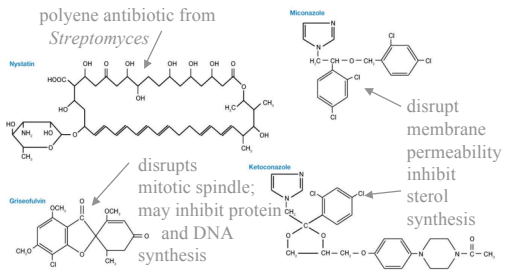
Preventing emergence of drug resistance

- ✓ give drug in high concentrations
- ✓ give two or more drugs at same time
- ✓ use drugs only when necessary
- ✓ possible future solutions
  - ✒ continued development of new drugs
  - ✒ use of bacteriophages to treat bacterial disease

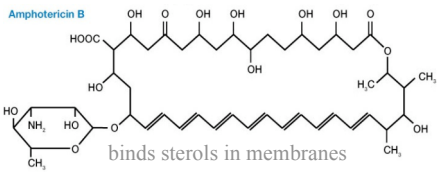
Antifungal Drugs

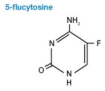
- ✓ fewer effective agents because of similarity of fungal cells and human cells
- ✓ easier to treat superficial mycoses than systemic infections

Treating superficial mycoses



Treating systemic infections





disrupts RNA function

Antiviral Drugs

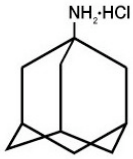
✓ relatively few because difficult to specifically target viral replication

Amantadine

✓ used to prevent influenza infections

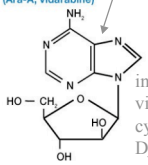
✓ blocks penetration and uncoating of influenza virus

Amantadine

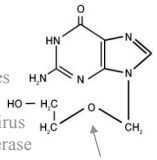


inhibits herpes virus enzymes involved in DNA and RNA synthesis and function

Adenine arabinoside (Ara-A, vidarabine)

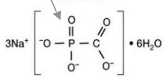


Acyclovir



inhibits herpes virus and cytomegalovirus DNA polymerase

Foscarnet

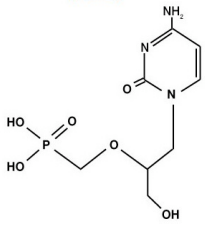


inhibits herpes virus DNA polymerase

Broad-spectrum anti-DNA virus drugs

inhibits viral DNA polymerase

Cidofovir (HPMPC)



papovaviruses, adenoviruses, herpesviruses iridoviruses, and poxviruses

Anti-HIV drugs

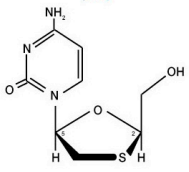
✓ two main targets

- ✒ HIV reverse transcriptase
- ✒ HIV protease

Reverse transcriptase inhibitors

Azidothymidine (AZT) or zidovudine

Lamivudine (3TC)



Protease inhibitors

Ritonavir