Antimicrobials Therapy

Pharmaceutical Microbiology

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Introduction

- The discovery of Antimicrobial drugs have successfully controlled the majority of bacterial, parasitical, fungal infections during the last 80 years or more.
- Sulfonamide 1934, Penicillin G 1941 (from Penicillium notatum), followed by Aminoglycosides (Streptomycin, Kanamycin,1946, from soil bacteria - Actinomyces).
- At present more than 100 antimicrobial drugs of different classes are available for use in humans.
- Clinically effective antimicrobial agents should exhibit selective toxicity toward the bacterium and not the host, few side effects and good pharmacokinetics.

Definition

- **Chemotherapeutic agent (drug): any chemical substance used in medical practice**
- **Antimicrobial chemotherapy: the utilization of chemical substances to treat microbial infections**
- **Antibiotic: chemical substances produced by microorganisms that inhibit the growth or destroy other microorganisms when used at low concentration**
- •Although the definition of antibiotic indicates natural substances, many antimicrobial compounds that are **semisynthetic (e.g. some β-lactams) or synthetic (e.g. sulfonamides & quinolones) are still called antibiotics in common practice**

Properties of Ideal Antimicrobial Agents

1. Selective toxicity:

- -**The drug should harm the microbe without significant harm on the host**. This means the toxic dosage level (the dose that causes damage to the host) should be much higher than the effective therapeutic dosage level (the dose needed to destroy the microbe).

- To evaluate toxicity of the drug, **chemotherapeutic index is used: the ratio between toxic dose & effective dose**

Properties of Ideal Antimicrobial Agents

2. Nonallergenic. The agent should not elicit an allergic reaction in the host.

3. Stability: maintenance of a constant, therapeutic concentration in blood and tissue fluids

4. **Resistance** by Microorganisms Not Easily Acquired.

5. Long Shelf-life.

6. Reasonable Cost.

The Spectrum of Activity

Spectrum: the range of microorganisms that a drug is effective against.

1. **Narrow spectrum** antibiotic: active against single or limited group of M.O. (Isoniazid)

2. **Extended spectrum :** is one that, as a result of chemical modification, affects additional types of bacteria, usually those that are gram-negative. active against types of bacteria G- & G+.

   (Ampicillin)

3. **Broad spectrum:** active against a wide variety of microbial species.

(Tetracyclines, quinolones, Chloramphenicol).

The Spectrum of Activity

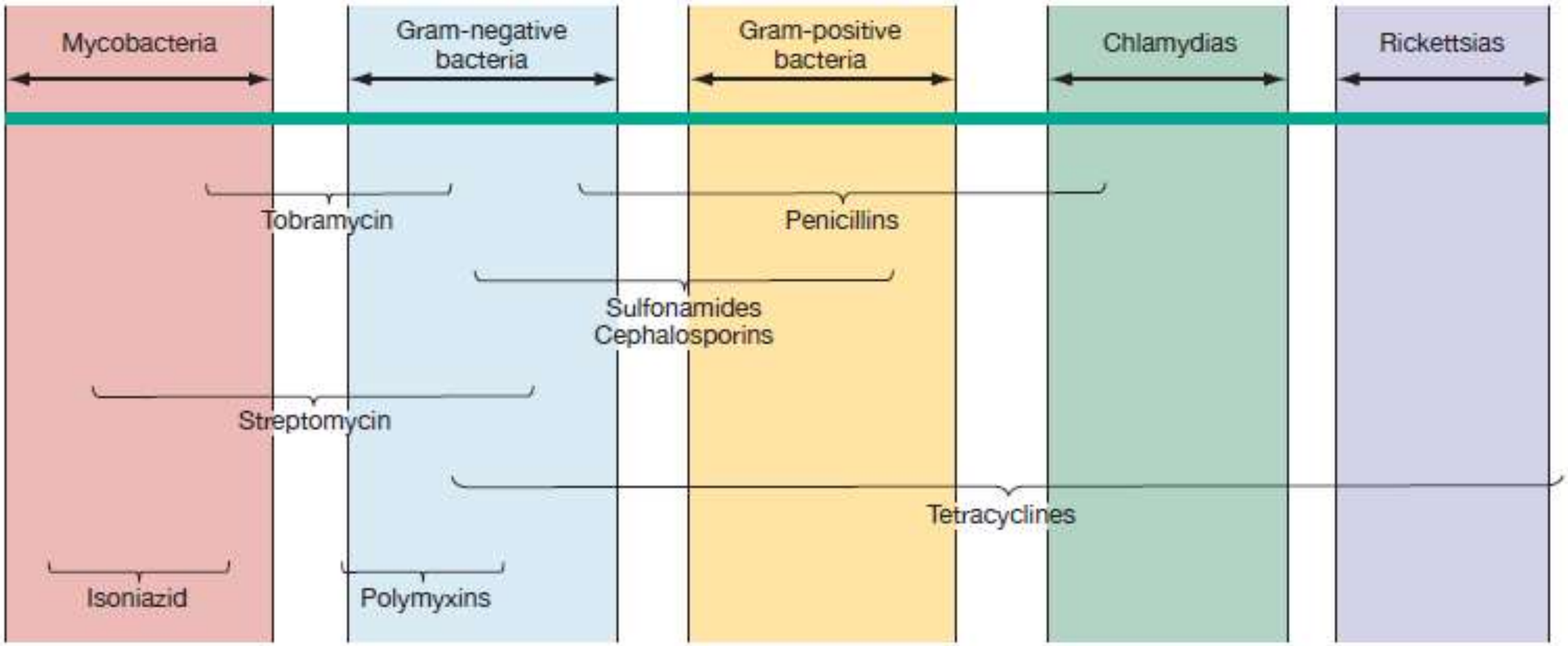
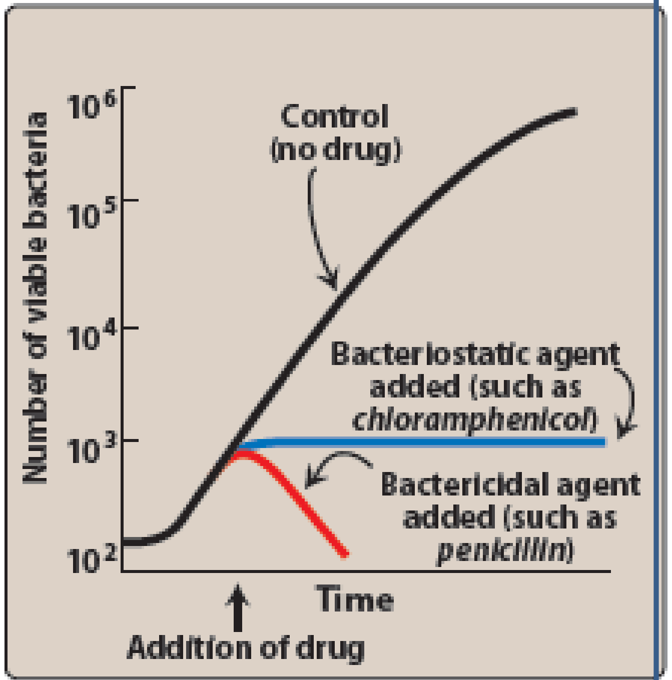


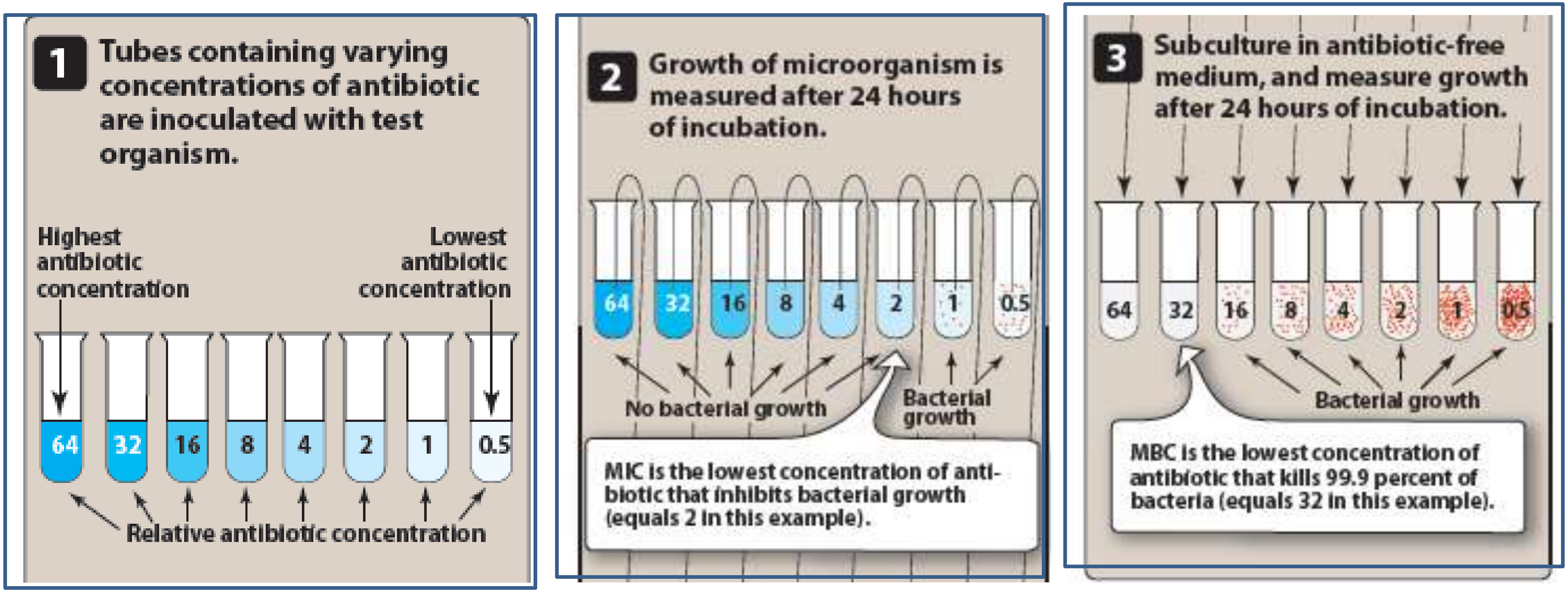
FIGURE 13.1 The spectrum of antibiotic activity. Broad-spectrum drugs, such as tetracycline, affect a variety of different organisms. Narrow-spectrum drugs, such as isoniazid, affect only a few specific types of organisms.

Antimicrobial Action

- Bacteriostatic: inhibit growth of microorganisms such as(Sulfonamides, Chloramphenicol and Tetracyclines). This is determined in vitro by testing a standardized concentration of organisms against a series of antimicrobial dilutions. The lowest concentration that inhibits the growth of the organism is referred to as the **Minimum Inhibitory Concentration (MIC).**

- Bactericidal: Kill microorganisms (such as Penicillins and Aminoglycosides). This is determined in vitro by exposing a standardized concentration of organisms to a series of antimicrobial dilutions. The lowest concentration that kills 99.9% of the population is referred to as the **Minimum Bactericidal Concentration (MBC).**





Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of an antibiotic.

Selection of Antimicrobial Agents

1- Making the diagnosis :

- ✓ To be sure that the patient is suffering from an bacterial infection.
- ✓ Know the site of infection(GI,RT,UT).
- ✓ Take the required specimen from the patient.(blood , CSF, mid stream urine ,ear swap, vaginal discharge)
- ✓ Identify the organism.

2-Remove the pathological barrier to cure

(abscess ,obstruction).

3-Select the best drug : So that it reach site of infection in the therapeutic conc.

- ➢ Drug properties: PK,TI.
- ➢ **Optimum dose & frequency**
- ➢ **the most appropriate route of administration**

4- The cost of therapy.

5-Patient factors:

1. Immune system.
2. Renal dysfunction.
3. Hepatic dysfunction.
4. Poor perfusion.
5. Age.
6. Pregnancy.
7. Lactation.
8. Concomitant medication.
9. Allergy.

- *Ideally*, the antimicrobial agent used to treat an infection is selected *after* the organism has been identified and its drug susceptibility established.

- However, in the critically ill patient, such a delay could prove fatal, and immediate *empiric* therapy is indicated.

- **Empiric therapy:** is treating the patient without knowing the causative organisms & their sensitivity test.
- Immediate administration of the drug prior to identification of bacteria and sensitivity test.(or the specimens is obtained but lab result not available)

- **Definitive therapy :**

treating exactly the causative agent depending on its sensitivity test (done after receiving the results of test)

- **Prophylactic therapy:**

Used drugs to prevent an infection rather than to treat , to maintain health and prevent the spread of disease.

Combination of antimicrobials

Advantages

1. To delay or avoid the development of resistance.

   (Ex. Tuberculosis)

2. To broaden the spectrum of activity. (Mixed infection , severe unknown infection,).

3. To obtain potentiation (synergistic effect).

   Ex: -B-lactams and aminoglycosides in endocarditis. Penicillin + Aminoglycosides

2separate IV bolus injection ,with time interval to avoid interaction.

- Co-trimoxazole.

Disadvantages:

1. Concomitant administration of a second agent is usually bacteriostatic and may interfere with the action of the first drug that is bactericidal

2. Suppression of normal flora, so give higher chance for opportunistic infection (**superinfection**).
3. Increased incidence of adverse reactions.
4. Highly cost

Problems with antimicrobial agents

1. Drug resistance. (the major problem)

(if the maximal level of that antibiotic that can be tolerated by the host does not halt bacterial growth).

2. Drug-drug interaction

3. Adverse effects.

Problems with antimicrobial agents

3. Adverse effects

<u>a. Hypersensitivity</u>; (not dose related)

e.g. Penicillin, cephalosporin.

<u>b. Toxic effect</u> (dose related)

High serum levels of certain antibiotics may cause Direct toxicity / Organ toxicity

e.g. Aminoglycosides(ototoxicity)

c. <u>Superinfections:</u>

alterations of the growth of normal flora of intestine, genitourinary tracts. Respiratory tract

Appearance of **<u>a new infection</u>** while treating an original infection (multiply C.difficile).

Mechanisms of Action of Antibacterial Drugs

Antimicrobial drugs affect various bacterial cellular targets:

1. Inhibit cell wall synthesis
2. Inhibit protein synthesis
3. Inhibit nucleic acid synthesis
4. Injury to plasma membrane
5. Inhibit synthesis of essential metabolites

Mechanisms of Action of Antibacterial Drugs

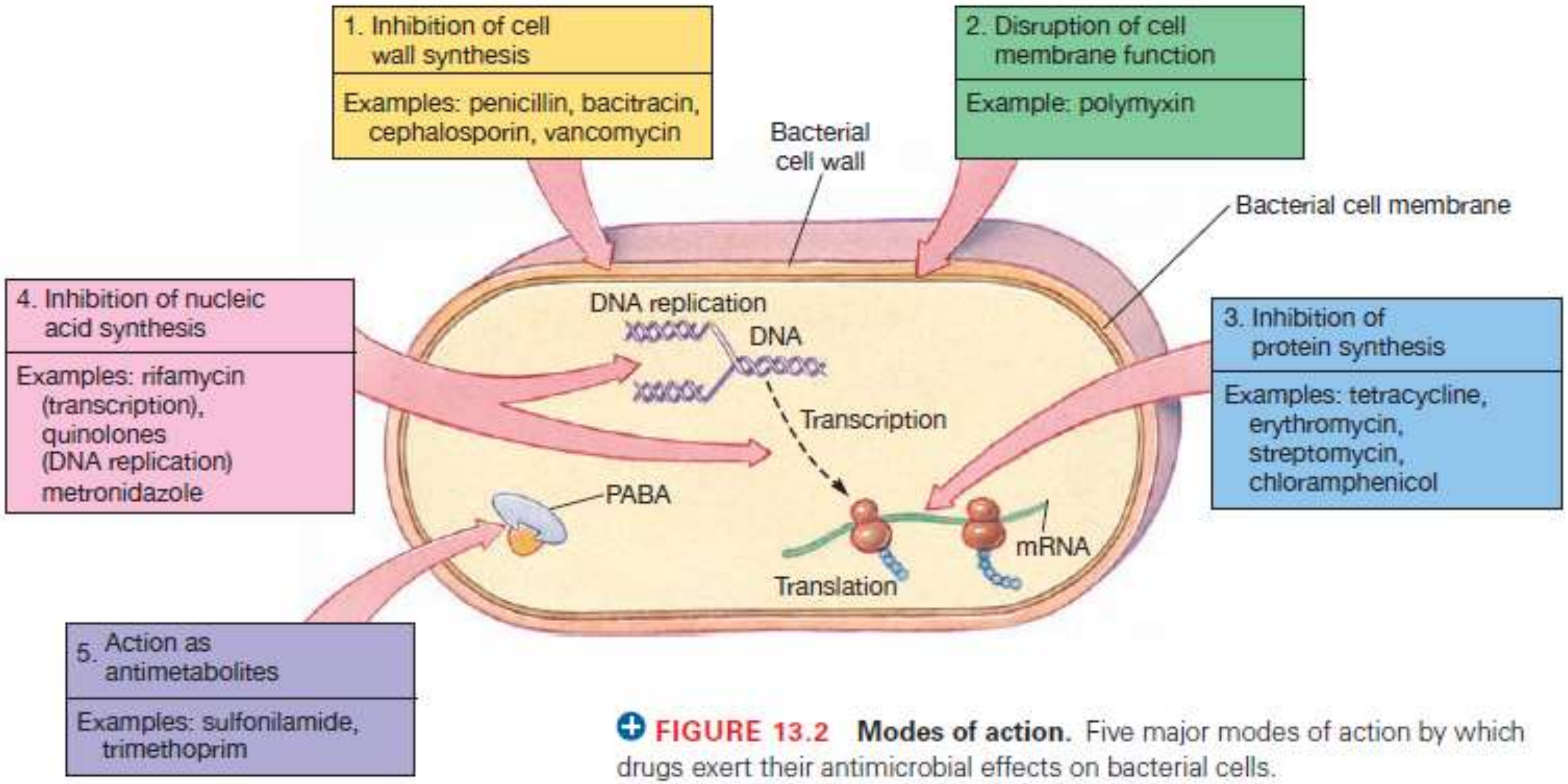
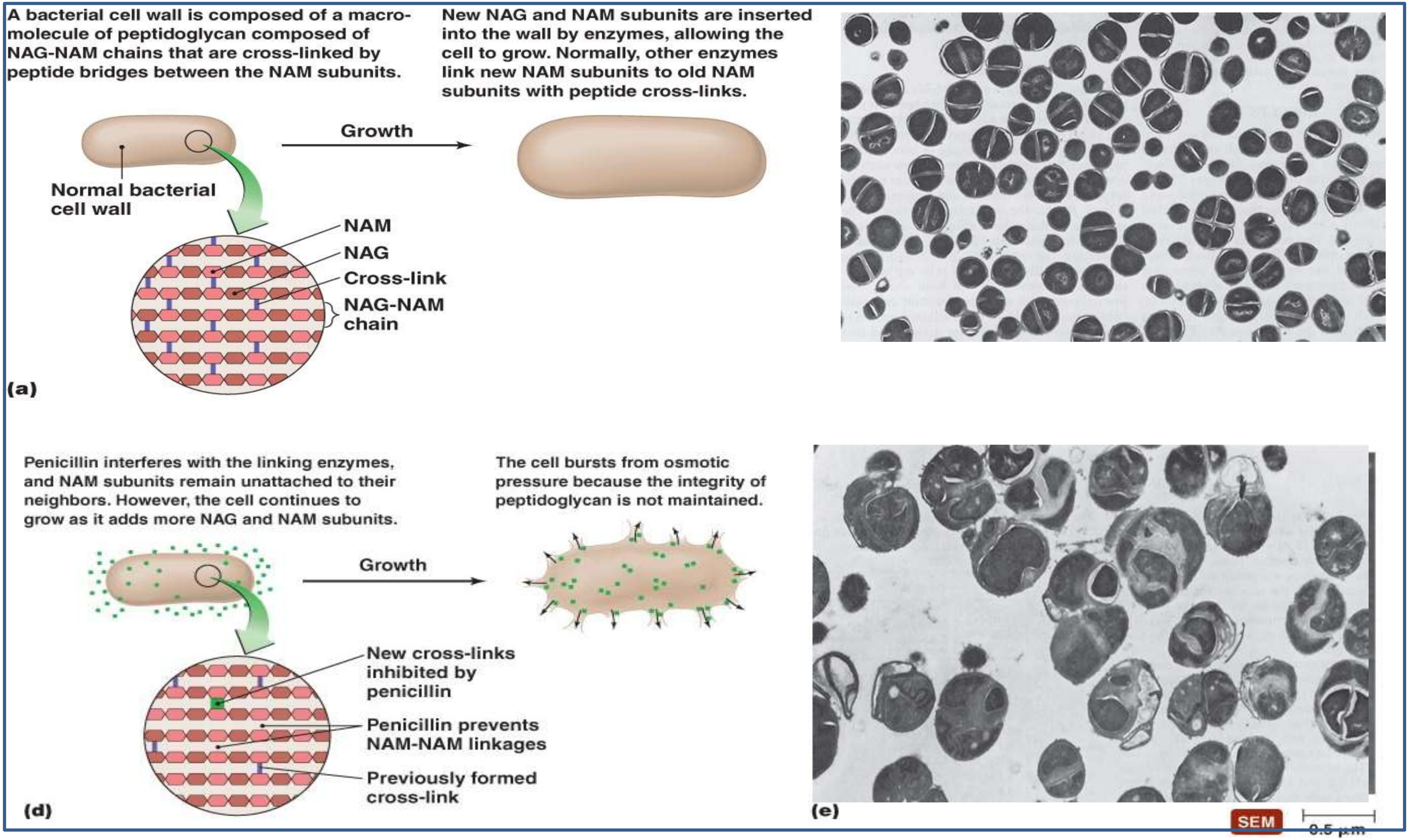


FIGURE 13.2 Modes of action. Five major modes of action by which drugs exert their antimicrobial effects on bacterial cells.

Inhibition of Cell Wall Synthesis

β-Lactam Drugs

- The main group of AB that act on bacterial cell wall is the ‘**beta lactams’; so called due to presence of a β-lactam ring.**
- Irreversibly inhibit enzymes involved in the final steps of cell wall synthesis
- These enzymes mediate formation of peptide bridges between adjacent stands of peptidoglycan (cross link, PBPs)
- Drug binds to enzyme, competitively inhibits enzymatic activity
- **β-lactam drugs include: Penicillins, Cephalosporins, Carbapenems and Monobactams**



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 cross-links.
Growth
Normal bacterial cell wall
NAM
NAG
Cross-link
NAG-NAM chain
(a)
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.
Growth
New cross-links inhibited by penicillin
Penicillin prevents NAM-NAM linkages
Previously formed cross-link
(d)
(e)
SEM
0.5 μm

ß-Lactams Antibiotics

ß-Lactams

Cephalosporins

- Cefalexin
- Cefuroxime
- Cefotaxime
- Ceftriaxone

Penicillins

Narrow Spectrum

- Benzylpenicillin (Penicillin G)
- Phenoxymethylpenicillin (Pen V)
- Flucloxacillin

Broad Spectrum

- Amoxicillin/Co-amoxiclav
- Ampicillin

Carbapenems

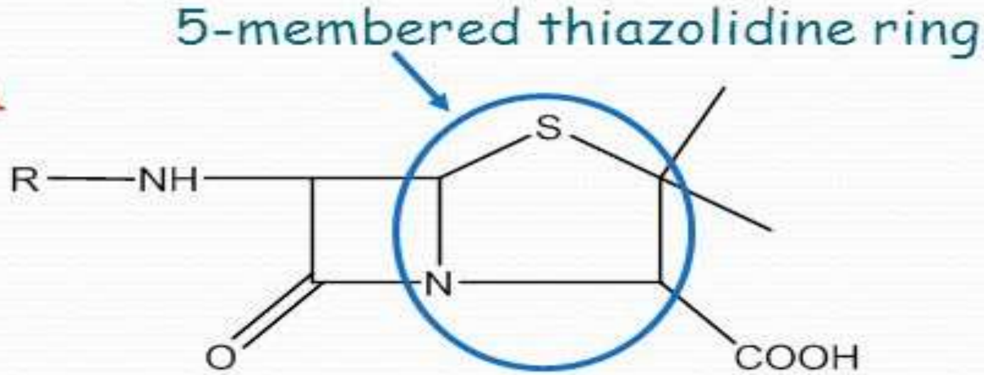
- Meropenem
- Imipenem
- Doripenem
- Ertapenem

Monobactams

- Aztreonam

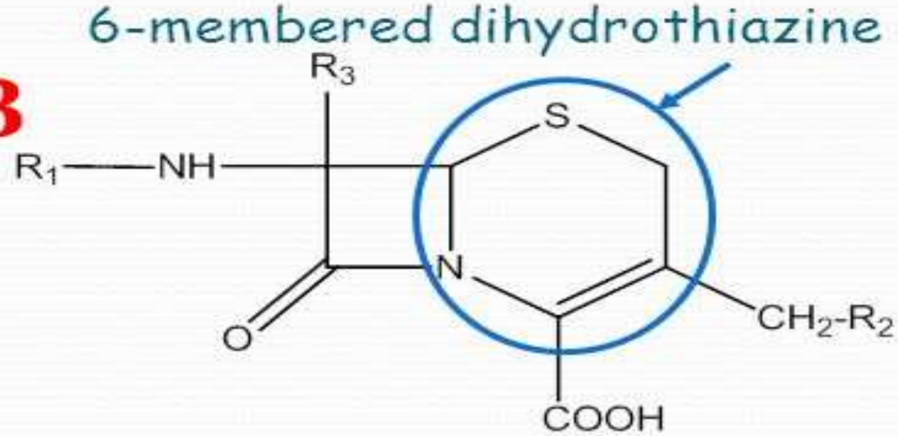
β-lactam antibiotics

A



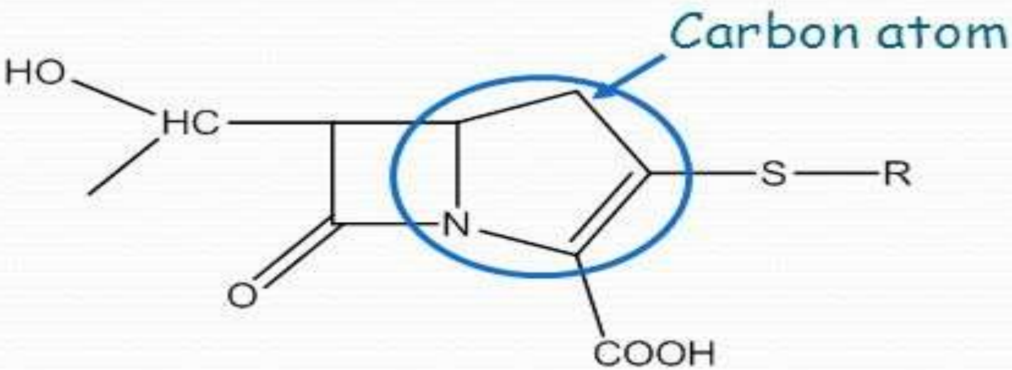
Penicillin nucleus

B



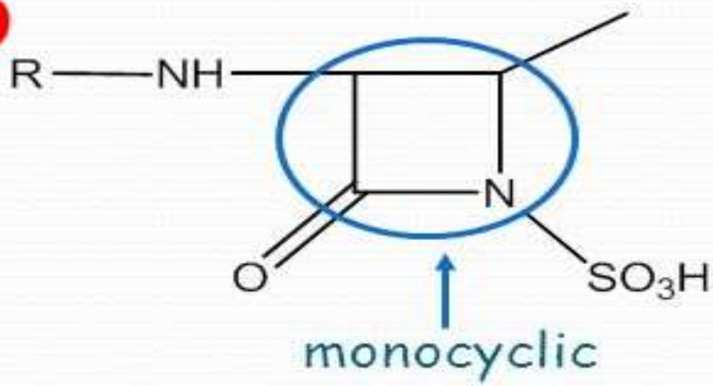
Cephalosporin nucleus

C



Carbapenem nucleus

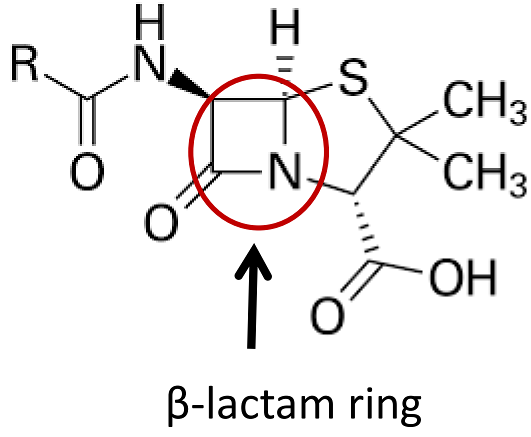
D



Monobactam nucleus

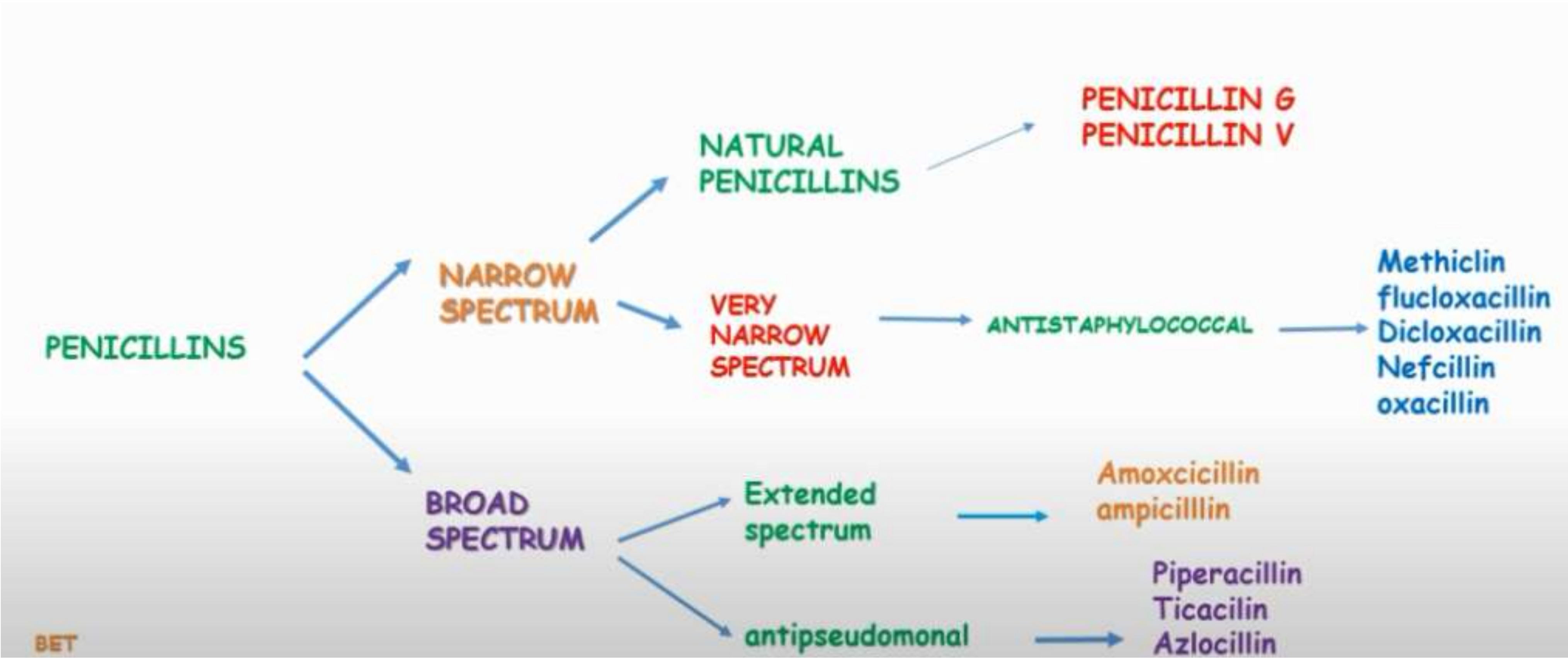
Penicillins

- Have β-lactam ring
- Bacteriocidal

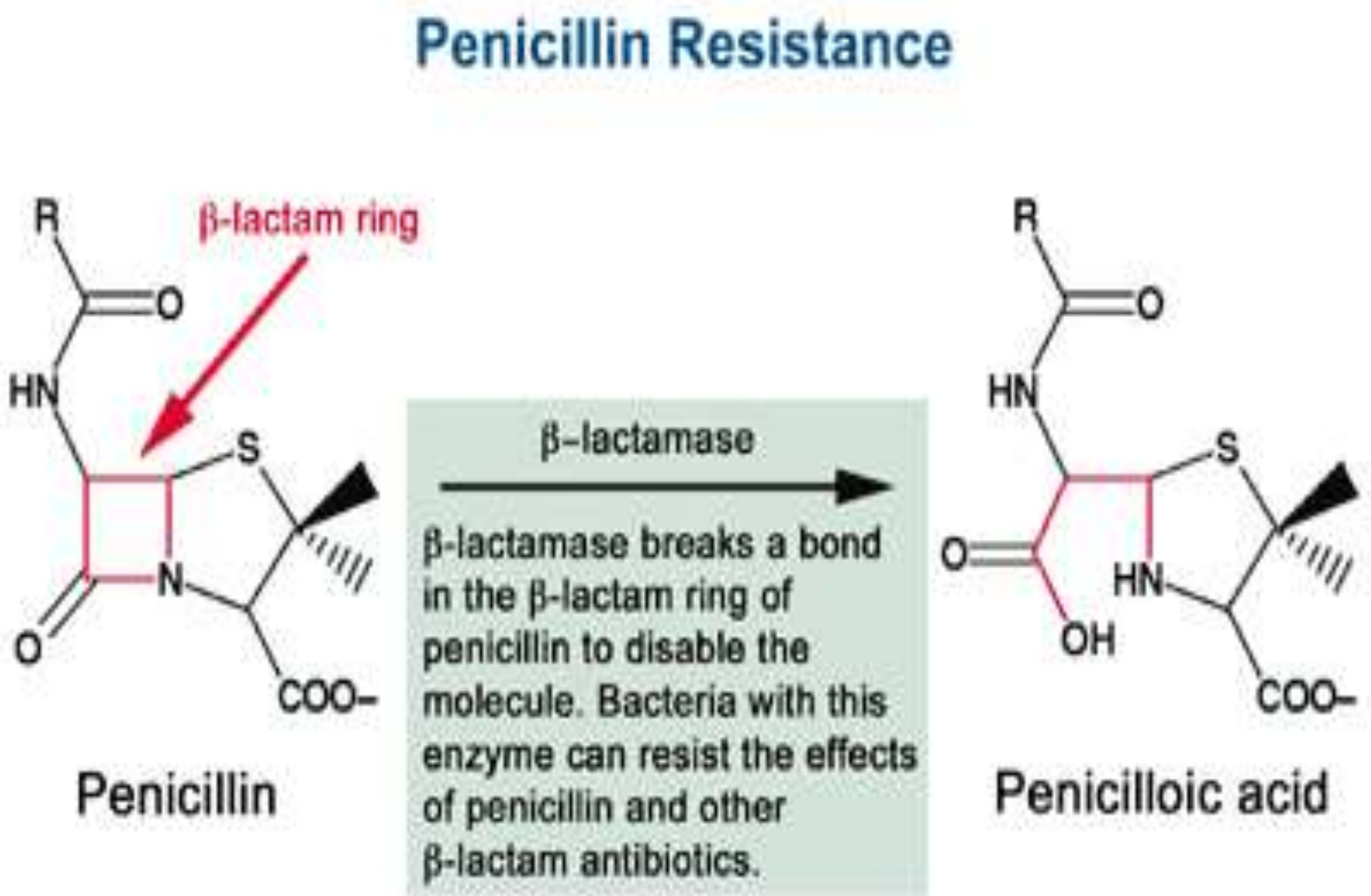


- Their official names usually include or end in "cillin"

Classification of Penicillins



Some bacteria produce β-lactamase enzyme that breaks the critical β-lactam ring



B-lactamase inhibitors

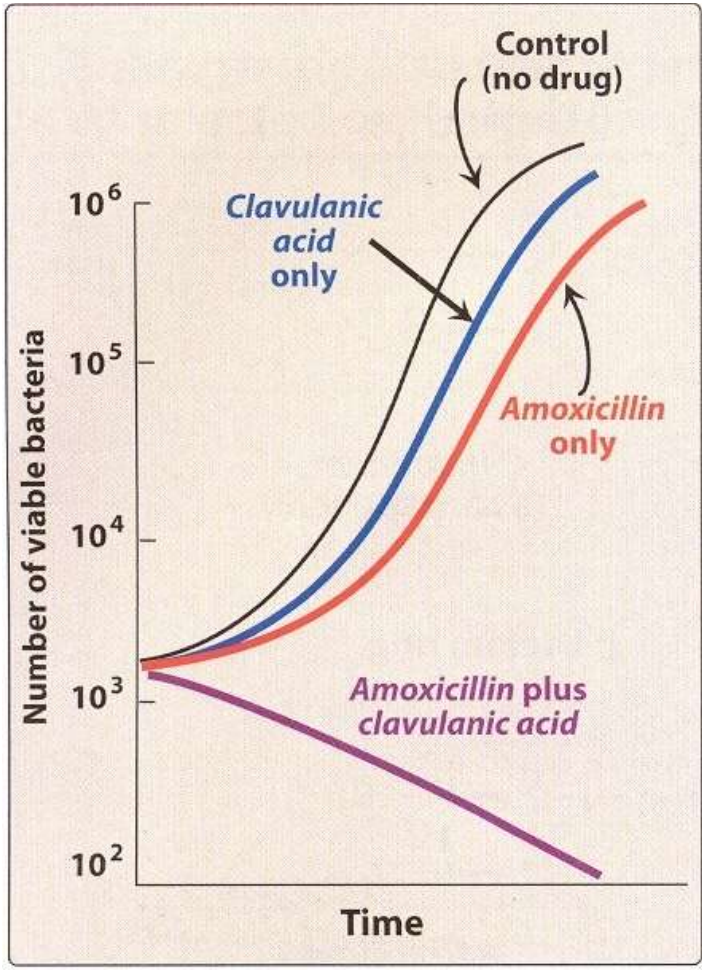
Substance **don't** have antibacterial activity but they have the ability to **inhibit** the B-lactamase enzyme….

Ex. Clavulanic acid

For example clavulanic acid Binds to beta-lactamase and competitively protects amoxicillin

*They potentiating amoxicillin against beta-lactamase producing bacteria.

* It is called "suicide inhibitor"



Penicillins adverse effects

- Allergy to penicillins is rare among children but occurs in 1 to 10% of adults.
  - Mild: rash
  - Severe: anaphylaxis & death

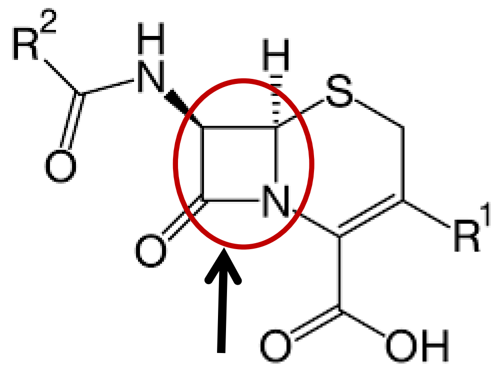
✓ There is cross-reactivity among all Penicillins

✓ Penicillins and cephalosporins ~5%

- Penicillins are generally nontoxic, but large doses can have toxic effects on the kidneys, liver, and central nervous system

Cephalosporins

- Different generations (1st, 2nd, third and fourth.)
- Bactericidal medications
- Safe in general
- 1st and 2nd generation drugs are not used for CNS Infections
- They are recognised by the inclusion of "cef" or "ceph"
- they tend to be more resistant than Penicillins to β-lactamases



β-lactam ring

Cephalosporins

- Since cephalosporins are structurally similar to penicillins, 4-15% of patients who are allergic to penicillins are also allergic to cephalosporins

- Penicillins & cephalosporions exert their activity against growing bacteria

WHY???

Other β-lactam antibiotics:

- **Monobactam/Aztreonam**
  - ➢ Against Gram-negative bacteria,
  - ➢ poor against gram positive
- **Carbapenem (Imipenem, Meropenem)**

➢ A group of β-lactams that are highly resistant to β –lactamases

➢ Active against G+ve & G-ve bacteria including *Pseudomonas aerogenosa & Enterococcus*

Vancomycin

- *is a large, complex molecule produced by* the soil actinomycete *Streptomyces orientalis.*
- It can be used to treat infections caused by methicillin-resistant staphylococci and enterococci. It is also the drug of choice against antibiotic-induced pseudomembranous colitis
- Vancomycin is fairly toxic, causing hearing loss and kidney damage
- Red man syndrome (red rash)

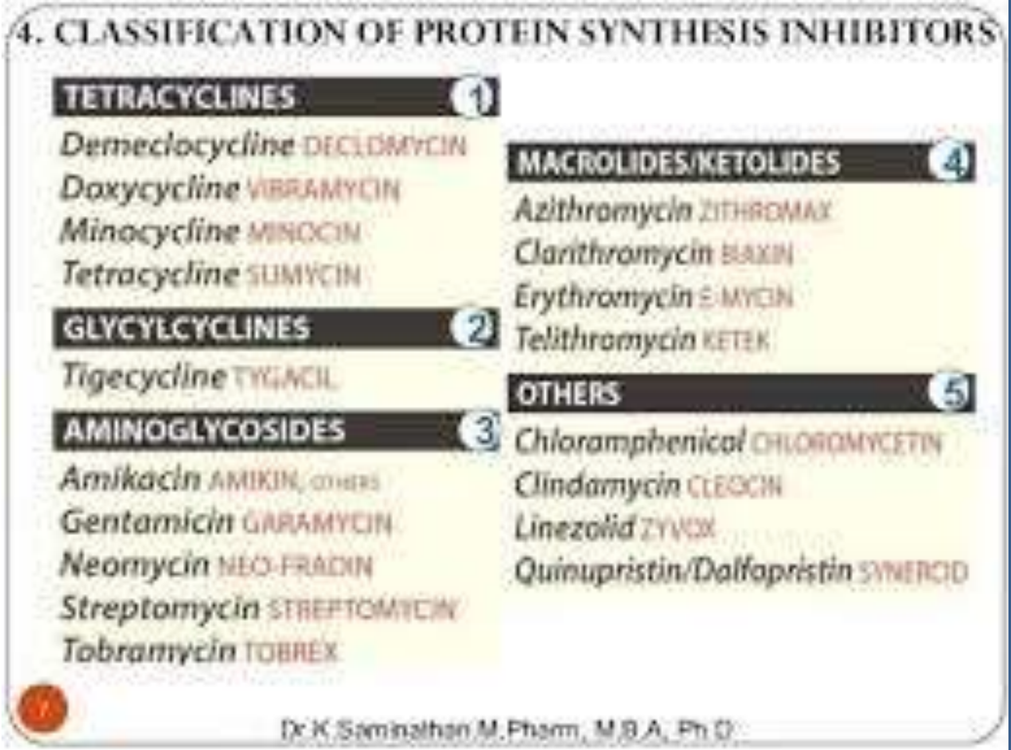
Protein Synthesis Inhibitors

- These drugs act on bacterial (70S) ribosomes
- Since bacterial ribosomes (30S+50S=70S) are different than animal ribosomes (60S+40S=80S), these antibiotics

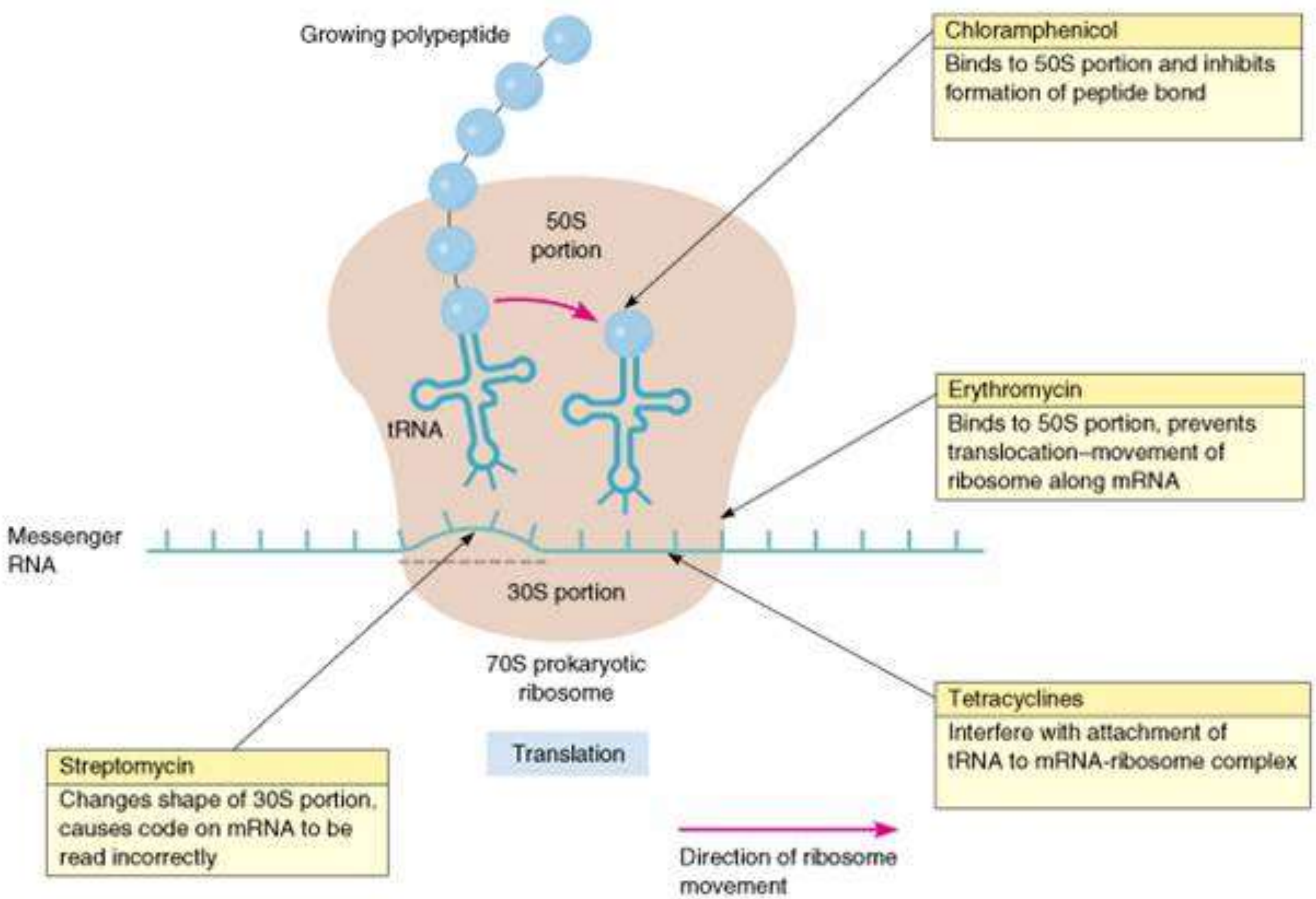
  have good **selectivity**

- They are **bacteriostatic agents except aminoglycosides which are bactericidal**

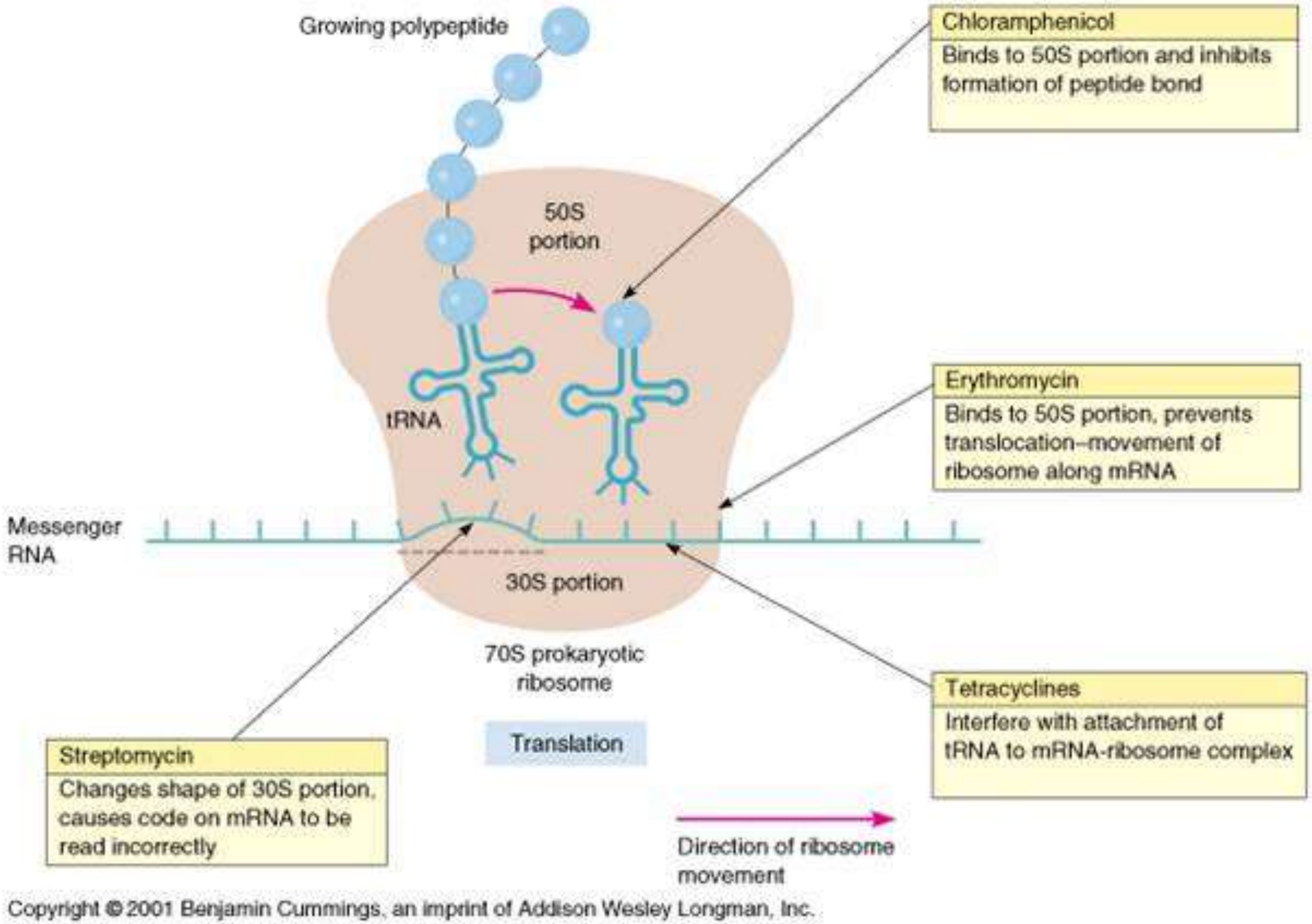
  at high concentrations



protein synthesis inhibitors antibiotics



protein synthesis inhibitors antibiotics



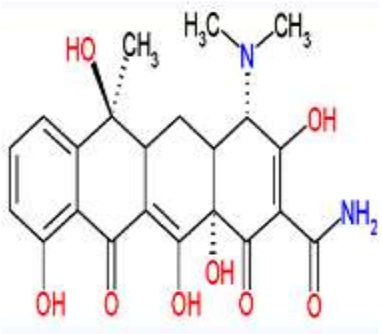
Aminoglycosides

- Antibacterial spectrum of aminoglycosides varies with the type of antibiotic.
- All aminoglycosides are active against aerobic, gram-negative bacteria.

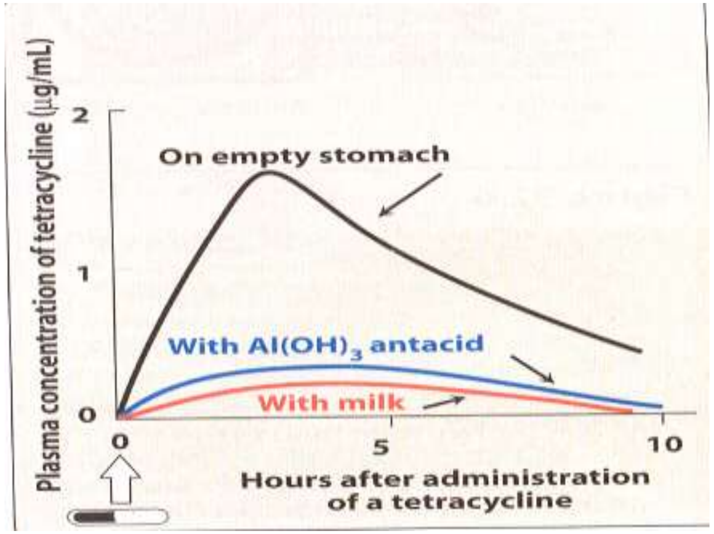
- Anaerobic bacteria are resistant.
- Aminoglycosides are transported across the bacterial membrane into cytoplasm by an OXYGEN dependent actively transport process. Therefore they have no activity against anaerobic bacteria.

- Have "mycin" or "micin" suffixes-----**e.g.:** Gentamicin, Tobramycin
- An important property of aminoglycosides is their ability to act synergistically with other drugs—an aminoglycoside and another drug together often control an infection better than either could alone

Tetracyclines



- Their names end in “cycline”
- Example: Doxycycline
- They are absorbed from the GIT and hence can be administered **orally**
- Tetracyclines form complexes with **calcium & become inactive, therefore milk & dairy products should not be taken with them.**



Tetracyclines

- Due to their binding with calcium, they also cause **teeth discoloration & abnormal bone formation so they are usually contraindicated during the second half of pregnancy or for children under the age of 8 .**

- Alteration in intestinal flora may result in Superinfection (overgrowth of nonsusceptible organisms such as Candida)


Doxycycline
100mg Capsules
50 capsules

Macrolides

- Generally bacteriostatic
- Their names end in “romycin” and mostly in “thromycin”
- E.g. Erythromycin, Clarithromycin

- They are active against most G+ve bacteria: *Streptococci, Pneumococci, Staphylococci & also Mycoplasma.*
- Macrolides have variable activity against G-ve bacteria

- Macrolides main use is for penicillin resistant infections & as alternative to penicillin for patients allergic to them

Lincosamides

- Bacteriostatic agents
- Include **clindamycin & lincomycin.**

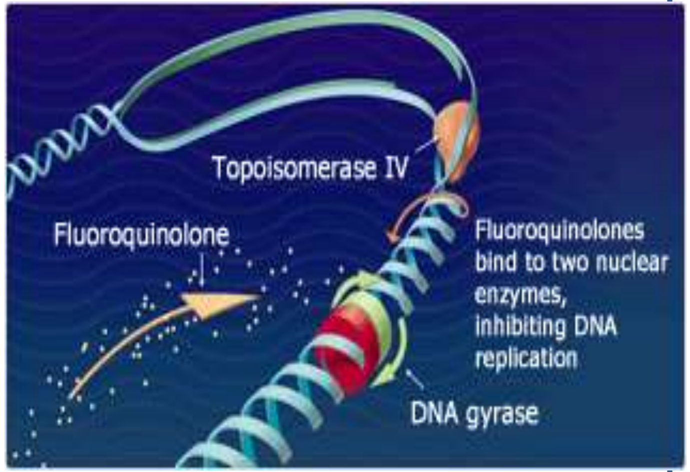
- Clindamycin is used to treat *Bacteroids & other* ***anaerobic bacteria***
- Also used for G+ve cocci (except *Enterococci) and in topical preparations for* ***acne***
- Clindamycin also has antiprotozoan activity (i.e. toxoplasmosis)
- Long term use of clindamycin causes **pseudomembranous colitis due to toxins from *Clostridium difficile***

Chloramphenicol

- It is a **bacteriostatic agent**
- It has a **broad spectrum of activity (excluding *Pseudomonas & Enterococcus)***
- But it has serious side effects such as **aplastic anemia and bone marrow suppression which could be fatal**
- Therefore, its use is considered as a **last choice when other effective agents are unavailable**
- Among its main uses is to treat **typhoid fever & meningitis for patients who are allergic to cephalosporins & penicillins**

Inhibitors of Nucleic Acid Synthesis

- Quinolones are a group of synthetic antibiotics that act by inhibiting **DNA gyrase (the enzyme which unwinds DNA double helix preparing it to replication) thus inhibiting DNA synthesis.**

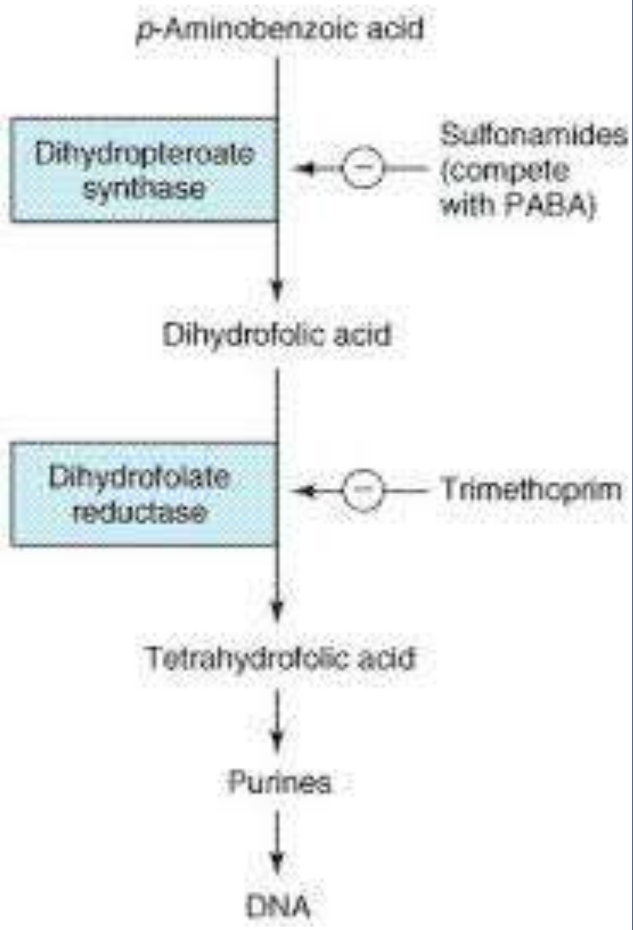


- The basic drug of this group is **nalidixic acid, but the majority of drugs in clinical use belong to the subgroup fluoroquinolones such as ciprofloxacin, norfloxacin.**
- They are active against **both G+ve & G-ve bacteria (including *Pseudomonas aeruginosa*)**
- Used mainly for traveler's diarrhea & urinary tract infections caused by bacteria that are resistant to other antibiotics

Antimetabolites

- The **sulfonamides, or *sulfa* *drugs*,** bacteriostatic agents
- They act by competitive inhibition to the enzyme that acts on **para amino benzoic acid (PABA) needed for folic acid synthesis**
- Folic acid is important for synthesizing nucleic acids & other metabolic products.
- Sulfonamides are chemically similar to PABA .

- Sulfamethoxazole is usually given **in combination with trimethoprim which inhibits another enzyme in the pathway of folic acid synthesis**
- This combination drug is called **co-trimoxazole**



Antiprotozoal agents

- Drugs used to treat infections caused by protozoa. Protozoa are unicellular animal like organisms.
- Examples: giardia, amoeba, plasmodium, leishmania, toxoplasma

Antiprotozoal agents

- Metronidazole (Falgyl®): causes breakage of DNA strands thus inhibit nucleic acid synthesis.
- Effective against intestinal infections caused by parasitic Giardia& amoebas. Also it is effective against anaerobic bacteria(responsible for intraabdominal abscess, peritonitis, joint infections, gingivitis) & Clostridium difficile.
- It is effective in treating Trichomonas infections, which typically cause a vaginal discharge and itching.

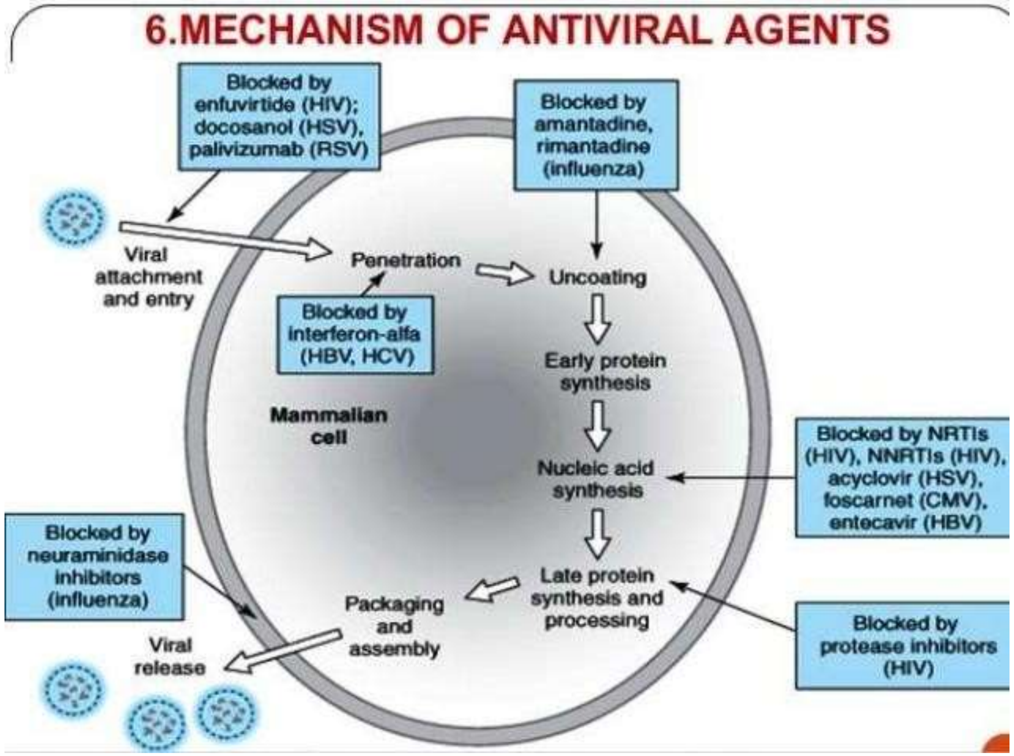
–Used as part of multidrug regime for peptic ulcer against Helicobacter pylori

Antiviral agents

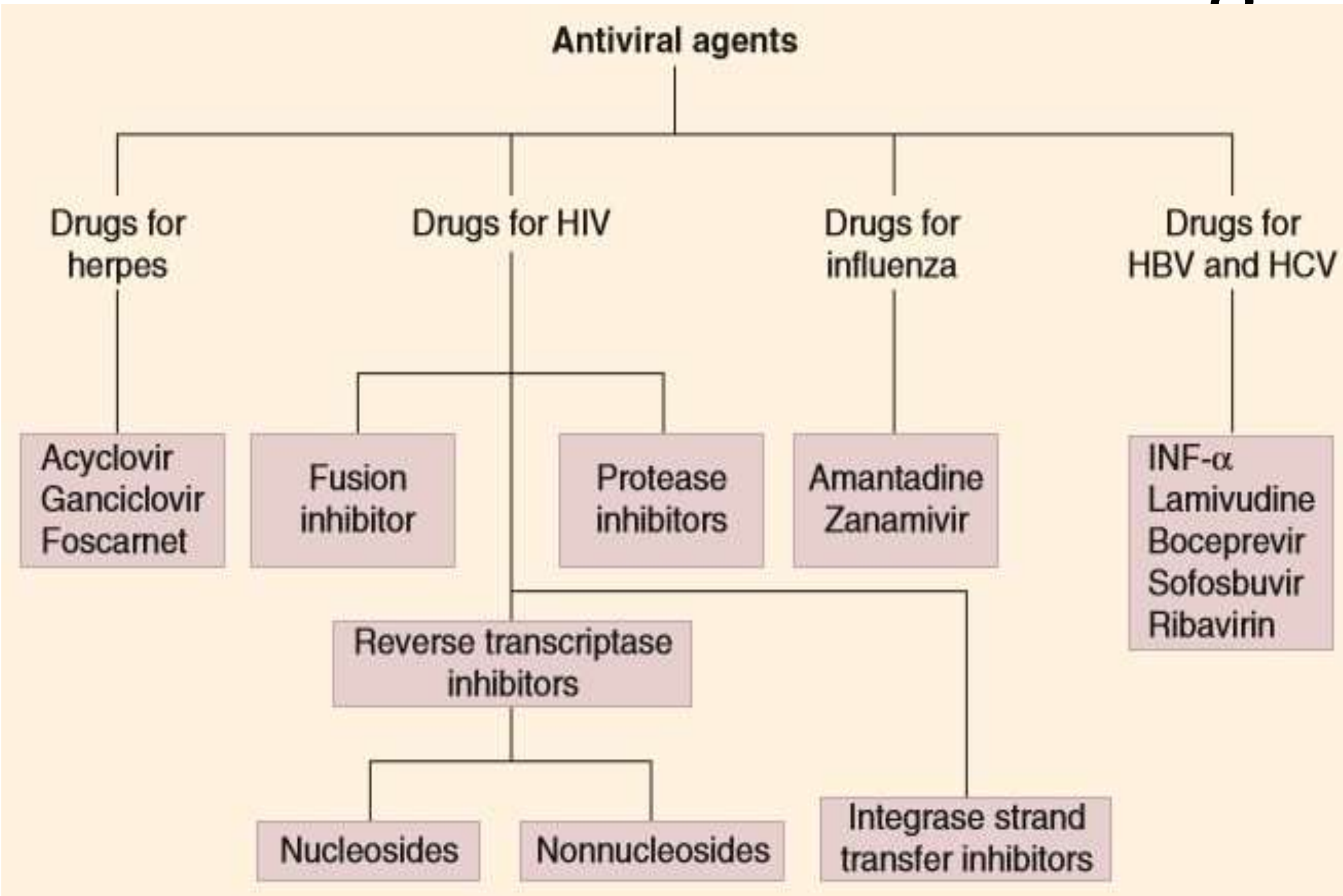
The difficulty in finding antiviral drugs is that the drug should attack viruses inside cells without affecting host cell.

- Antiviral agents act by interfering on some phase of viral replication but they don't kill viruses.

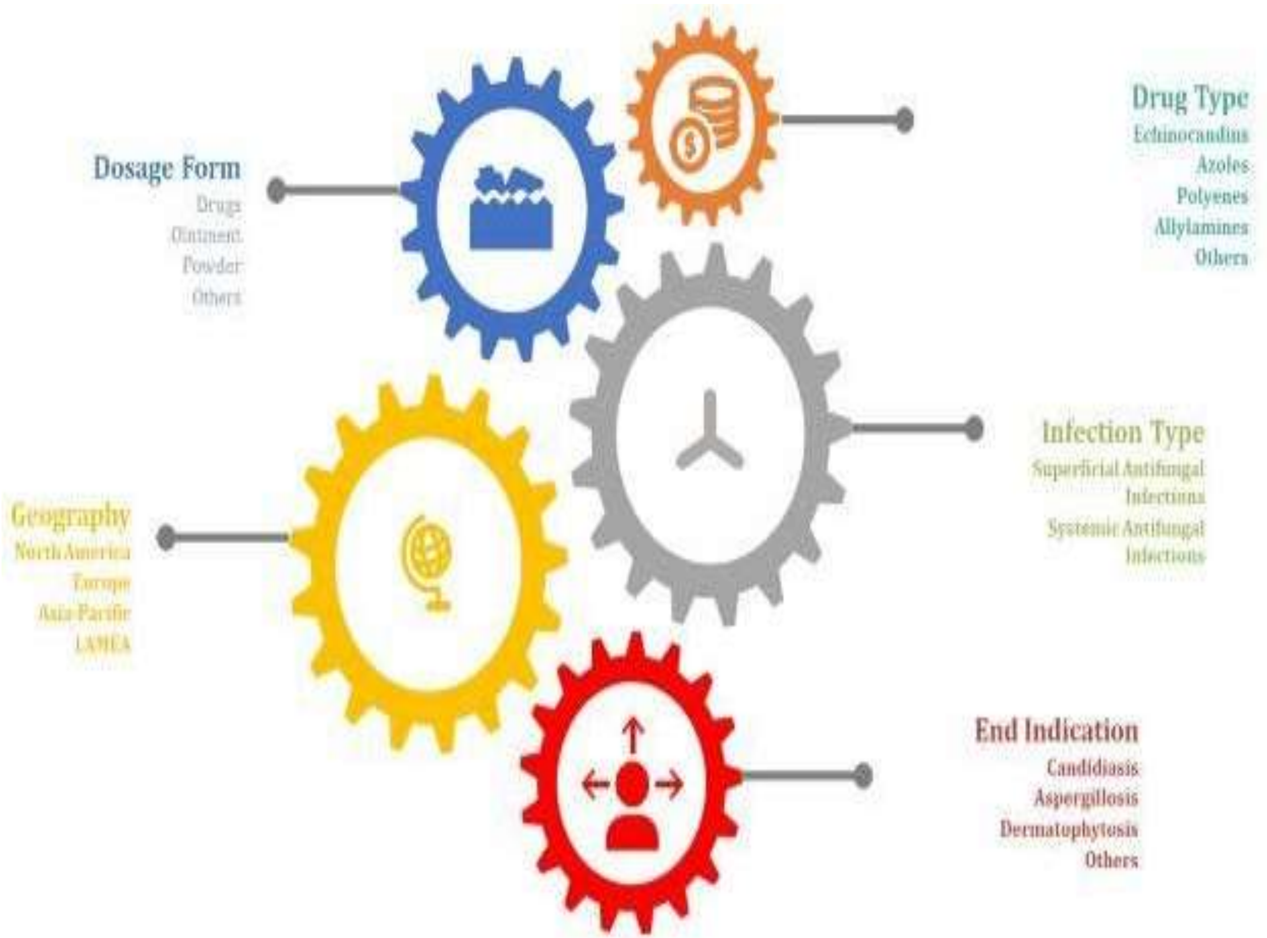
  Therefore, latent non replicating viruses can't be destroyed.



Classification of antiviral agents



Antifungal Drugs



By Infection Type

Superficial Infections

Systemic Infections

By Therapeutic Indications

Aspergillosis

Dermatophytosis

Candidiasis

Others

By Dosage Forms

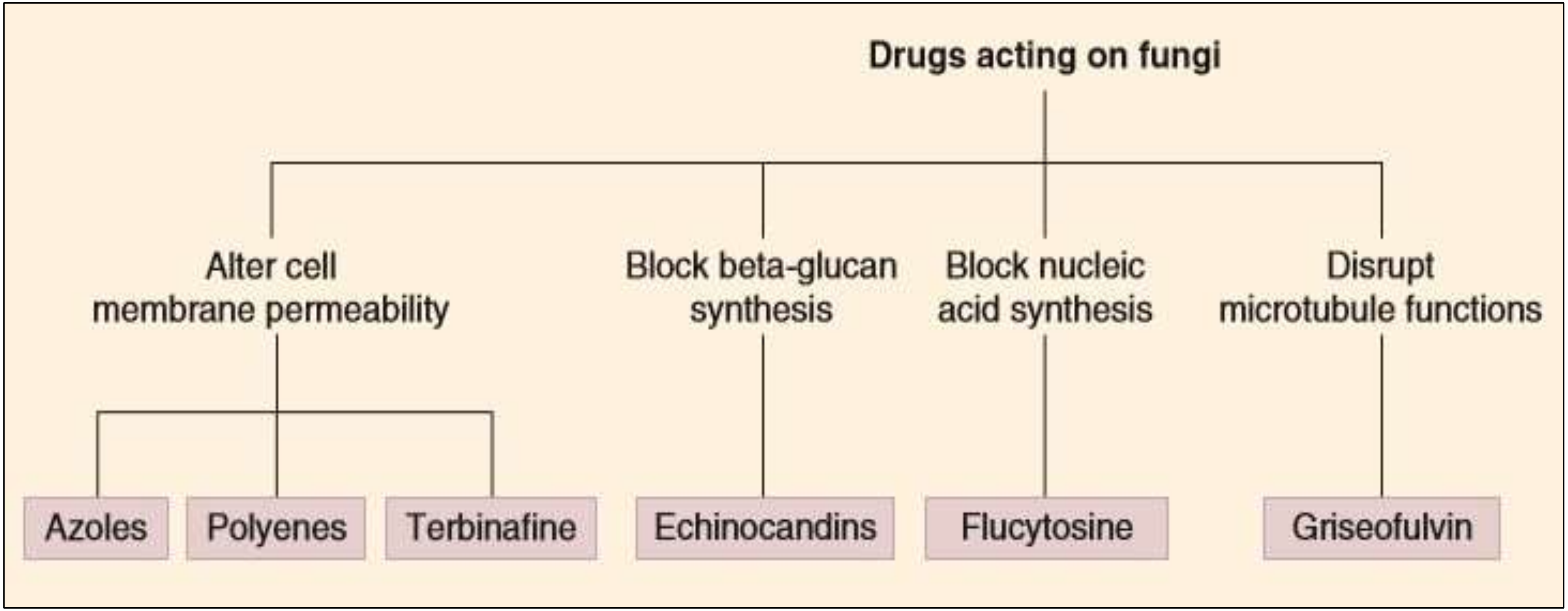
Ointments

oral

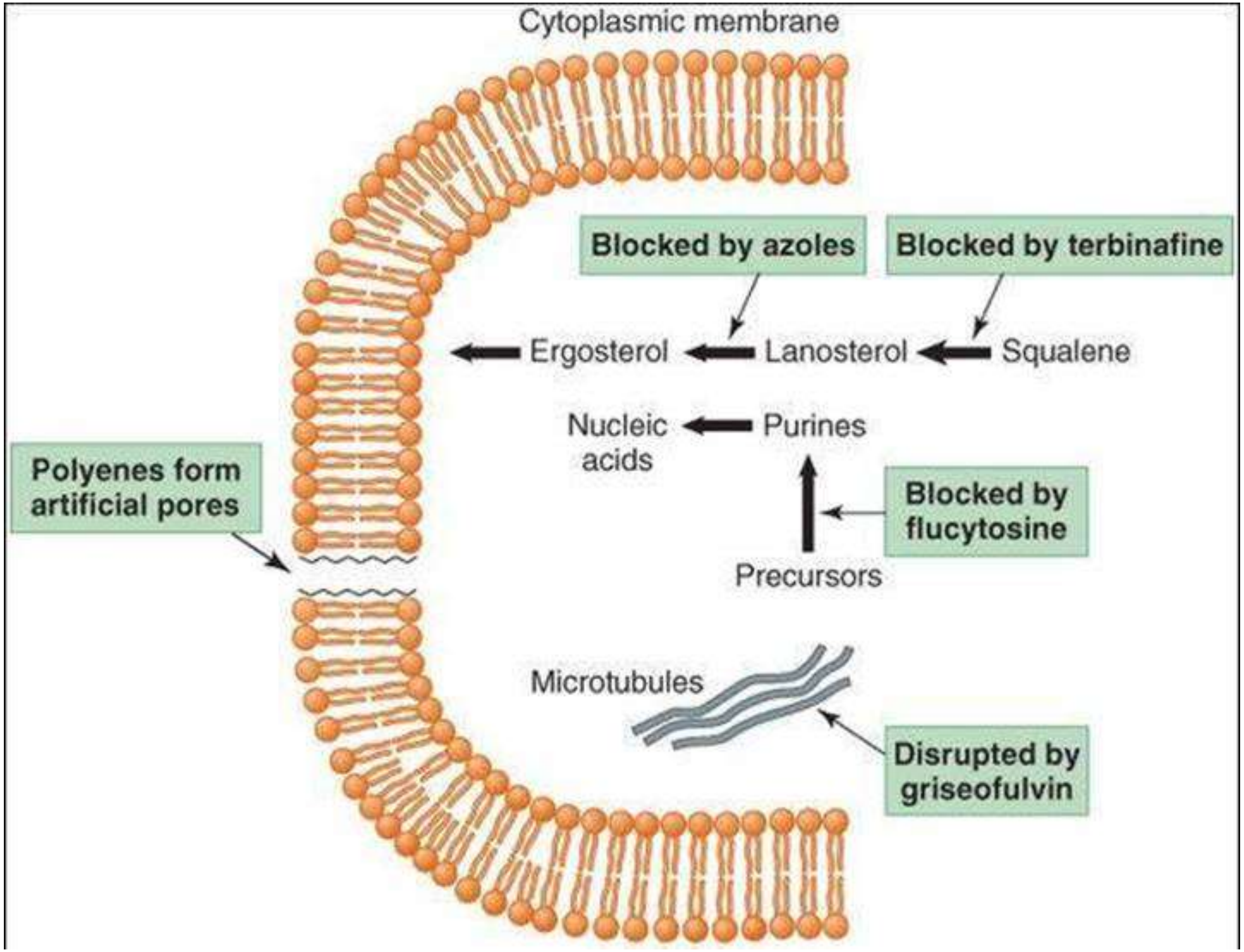
Parentral

others

Antifungals are selectively toxic to fungi because they interact with or inhibit the synthesis of ergosterol, a sterol unique to fungal cell membranes.



Mechanisms of antifungals



LABORATORY TESTING OF ANTIMICROBIAL SUSCEPTIBILITY

- **Drug efficacy** determined based on clinical and laboratory parameters

- Antibiotic sensitivity testing or antibiotic susceptibility testing is the measurement of the susceptibility of bacteria to antibiotics. It is used because bacteria may have resistance to some antibiotics.

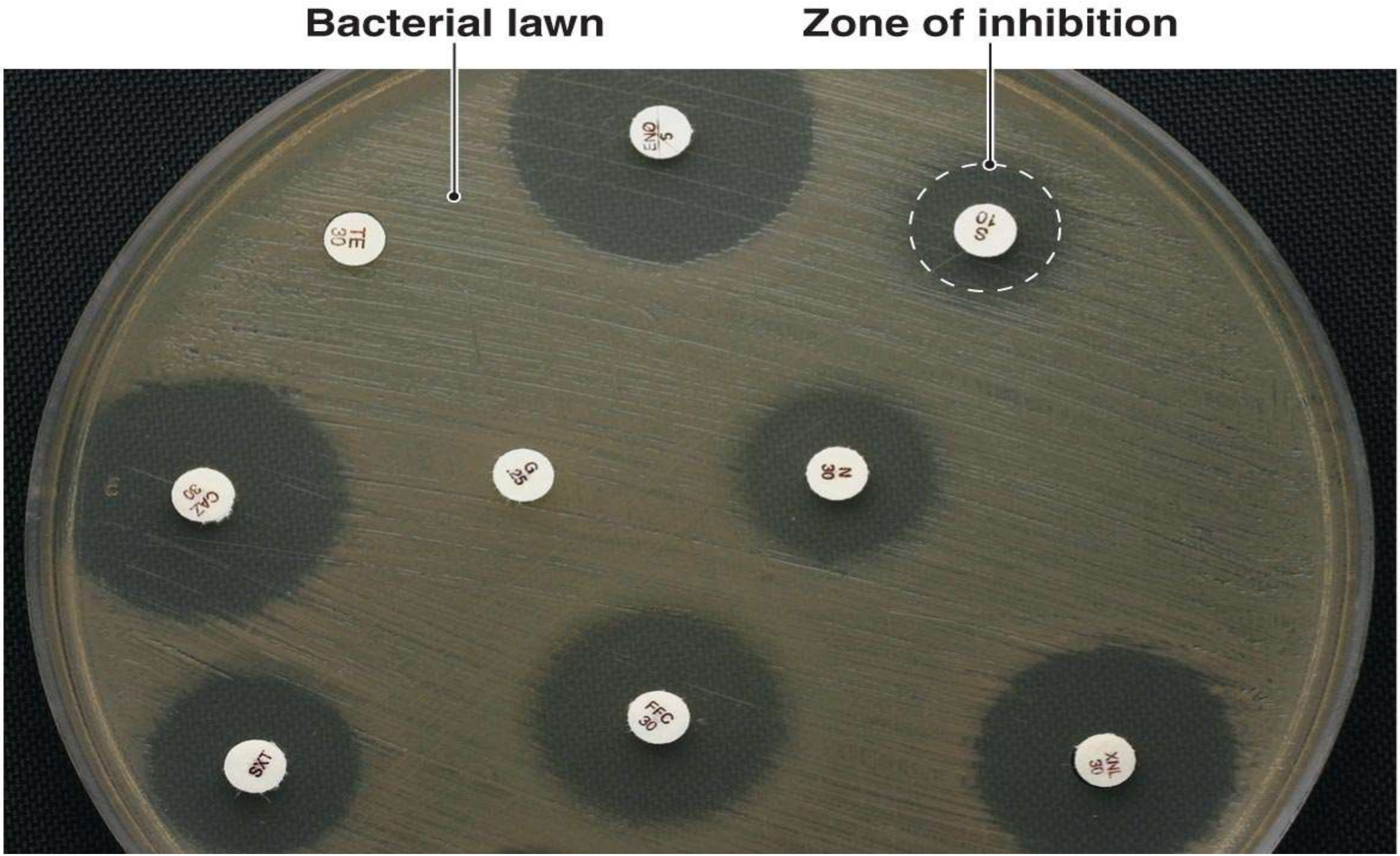
- Drug efficacy can be measured by susceptibility testing including:

1. Kirby-Bauer Method (diffusion test)
2. Broth dilution test
3. E-test

Kirby-Bauer Method (diffusion test)

- In diffusion testing (often called the Kirby-Bauer technique), the inoculum is seeded onto the surface of an agar plate, and filter paper disks containing defined amounts of antimicrobials are applied.

- The antimicrobial diffuses into the medium to produce a circular gradient around the disk. After incubation overnight, the size of the zone of growth inhibition around the disk can be used as an indirect measure of the MIC of the organism.
- The diameters of the zones of inhibition obtained with the various antibiotics are converted to “susceptible,” “intermediate,” or “resistant” categories by referring to a table.


Bacterial lawn
Zone of inhibition
ENO 5
TE 30
S 10
G .25
N 30
CAZ 30
SXT
FFC 30
XNL 30

E(epsilometer) test

- A newer version of the diffusion test, called an **E(epsilometer) test uses a plastic strip containing** a gradient of concentration of antibiotic.
- Printed on the strips are concentration values, allowing the laboratory technician to directly read off the minimum concentration needed to inhibit growth.

Diffusion tests

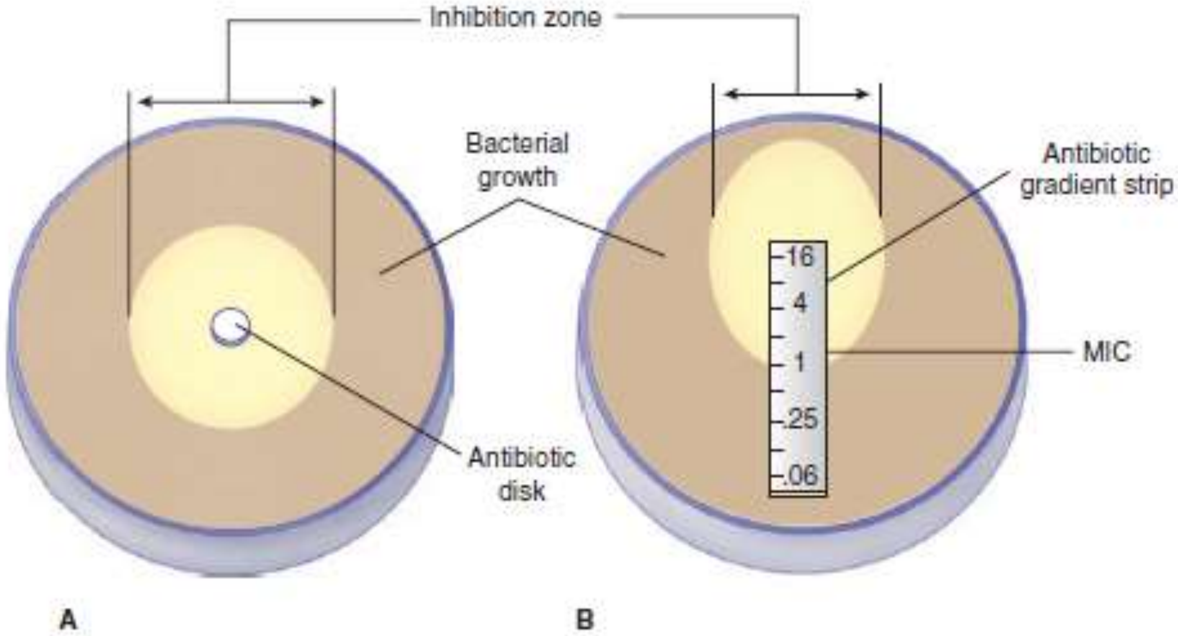


FIGURE 23–6. Diffusion tests. **A.** Disk diffusion. The diameter of the zone of growth inhibition around a disk of fixed antimicrobial content is inversely proportional to the minimum inhibitory concentration (MIC) for that antimicrobial; that is, the larger the zone, the lower the MIC. **B.** The E test. A strip containing a gradient of antimicrobial content creates an elliptical zone of inhibition. The conditions are empirically adjusted so that the MIC endpoint is where the growth intersects the strip.

Broth dilution test

Quantitative subculture of the clear tubes in the broth dilution test and comparison of the number of viable bacteria at the beginning and end of the test.