9

Antimicrobial Chemotherapy

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(سريعة التأثر) microbes or inhibit their growth

Penicillin

- First discovered by Ernest Duchesne (1896), but discovery lost
- 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)
- Fleming, Florey, and Chain received Nobel Prize in 1945 for discovery and production of penicillin

Christine L. Case, Skyline College

Later Discoveries

- Streptomycin, an antibiotic active against tuberculosis(مرض السل), was discovered by Selman Waksman (1944)
  - Nobel Prize was awarded to Waksman in 1952 for this discovery
- By 1953 chloramphenicol, terramycin, neomycin, and tetracycline isolated

General Characteristics 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

General Characteristics of Antimicrobial Drugs…

- Side effects – undesirable effects of drugs on host cells
- Narrow-spectrum drugs – attack only a few different pathogens
- Broad-spectrum drugs – attack many different pathogens
- Cidal agent - kills microbes
- Static agent - inhibits growth of microbes

Table 9.1 Properties of Some Common Antibacterial Drugs

Antibiotic Group	Primary Effect	Mechanism of Action	Members	Spectrum	Common Side Effects
Cell Wall Synthesis Inhibition					
Penicillins	Cidal	Inhibit transpeptidation enzymes involved in cross-linking the polysaccharide chains of peptidoglycan Activate cell wall lytic enzymes	Penicillin G, penicillin V, methicillin Ampicillin, carbenicillin	Narrow (Gram-positive) Broad (Gram-positive, some Gram-negative)	Allergic reactions (diarrhea, anemia, hives, nausea, renal toxicity)
Cephalosporins	Cidal	Same as above	Cephalothin, cefoxitin, cefaperazone, ceftriaxone	Broad (Gram-positive, some Gram-negative)	Allergic reactions, thrombophlebitis, renal injury
Vancomycin	Cidal	Prevents transpeptidation of peptidoglycan subunits by binding to D-Ala-D-Ala amino acids at the end of peptide side chains. Thus it has a different binding site than that of the penicillins.	Vancomycin	Narrow (Gram-positive)	Ototoxic (tinnitus and deafness), nephrotoxic, allergic reactions
Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly inhibiting synthesis and causing misreading of mRNA	Neomycin, kanamycin, gentamicin Streptomycin	Broad (Gram-negative, mycobacteria) Narrow (aerobic Gram-negative)	Ototoxic, renal damage, loss of balance, nausea, allergic reactions
Tetracyclines	Static	Same as aminoglycosides	Oxytetracycline, chlortetracycline	Broad (including rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration, renal and hepatic injury
Macrolides	Static	Bind to 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin, clindamycin	Broad (aerobic and anaerobic Gram-positive, some Gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic reactions
Chloramphenicol	Static	Same as macrolides	Chloramphenicol	Broad (Gram-positive and -negative, rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions
Nucleic Acid Synthesis Inhibition					
Quinolones and Fluoroquinolones	Cidal	Inhibit DNA gyrase and topoisomerase II, thereby blocking DNA replication	Norfloxacin, ciprofloxacin, Levofloxacin	Narrow (Gram-negatives better than Gram-positives) Broad spectrum	Tendonitis, headache, light-headedness, convulsions, allergic reactions
Rifampin	Cidal	Inhibits bacterial DNA-dependent RNA polymerase	R-Cin, rifacilin, rifamycin, rimactane, rimpin, siticox	*Mycobacterium* infections and some Gram-negatives (e.g., *Neisseria meningitidis* and *Haemophilus influenzae* b)	Nausea, vomiting, diarrhea, fatigue, anemia, drowsiness, headache, mouth ulceration, liver damage

Continued

Table 9.1 Properties of Some Common Antibacterial Drugs (*continued*)

Cell Membrane Disruption					
Polymyxin B	Cidal	Binds to plasma membrane and disrupts its structure and permeability properties	Polymyxin B, polymyxin topical ointment	Narrow—mycobacterial infections, principally leprosy	Can cause severe kidney damage, drowsiness, dizziness
Antimetabolites					
Sulfonamides	Static	Inhibit folic acid synthesis by competing with *p*-aminobenzoic acid (PABA)	Silver sulfadiazine, sulfamethoxazole, sulfanilamide, sulfasalazine	Broad spectrum	Nausea, vomiting, and diarrhea; hypersensitivity reactions such as rashes, photosensitivity
Trimethoprim	Static	Blocks folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase	Trimethoprim (in combination with a sulfamethoxazole)	Broad spectrum	Same as sulfonamides but less frequent
Dapsone	Static	Thought to interfere with folic acid synthesis	Dapsone	Narrow—mycobacterial infections, principally leprosy	Back, leg, or stomach pains; discolored fingernails, lips, or skin; breathing difficulties, fever, loss of appetite, skin rash, fatigue
Isoniazid	Cidal if bacteria are actively growing, static if bacteria are dormant	Exact mechanism is unclear but thought to inhibit lipid synthesis (especially mycolic acid); putative enoyl-reductase inhibitor	Isoniazid	Narrow—mycobacterial infections, principally tuberculosis	Nausea, vomiting, liver damage, seizures, "pins and needles" in extremities (peripheral neuropathy)

General Characteristics of Antimicrobial Drugs…

- Effect of an agent may vary
  - with concentration, microbe, host
- 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

9.3 Determining the level of antimicrobial activity

1. Explain how to determine the level of antibacterial drug activity using the dilution susceptibility test, the disk diffusion test, and the Etest
2. Predict antimicrobial drug levels *in vivo* from *in vitro* data

Determining the Level of Antimicrobial Activity

- Dilution susceptibility tests for MIC
- Disk diffusion tests – Kirby Bauer
- The E-test MIC and diffusion

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

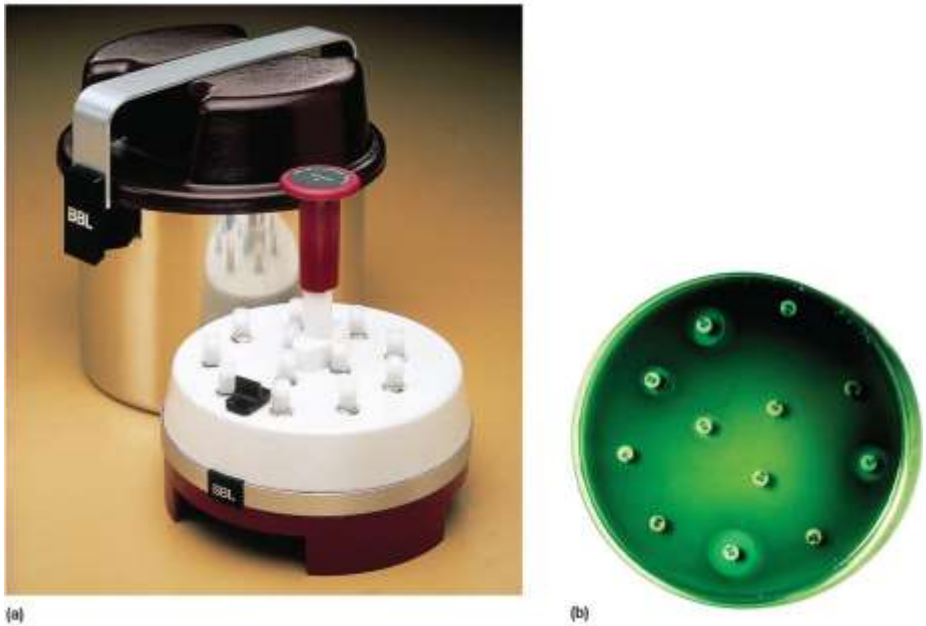
Table 9.2 Inhibition Zone Diameter of Selected Chemotherapeutic Drugs

Chemotherapeutic Drug	Disk Content	ZONE DIAMETER (NEAREST MM)		
		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
Erythromycin	15 µg	≤13	14–17	≥18
Penicillin G (with staphylococci)	10 U[1]	≤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

1 One milligram of penicillin G sodium = 1,600 units (U).

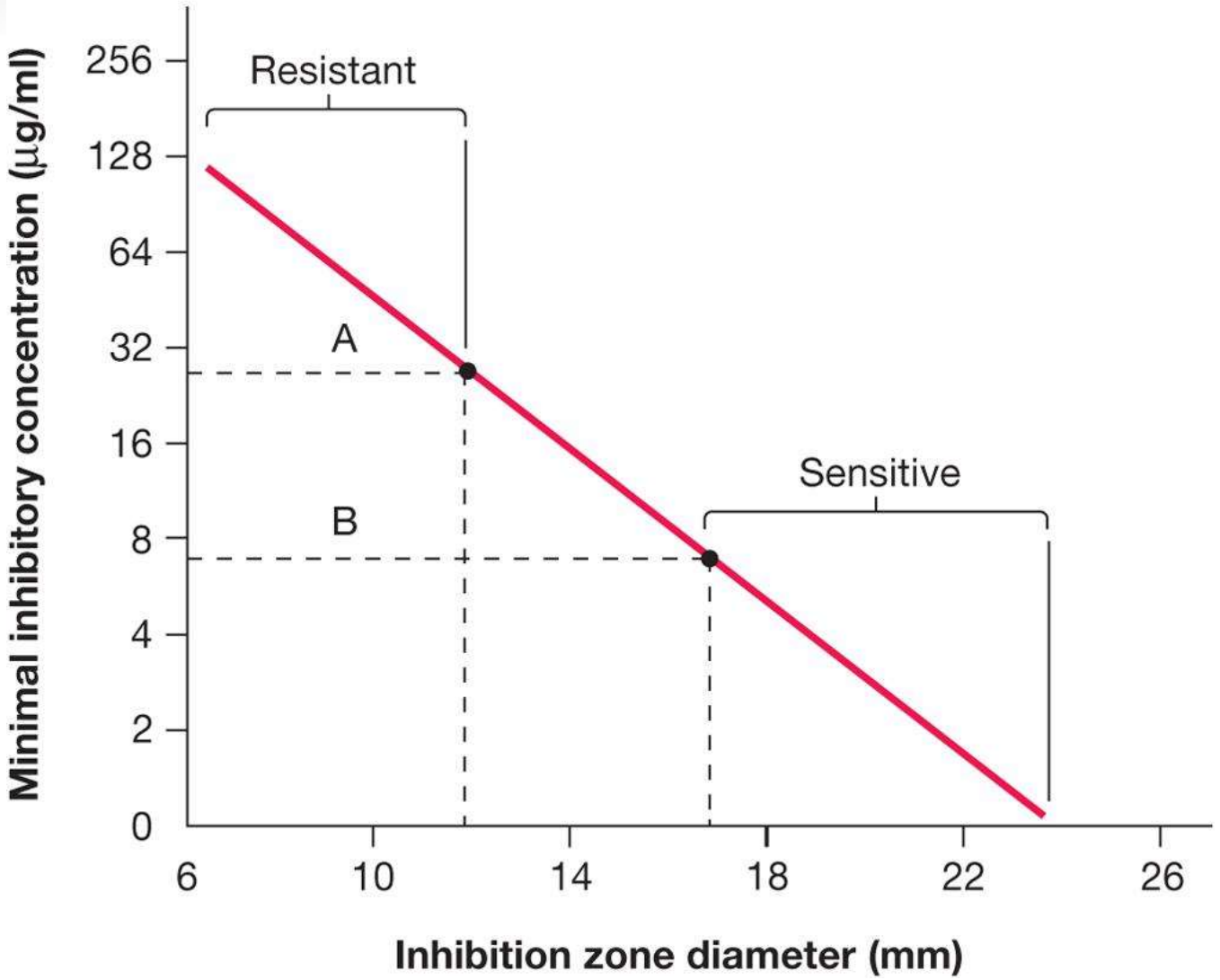
Kirby-Bauer Method

- Standardized method for disk diffusion test
- Sensitivity/resistance determined using tables relating zone diameter with microbial resistance
- Table values plotted, used to determine if effective concentration of drug in body can be reached



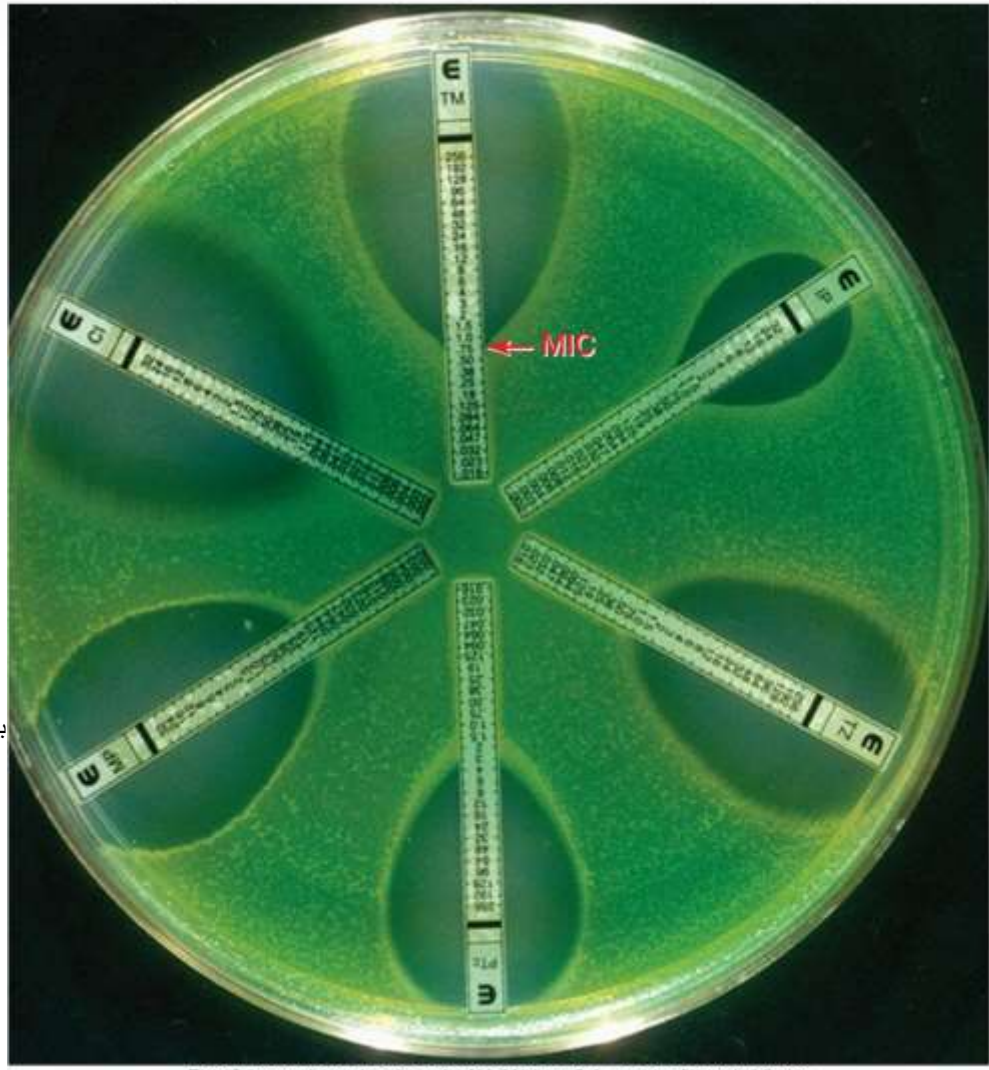
(a) (b)

a: Courtesy and © Becton, Dickinson and Company. b: © Laurtz Jensen/Visuals Unlimited



The E Test

- Convenient(مناسب) 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(تداخل) of elliptical(بيضاوي الشكل) zone of inhibition with strip indicates MIC



9.4 Antibacterial drugs

1. Compare antibacterial drug mechanisms of action
2. Relate side effect toxicity of antibacterial drugs to mechanism of action
3. Explain the relative effectiveness of various antibacterial agents based on drug target

Antimicrobial Drugs

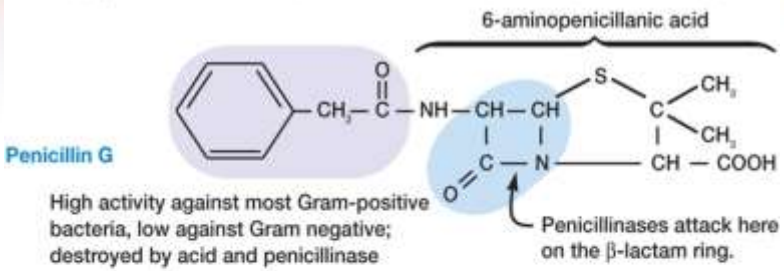
- Inhibitors of cell wall synthesis
- Protein synthesis inhibitors
- Metabolic antagonists
- Nucleic acid synthesis inhibition

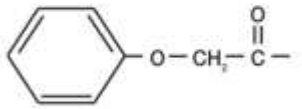
Inhibitors of Cell Wall Synthesis

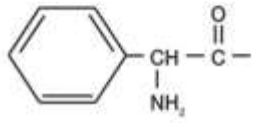
- Penicillins
  - most are 6-aminopenicillanic acid derivatives and differ in side chain attached to amino group
  - most crucial(مهمة) feature of molecule is the β-lactam ring
    - essential for bioactivity
    - many penicillin resistant organisms produce β-lactamase (penicillinase) which hydrolyzes a bond in this ring

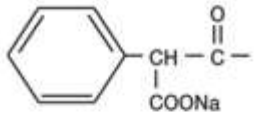
Penicillins…

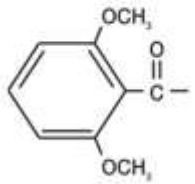
- Mode of action
  - blocks the enzyme that catalyzes transpeptidation (formation of cross-links in peptidoglycan)
  - prevents the synthesis of complete cell walls leading to lysis of cell
  - acts only on growing bacteria that are synthesizing new peptidoglycan

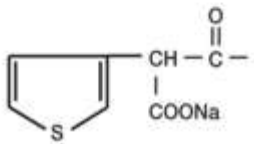












Other Actions of Penicillins

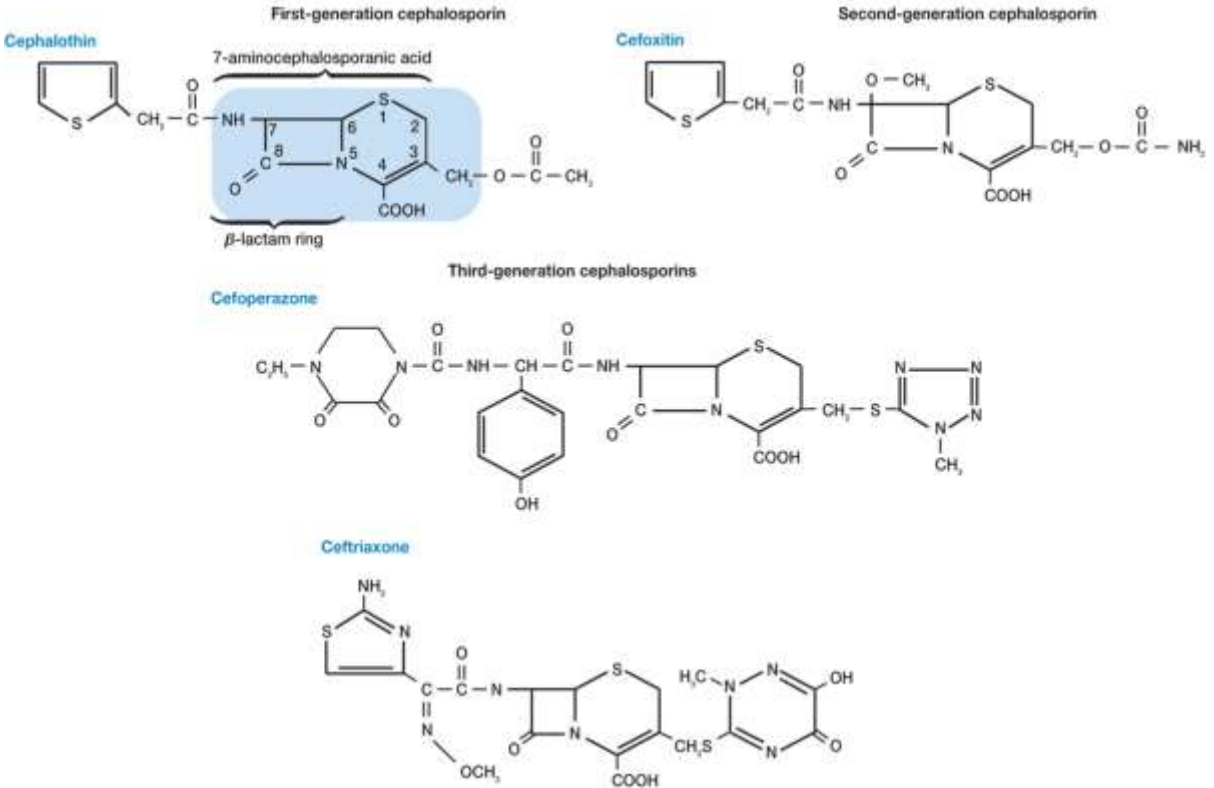
- Binds to periplasmic proteins (penicillin-binding proteins, PBPs)
- May activate bacterial autolysins and murein hydrolases
- Stimulate bacterial holins to form holes or lesions in the plasma membrane

Penicillins…

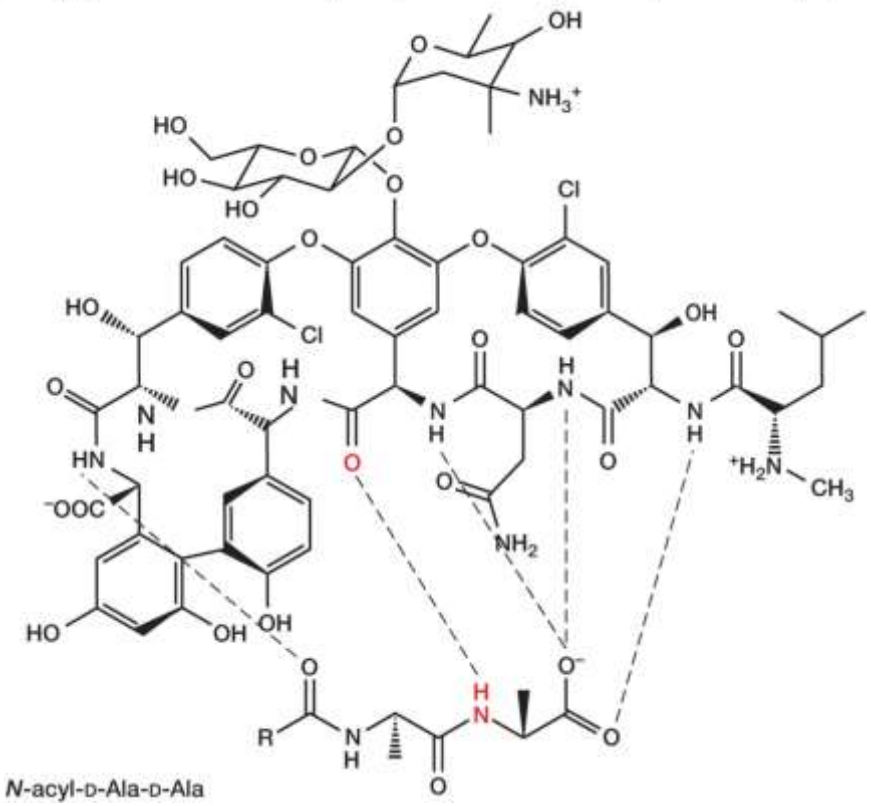
- Naturally occurring penicillins
  - penicillin V and G are narrow spectrum
- Semisynthetic penicillins have a broader spectrum than naturally occurring ones
- Resistance to penicillins, including the semisynthetic analogs, continues to be a problem
- ~1–5% of adults in U.S. are allergic to penicillin
  - allergy can lead to a violent allergic response and death

Cephalosporins

- Structurally and functionally similar to penicillins
- Broad-spectrum antibiotics that can be used by most patients that are allergic to penicillin
- Four categories based on their spectrum of activity



Vancomycin and Teicoplanin

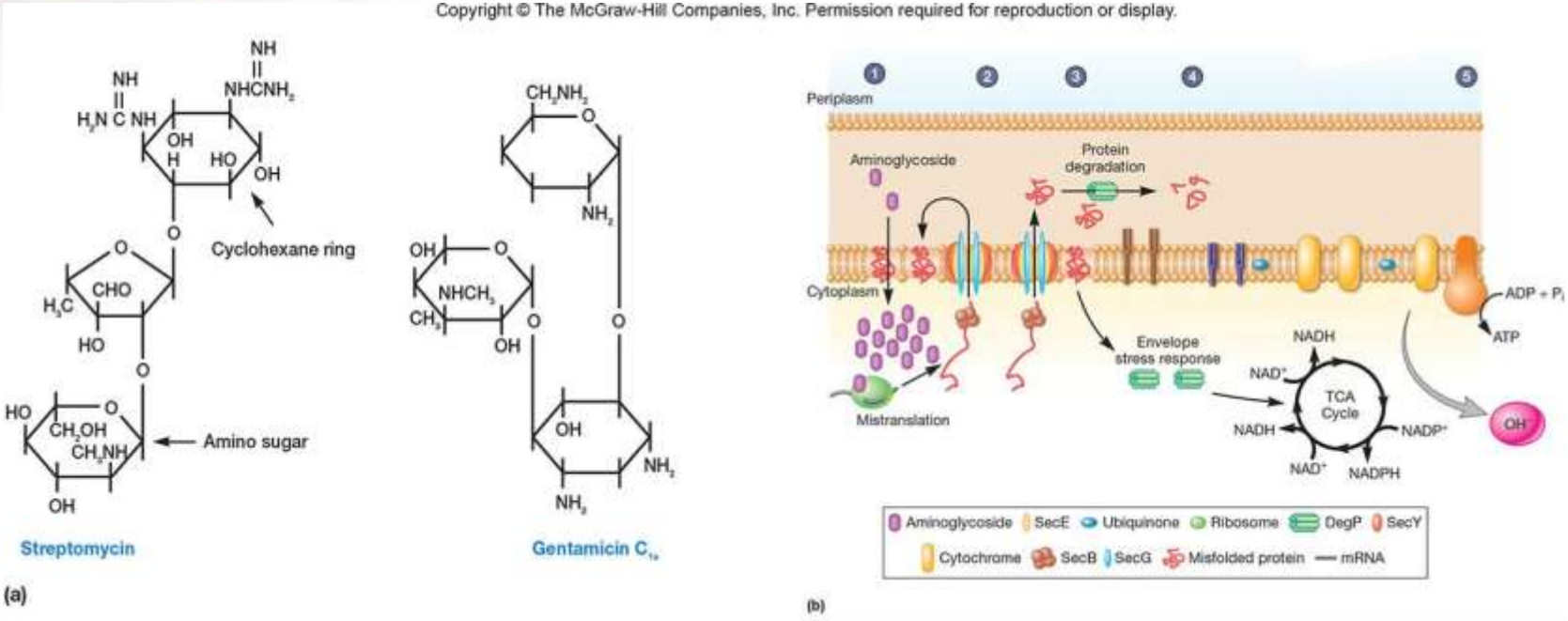


- Glycopeptide antibiotics
- Inhibit cell wall synthesis
- Vancomycin - important for treatment of antibiotic-resistant staphylococcal and enterococcal infections
  - previously considered “drug of last resort” so rise in resistance to vancomycin is of great concern(كبير/قلق عظيم)

Protein Synthesis Inhibitors

- Many antibiotics bind specifically to the bacterial ribosome
  - binding can be to 30S (small) or 50S (large) ribosomal subunit
- Other antibiotics inhibit a step in protein synthesis
  - aminoacyl-tRNA binding
  - peptide bond formation
  - mRNA reading
  - translocation

Aminoglycoside Antibiotics

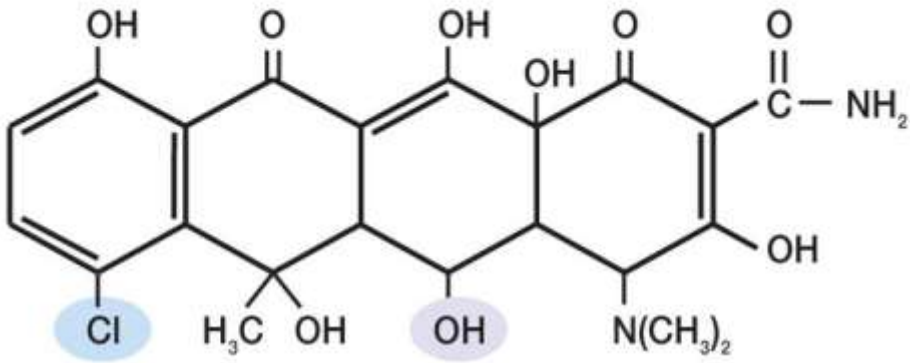


- Large group, all with a cyclohexane ring, amino sugars
- Bind to 30S ribosomal subunit, interfere with protein synthesis by directly inhibiting the process and by causing misreading of the messenger RNA
- Resistance and toxicity

Tetracyclines

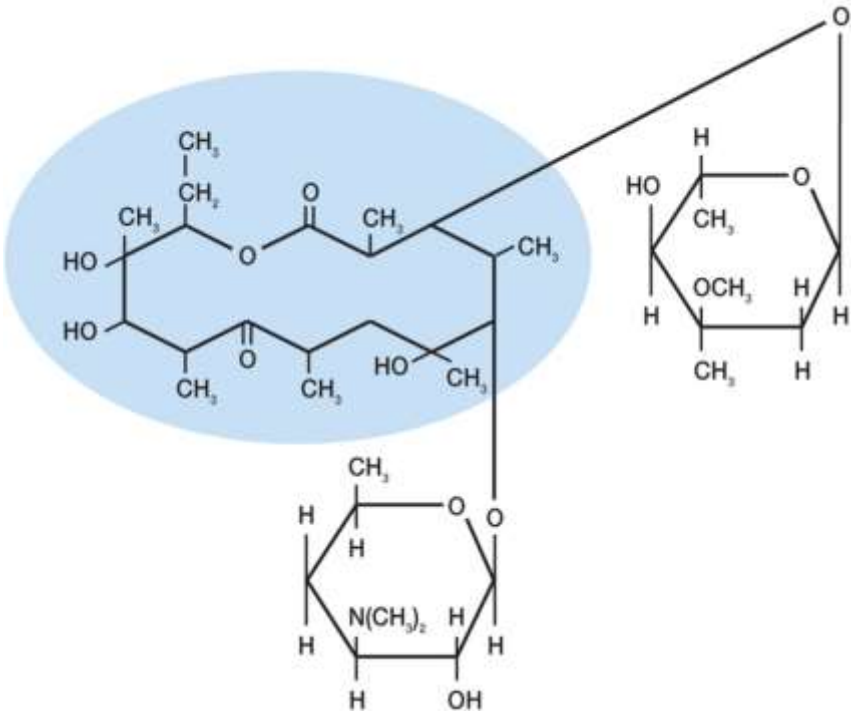
- All have a four-ring structure to which a variety of side chains are attached
- Are broad spectrum, bacteriostatic
- Combine with 30S ribosomal subunit
  - inhibits bind of aminoacyl-tRNA molecules to the A site of the ribosome
- Sometimes used to treat acne(حب الشباب)

Tetracycline (chlortetracycline, doxycycline)



Macrolides

- Contain 12- to 22-carbon lactone rings linked to one or more sugars
- e.g., erythromycin
  - broad spectrum, usually bacteriostatic
  - binds to 23S rRNA of 50S ribosomal subunit
    - inhibits peptide chain elongation
- Used for patients allergic to penicillin



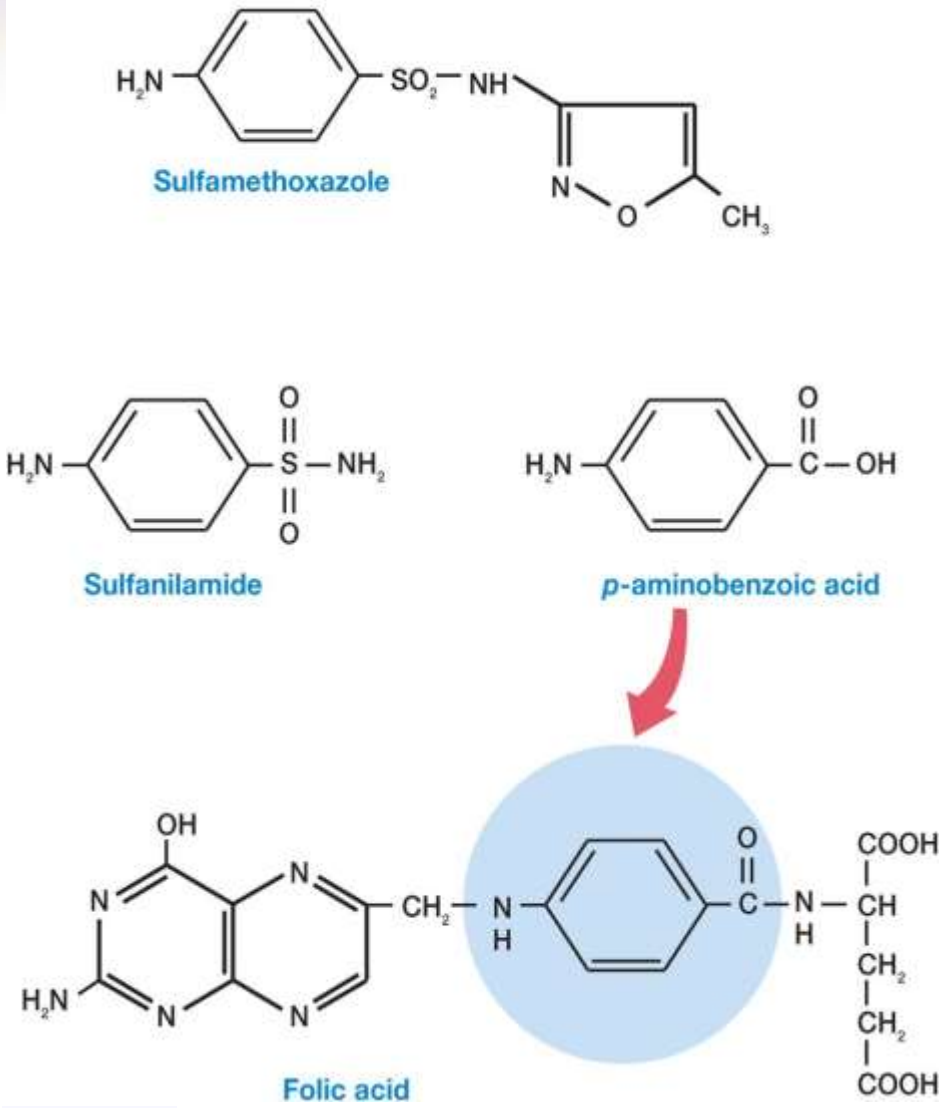
Chloramphenicol

- Now is chemically synthesized
- Binds to 23s rRNA on 50S ribosomal subunit and inhibits peptidyl transferase reaction
- Toxic with numerous side effects so only used in life-threatening situations

Metabolic Antagonists

- Act as antimetabolites
  - antagonize or block functioning of metabolic pathways by competitively inhibiting the use of metabolites by key enzymes
- Are structural analogs
  - molecules that are structurally similar to, and compete with, naturally occurring metabolic intermediates
    - block normal cellular metabolism

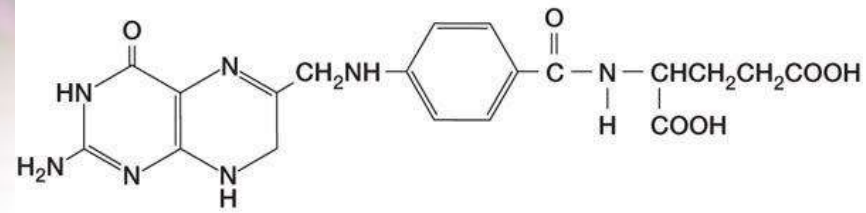
Sulfonamides or Sulfa Drugs



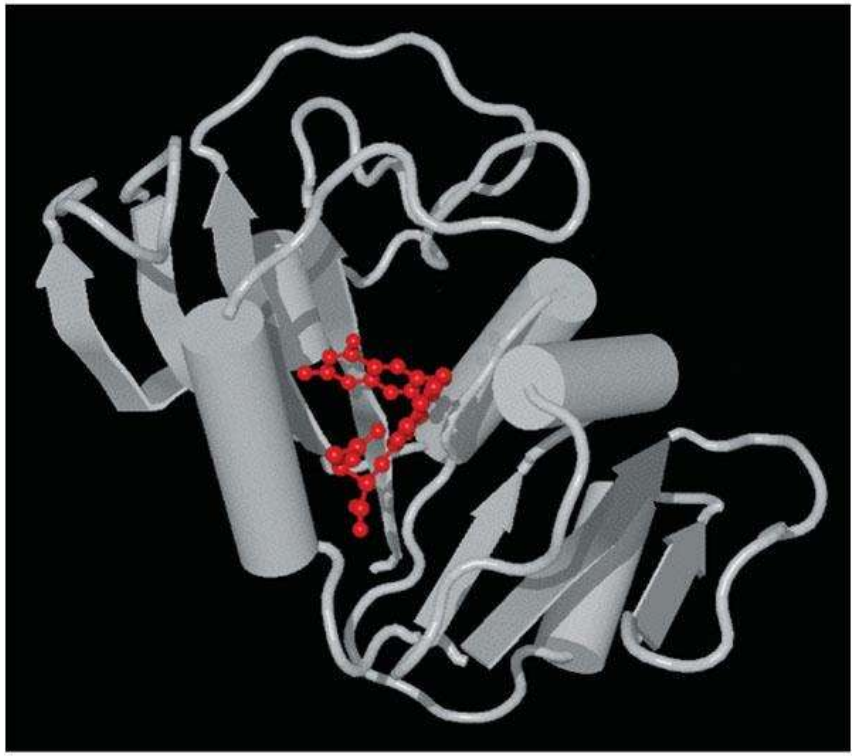
- Structurally related to sulfanilamide, a paminobenzoic acid (PABA) analog
- PABA used for the synthesis of folic acid and is made by many pathogens
  - sulfa drugs are selectively toxic due to competitive inhibition of folic acid synthesis enzymes

Trimethoprim

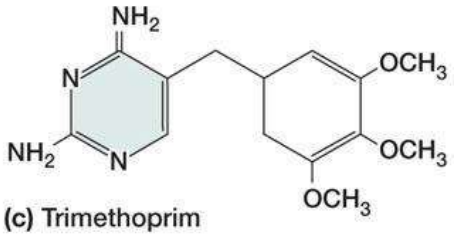
- Synthetic antibiotic that also interferes with(يتداخل مع) folic acid production
- Broad spectrum
- Can be combined with sulfa drugs to increase efficacy of treatment
  - combination blocks two steps in folic acid pathway
- Has a variety of side effects including abdominal pain and photosensitivity reactions (الحساسية للضوء)



(a) Dihydrofolic acid (DFA)



(b) Dihydrofolate reductase



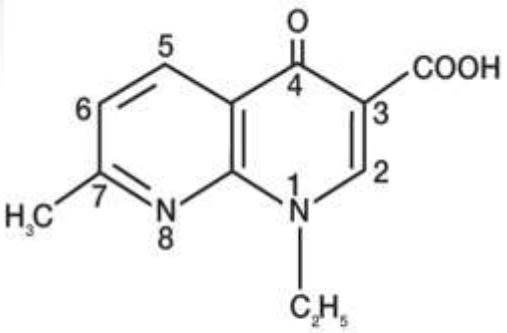
(c) Trimethoprim

Alex MacKerell, Ph.D.

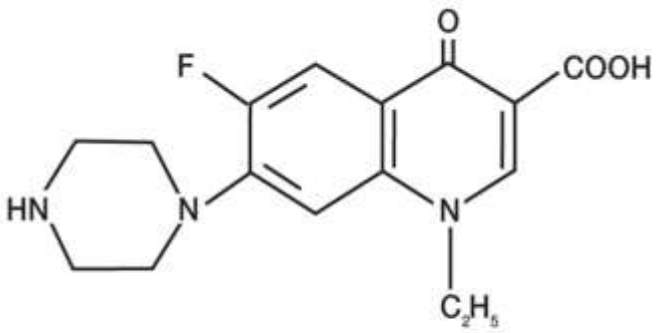
Nucleic Acid Synthesis Inhibition

- A variety of mechanisms
  - block DNA replication
    - inhibition of DNA polymerase
    - inhibition of DNA helicase
  - block transcription
    - inhibition of RNA polymerase
- Drugs not as selectively toxic as other antibiotics because bacteria and eukaryotes do not differ greatly in the way they synthesize nucleic acids

Quinolones



Nalidixic acid



Norfloxacin

- Broad-spectrum, synthetic drugs containing the 4-quinolone ring
- Nalidixic acid first synthesized quinolone (1962)
- Act by inhibiting bacterial DNA-gyrase and topoisomerase II
- Broad spectrum, bactericidal, wide range of infections

- **Type II topoisomerases** cut both strands of the DNA helix simultaneously in order to manage DNA tangles and supercoils. They use the hydrolysis of ATP, unlike Type I topoisomerase. In this process, these enzymes change the linking number of circular DNA by ±2.

- **DNA gyrase**, or simply **gyrase**, is an enzyme within the class of topoisomerase (Type II topoisomerase) [1] that relieves strain(يخفف من الضغط) while double-stranded DNA is being unwound by helicase.[2][3] The enzyme causes negative supercoiling of the DNA or relaxes positive supercoils.

9.8 Factors influencing antimicrobial drug effectiveness

1. Predict the effects of (1) delivery route, (2) metabolism, and (3) local concentration on the effectiveness of an antimicrobial drug
2. Correlate the sensitivity of a microorganism to an antimicrobial agent with microbial growth in the presence of that agent
3. Identify the practices that lead to antimicrobial drug resistance and suggest countermeasures

Factors Influencing 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(يتجاوز) 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
    - nonoral routes of administration
- Drug can be excluded by blood clots or necrotic tissue(الأنسجة الميتة)

Factors Influencing Ability of Drug to Reach Concentrations Exceeding MIC

- Amount administered
- Route of administration
- Speed of uptake
- Rate of clearance (elimination) from body

Drug Resistance

- An increasing problem
  - once resistance originates(ينشأ) in a population it can be transmitted to other bacteria
  - a particular type of resistance mechanism is not confirmed to a single class of drugs
- Microbes in abscesses or biofilms may be growing slowly and not be susceptible(سريع التأثر)
- Resistance mutants arise spontaneously and are then selected

Overcoming Drug Resistance

- Give drug in appropriate concentrations to destroy susceptible
- 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