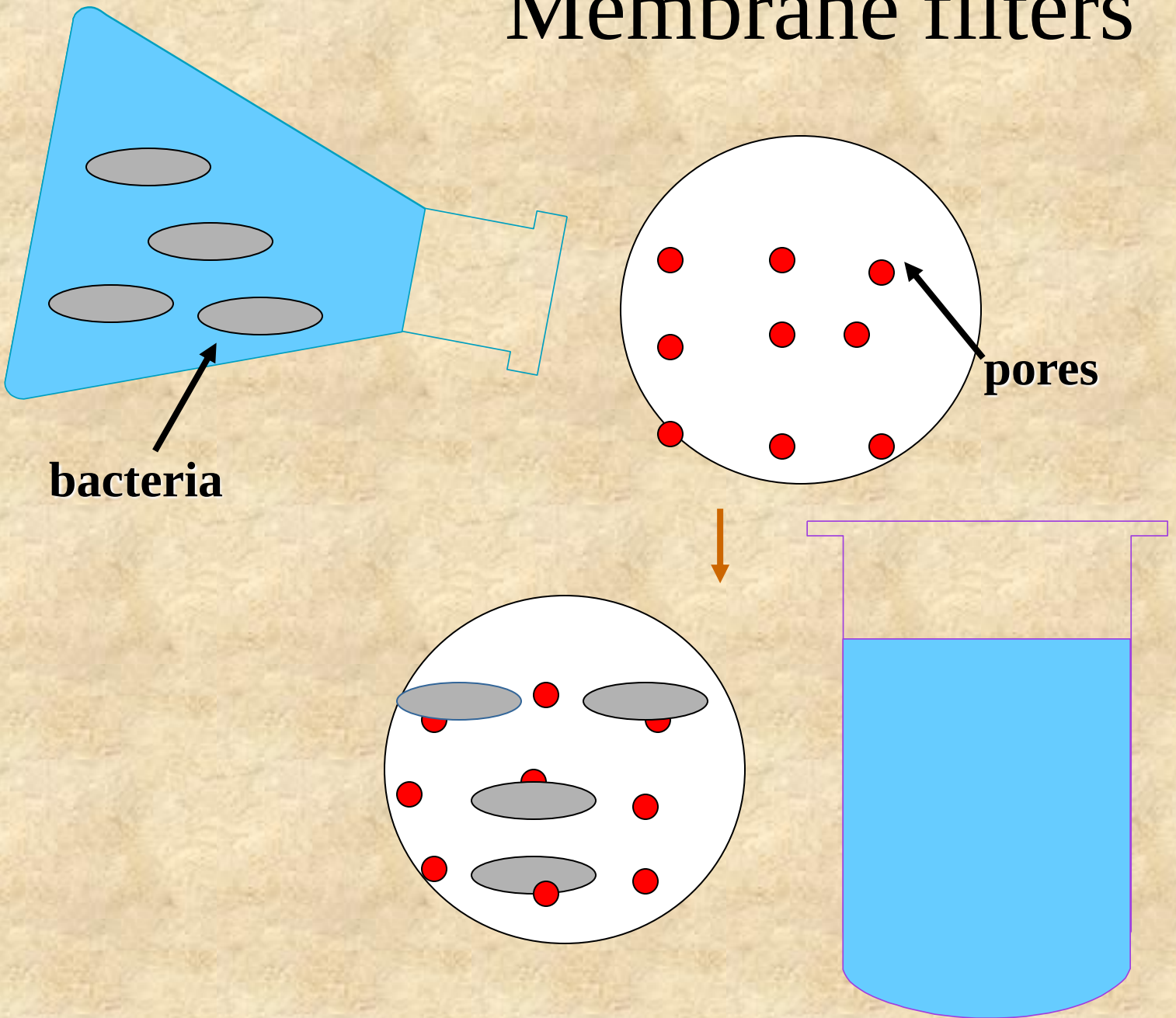
STERILIZATION

- All killed
- non-selective

Sterilization

- **autoclaving**
  - 121°C (heat/pressure)
    - * Heat resistant materials
- **ethylene oxide**
  - Non heat resistant
  - usually equipment
- **ultra-violet light**
  - surfaces (e.g operating rooms)
  - not totally effective

Membrane filters



Disinfection

- Liquids that kill bacteria
  - e.g. phenol based
  - too toxic for skin surfaces

Antiseptics

- Topical (e.g. skin)
  - e.g. iodine or 70% alcohol
  - "reduce" bacterial load

ANTIBIOTICS

- Selectively toxic for bacteria
  - bactericidal (killing)
  - bacteriostatic (growth inhibition)
- no harm to patient
- destroy structures
  - ✓ present in bacteria
  - ✓ not present in host
  - **Antibiotics work together with immune system**

Terminology :

- **Antibacterial spectrum**: range of activity of a compound against microorganisms.
  - ✓**A broad – spectrum antibacterial drug** can inhibit a wide variety of both gram-positive and gram-negative bacteria,
  - ✓ whereas a **narrow spectrum drug** is active only against selected organisms;
  - ✓**Natural spectrum-**when the Ab was discovered
  - ✓**Actual** spectrum–in a specific area, moment,
- **antimicrobial activity**:
  - **bacteriostatic activity** of a chemotherapeutic agent tested in the laboratory and expressed as the lowest concentration at which the drug inhibits multiplication of the organism (minimum inhibitory concentration, or MIC);
  - **bactericidal activity**: ability of a chemotherapeutic agent to kill a microorganism; expressed as the minimum bactericidal concentration (MBC);

Minimal inhibitory concentration (MIC)

- lowest level stopping growth
- e. g. zone of inhibition around a disk impregnated with antibiotic

History:

- **1928-Fleming** - first noted that the mold *Penicillium* prevented the multiplication of staphylococci. A concentrate from a culture of this mold was prepared, and the remarkable activity and lack of toxicity of the first antibiotic, penicillin, was demonstrated. Later, in the 1940s and 1950s, streptomycin and the tetracyclines were developed and were followed rapidly by additional aminoglycosides, semisynthetic penicillins, cephalosporins, quinolones and other antimicrobials. All greatly increased the range and effectiveness of antibacterial agents.
- **1935- the red azo dye protosil** was shown to protect mice against systemic streptococcal infection and was curative in patients suffering from such infections. These observations with the first sulfa drug initiated a new era in medicine. Compounds (antibiotics) produced by microorganisms were eventually discovered to inhibit the growth of other microorganisms.

- Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable **ability to develop resistance to these agents. Thus antibiotic therapy will not be the predicted magic bullet against infections**.
- **The results of in vitro antimicrobial susceptibility testing are valuable for selecting chemotherapeutic agents active against the infecting organism.**
- **Extensive work has been performed to standardize the testing methods and improve the clinical predictive value** of the results.
- Despite these efforts, the in vitro tests are simply a measurement of the effect of the antibiotic against the organism.
- **Selection of an antibiotic and the patient's outcome are influenced by a variety of interrelated factors, including**
  - **the pharmacokinetic properties of the antibiotic,**
  - **drug toxicity,**
  - **and the patient's general medical status.**

The five basic sites of antibiotic activity are summarized:

- **inhibition of cell wall synthesis** (penicillins, cephalosporins, cephamycins, carbapenems, monobactams, β-lactamase inhibitors, vancomycin, bacitracin, isoniazid, cycloserine, ethionamide);
- **alteration of cell membranes** (polymixins);
- **inhibition of protein synthesis** (aminoglycosides, tetracyclines, chloramphenicol, macrolides, clindamycin);
- **inhibition of nucleic acid synthesis** (rifampin, quinolones, metronidazole);
- **antimetabolites** (sulfonamides, trimethoprim, dapsone).

Antibiotics that affect the Cell Envelope

I. Inhibition of cell wall synthesis

Antibiotics that inhibit cell wall biosynthesis are bactericidal

- **Without cell wall osmotic pressure causes bacteria to burst**

- most common site of antibiotic activity. The majority of the cell wall active antibiotics are classified as:

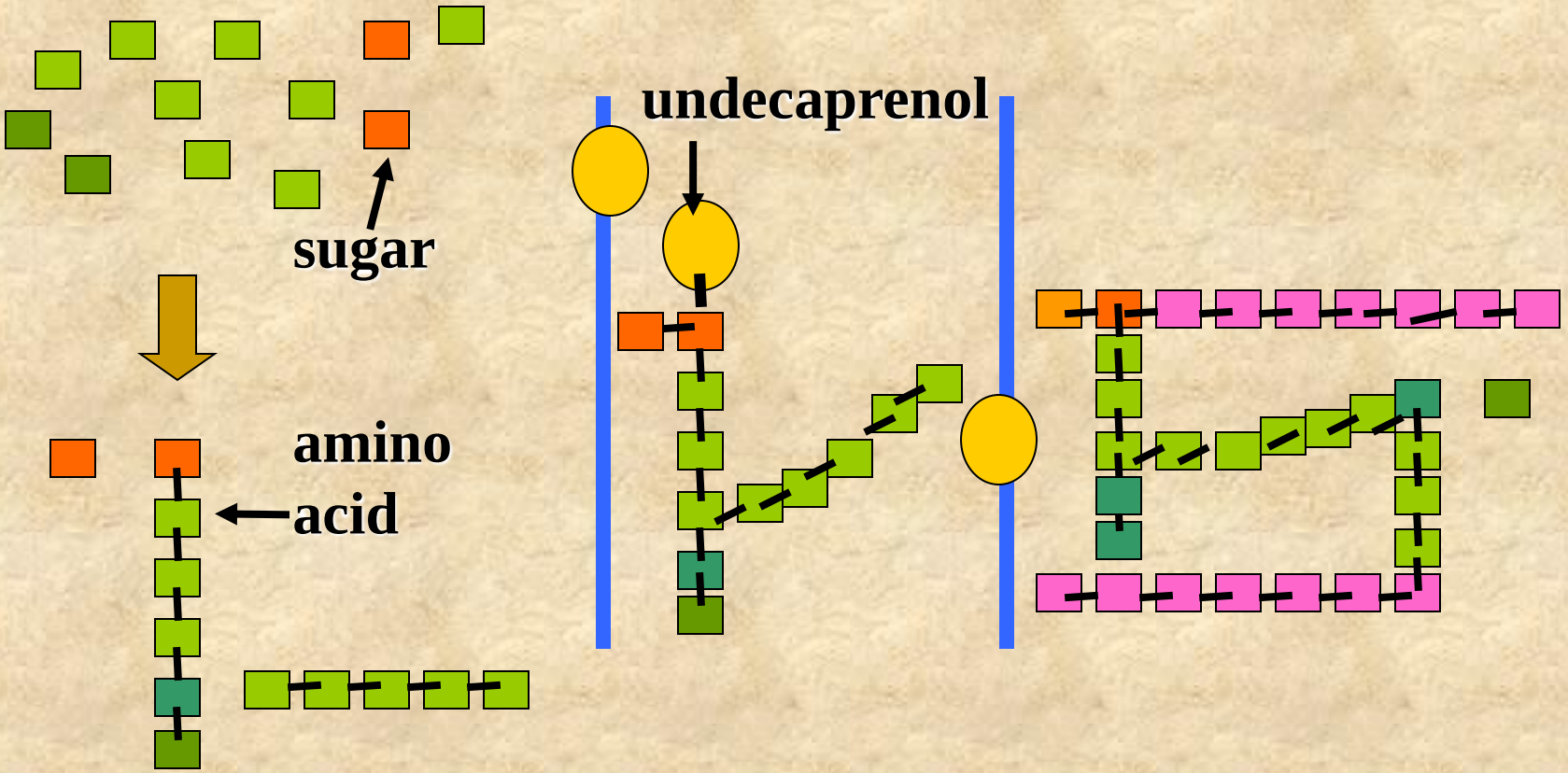
1. β-lactam antibiotics (e.g., penicillins, cephalo-sporins, cephamycins, carbapenems, monobactams, and β-lactamase inhibitors), so named because they share a common β-lactam ring structure.

2. vancomycin,

3. bacitracin,

4. and the antimycobacterial agents: isoniazid, cycloserine, and ethionamide.

Peptidoglycan synthesis



4. Cycloserine Ethionamide, and Isoniazid

Modes of Action, Spectrum of Activity, and Mechanisms of Resistance

- are antibiotics useful for the management of some mycobacterial infections.

-Cycloserine inhibits two enzymes that catalyze cell wall synthesis, D-alanyl-D-alanine synthetase and alanine racemase.

- Ethionamide and isoniazid interfere with mycobacterial replication at multiple levels.

-Resistance is mediated by either reduced drug uptake into the bacterial cell or alteration of the target sites.

3. Bacitracin

- Bacitracin, another cell wall-active antibiotic, is a mixture of polypeptides used topically for skin infections caused by gram-positive bacteria.
- It inhibits cell wall synthesis by interfering with dephosphorylation of the lipid carrier responsible for moving the peptidoglycan precursors through the cytoplasmic membrane to the cell wall.
- It may also damage the bacterial cytoplasmic membrane and inhibit RNA transcription.
- Resistance is most likely due to failure of the antibiotic to penetrate into the bacterial cell.

2. Vancomycin

- Vancomycin, obtained from an actinomycete, is a complex glycopeptide that interferes with cell wall synthesis in growing gram-positive bacteria.
- Vancomycin acts by interfering with elongation of the peptidoglycan chain
- Vancomycin is inactive against gram-negative bacteria because the molecule is too large to pass through the outer membrane and reach the peptidoglycan target site.

•Vancomycin is used for the management of infections with **oxacillin-resistant staphylococci (MRSA)**, *Clostridium difficile*, and other gram-positive bacteria that are resistant to β-lactam antibiotics. Resistance among gram-positive bacteria is uncommon but has been reported for *Leuconostoc*, *Lactobacillus*, *Pediococcus*, *Erysipelothrix*, and for rare isolates of *Enterococcus* and *Staphylococcus*.

•Vancomycin resistance occurs in bacteria with alterations of the terminal side chain (presence of L-alanine rather than D-alanine) or in strains producing a protein that interferes with vancomycin binding to its target site.

1. Beta lactam antibiotics

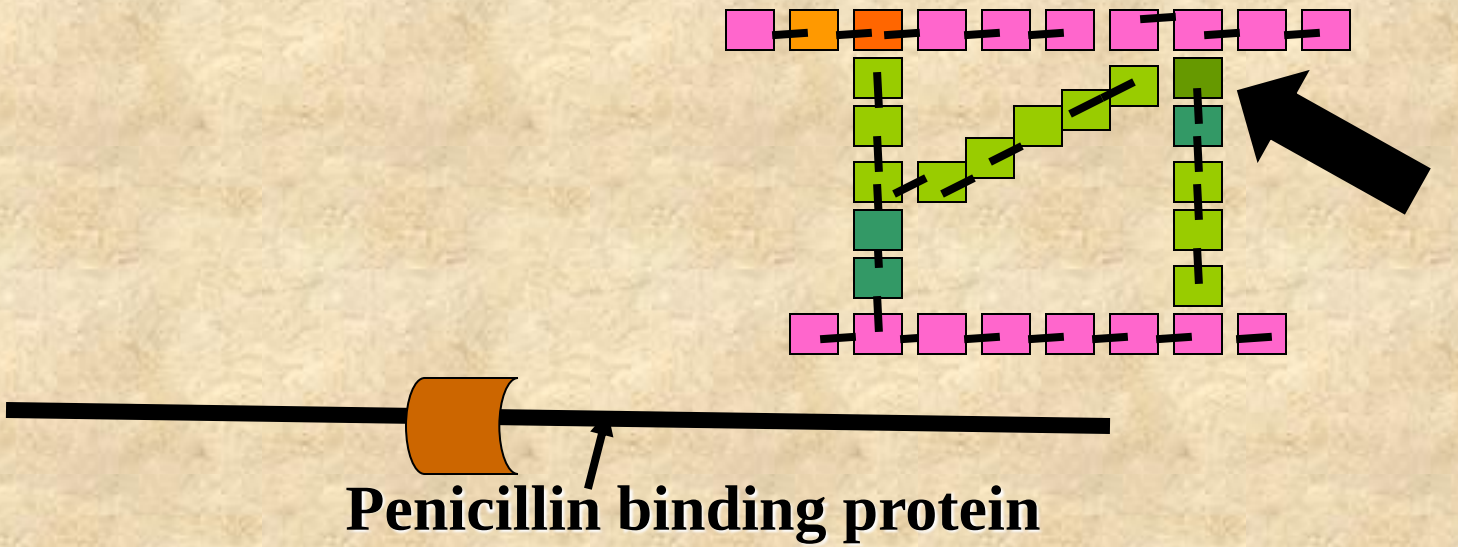
- penicillins
- cephalosporins
- monobactams

✓inhibit penicillin binding proteins

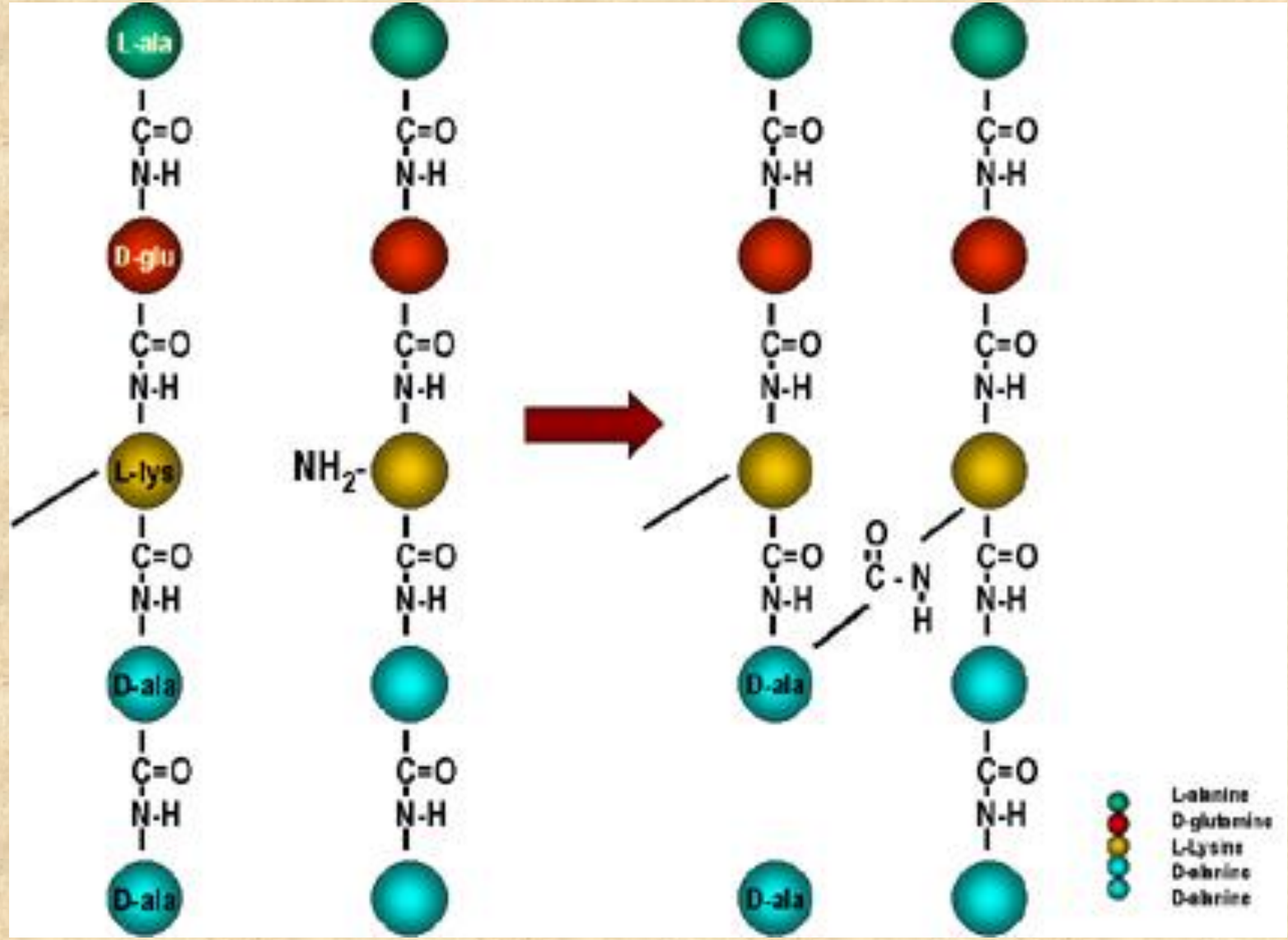
✓stop cross-linking

Beta lactams

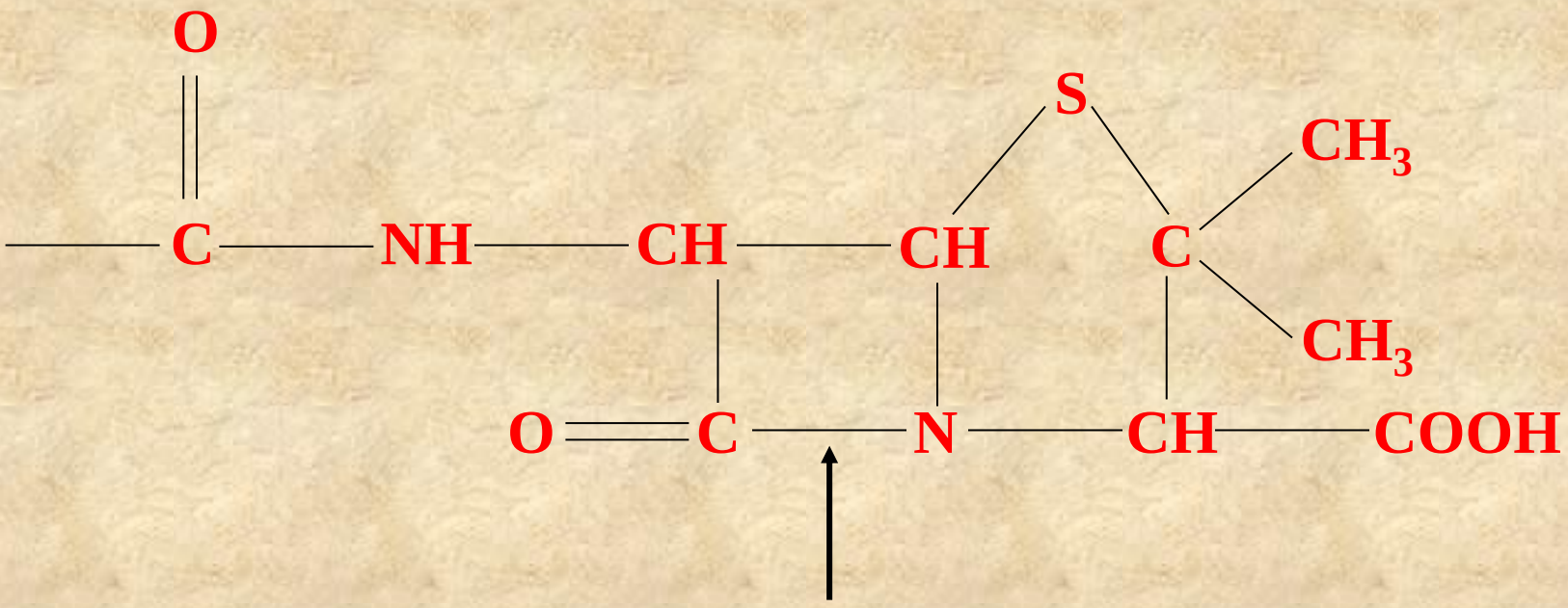
Cell wall



Cross-linking of peptidoglycan



STRUCTURE OF PENICILLIN



Site of penicillinase action.

Breakage of the beta lactam ring.

Attached to lactam ring

- penicillins
  - 5 membered ring
- cephalosporins
  - 6 membered ring
- monobactams
  - no second ring

Mode of Action

- Synthesis of the bacterial cell wall is catalyzed by specific enzymes (e.g., transpeptidases, carboxy-peptidases, and endopeptidases). These regulatory proteins are also called **penicillin binding proteins** (PBPs) because they can be bound by β-lactam antibiotics. When growing bacteria are exposed to these antibiotics, the antibiotic binds to the PBPs in the cell membrane,
  - synthesis of the cell wall peptidoglycan layer is inhibited,
  - and autolytic enzymes are released that degrade the preformed cell wall, resulting in bacterial cell death.
  - thus the β-lactam antibiotics generally act as bactericidal agents.

Spectrum of Activity

- **Penicillins.** Penicillin compounds are highly effective antibiotics with extremely low toxicity.
  - ✓**Penicillin G** is incompletely absorbed because it is inactivated by gastric acid. Thus it is used mainly as an intravenous drug for serious infections with penicillin-sensitive organisms (e.g., streptococci, gonococcus).
  - ✓**Penicillin V** is more resistant to acid and is the preferred oral form for treatment of susceptible streptococci.
  - ✓**Penicillinase-resistant penicillins** such as nafcillin and cloxacillin are used to treat infections caused by penicillinase-producing staphylococci.
  - ✓**Ampicillin** was the first penicillin active against GNBi
  - ✓**Parenteral penicillins** (e.g., carbenicillin, ticarcillin, piperacillin) have been now been developed that can be effective against a broad spectrum of gram-negative bacteria (*Klebsiella*, *Enterobacter*, *Pseudomonas).*

▪**Cephalosporins and Cephamycins**. The cephalosporins are β-lactam antibiotics derived from 7-aminocephalosporanic acid, which was originally isolated for a *Cephalosporium* mold; the cephamycins are closely related. The cephalosporins and cephamycins have the same mechanism of action as the penicillins but have a wider antibacterial spectrum, are resistant to many β-lactamases, and have improved pharmacokinetic properties.

- ✓ The activity of the **first-generation antibiotics(Cefalexin, Cephalotin)** is similar to that of ampicillin.
- ✓ Many of the **second-generation antibiotics (Cefaclor, Cefuroxime,** have expanded activity to include *Haemophilus influenzae*, an important pediatric pathogen, and **Cefoxitin and Cefotetan** are active against *Bacteroides fragilis*, an important anaerobic pathogen.
- ✓ The **third-generation antibiotics (Ceftazidime, Cefotaxime, Cefoperazona)**further extend the antibacterial spectrum to include virtually all Enterobacteriaceae and *P. aeruginosa*.

✓Unfortunately, the second- and third-generation antibiotics were frequently less active against gram-positive cocci.

✓Furthermore, all cephalosporin-type antibiotics are ineffective against penicillin-resistant *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus*, as well as *Enterococcus*, and *Listeria*.

✓In addition, organisms such as *Enterobacter*, *Serratia*, and *Pseudomonas* can develop resistance during therapy with the cephalosporins and then display cross-resistance to all β-lactam.

- **Other β-Lactam Antibiotics**. Several β-lactam antibiotics have slightly different biochemical structures from the penicillins and cephalosporins but have similar potent antibacterial activity.

  ✓**Imipenem is a carbapenem** with excellent in vitro and in vivo activity against aerobic and anaerobic gram-positive and gram-negative bacteria.

  ✓ **Aztreonam, a monobactam**, is a narrow-spectrum antibiotic with activity specific for GNB (Enterobacteriaceae, *Pseudomonas*).

•Finally, **β-lactamase inhibitors (e.g., clavulanic acid,sulbactam)**

•-**binds strongly beta lactamases inhibits activity**

–are relatively inactive by themselves but have been combined with some penicillins (e.g., ampicillin, amoxicillin, ticarcillin) to treat infections caused by β-lactamase producing bacteria. This latter group of antibiotics will irreversibly bind and inactivate bacterial β-lactamases, permitting the companion drug to enter the cell and disrupt bacterial cell wall synthesis.

Resistance mechansims

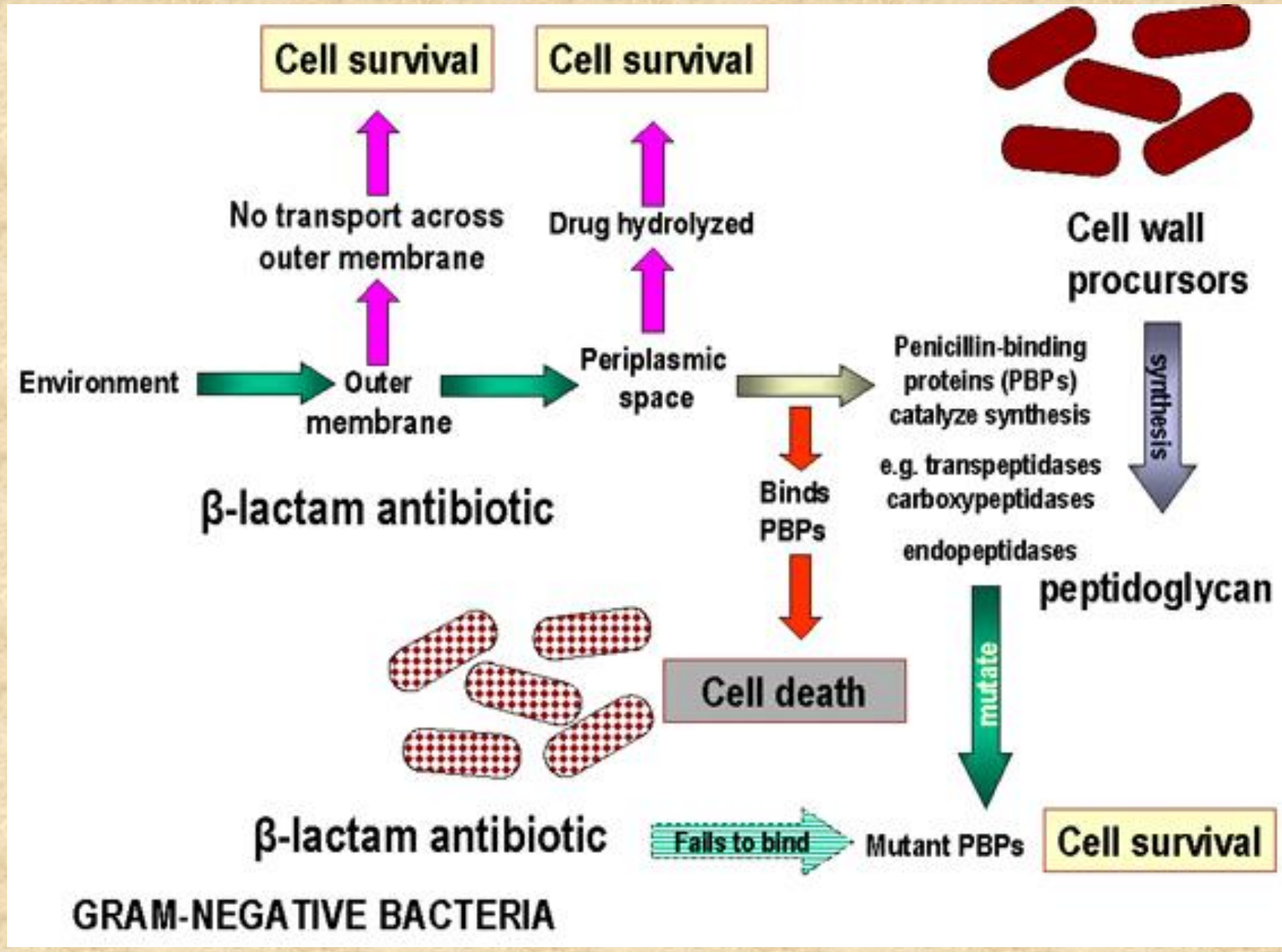
- ✓ Produce beta lactamase (penicillinase)-destroys antibiotic
- ✓ modified penicillin binding proteins (PBP)- don't bind antibiotic
- ✓ modified porins- no internalization of antibiotic

Mechanisms of Resistance: three general mechanisms of resistance occur:

✓**failure of the antibiotic to penetrate through the outer membrane-**mutation of the porin proteins can render the organism resistant to the β-lactam antibiotic (e.g., resistance of *Pseudomonas aeruginosa* to Imipenem).

✓**failure to bind to the target site (penicillin binding proteins)** - mutation in the penicillin binding proteins can lead to antibiotic resistance. This mechanism is responsible **for oxacillin resistance in staphylococci (MRSA**)and penicillin resistance in *Streptococcus pneumoniae*.

✓**hydrolysis of the antibiotic by β-lactamases-**despite the initial success of penicillin G against staphylococci, resistance mediated by β-lactamase hydrolysis developed rapidly. Unfortunately this resistance was not restricted to the early β-lactam antibiotics. Simple point mutations in the genes for the initial β-lactamases (enzymes with a narrow spectrum of activity and present in many bacteria) have now rendered these enzymes active against most penicillins and cephalosporins, including the broad spectrum agents. **Because these potent β-lactamases are present on plasmids and can be exchanged among bacterial species, the utility of β-lactam antibiotics may be severely limited in the future.**


Cell survival
Cell survival
No transport across outer membrane
Drug hydrolyzed
Cell wall procursors
Environment
Outer membrane
Periplasmic space
Penicillin-binding proteins (PBPs) catalyze synthesis
synthesis
e.g. transpeptidases carboxypeptidases endopeptidases
peptidoglycan
β-lactam antibiotic
Binds PBPs
Cell death
mutate
β-lactam antibiotic
Fails to bind
Mutant PBPs
Cell survival
GRAM-NEGATIVE BACTERIA

Cell wall procursors

synthesis

peptidoglycan

Penicillin-binding proteins (PBPs) catalyze synthesis

e.g. transpeptidases
carboxypeptidases
endopeptidases

mutate

Mutant PBPs

Falls to bind

Cell survival

β-lactam antibiotic

Binds to PBPs and inhibits peptidoglycan synthesis

Cell death

Drug hydrolyzed

Cell survival

GRAM-POSITIVE BACTERIA

II. Alteration of cell membranes

•**The polymyxin class** of antibiotics is an important example of this activity. These antibiotics consist of cationic branched cyclic decapeptides that destroy the cytoplasmic membranes of susceptible bacteria.

•Members of this class of antibiotics include **polymyxin B and colistin**.

•These antibiotics are active against gram-negative bacteria;

• however, serious nephrotoxicity has limited their use to the external treatment of localized infections such as external otitis, eye infections, and skin infections with sensitive organisms.

•The detergent-like activity of the polymyxins is prevented when the antibiotic is unable to penetrate through the outer cell wall to the inner cytoplasmic membrane.

•Other antibiotics acting on the cell membrane include the **antifungal polyene antibiotics (e.g., amphotericin B, nystatin).**

Polymyxin B

- binds
  - lipid A
  - phospholipids

- disrupts outer membrane, Gram negative bacteria
- toxic to human cells

III. Inhibition of protein synthesis

Protein Synthesis Inhibitors

- Mostly bacteriostatic
- Selectivity due to differences in prokaryotic and eukaryotic ribosomes
- Some toxicity - eukaryotic 70S ribosomes

1. Antimicrobials that Bind to the 30S Ribosomal Subunit

•Aminoglycosides (bactericidal)

streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin, neomycin (topical)

- **Mode of action -** exert their effect by passing through the bacterial membranes and cell wall to the cytoplasm, where they inhibit bacterial protein synthesis by irreversibly binding to the ribosomes. Aminoglycosides can bind to several sites on the ribosome including the interface between the 30S and 5OS subunits as well as to the individual subunits..
- **Spectrum of Activity** - are bactericidal antibiotics due to irreversible binding to ribosomes and are commonly used to treat serious infections caused by many GNB and some CPB.
- Streptococci and anaerobes are resistant to aminoglycosides.

✓ Gentamicin and tobramycin have a broad spectrum of activity, with tobramycin being slightly more active against *Pseudomonas*

✓ Netilmicin- less ototoxic than gentamicin or tobramycin-but less antibacterial activity.

✓All three aminoglycosides are used to treat systemic infections caused by susceptible gram-negative bacteria, including the Enterobacteriaceae and *Pseudomonas*.

✓Because enzymatic modification of amikacin is rare, it is used to treat infections caused by gram-negative bacteria that are resistant to other aminoglycosides.

✓ **Streptomycin** has been used for the treatment of tuberculosis, tularemia, and streptococcal endocarditis (when combined with a penicillin).

✓ Although **kanamycin** was one of the first aminoglycosides with broad activity against gram-negative bacteria, it is now rarely used

•**Resistance -** can develop in one of three ways:

- mutation of the ribosome binding site,
- decreased antibiotic uptake into the bacterial cell,
- and enzymatic modification (e.g., acetylation, phosphorylation)

•**Synergy** - The aminoglycosides synergize with beta-lactam antibiotics- inhibit cell wall synthesis and increase the permeability of the aminoglycosides.

•Spectinomycin (bacteriostatic)

- **Mode of action -** Spectinomycin reversibly interferes with m-RNA interaction with the 30S ribosome. It is structurally similar to the aminoglycosides but does not cause misreading of mRNA.
- **Spectrum of activity -** Used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*
- **Resistance -** Rare in *Neisseria gonorrhoeae*

•Tetracyclines (bacteriostatic)

tetracycline, minocycline and doxycycline

- **Mode of action** - reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.
- **Spectrum of activity** - Broad spectrum; Useful against ic bacteria
- bacteriostatic antibiotics that inhibit protein synthesis in bacteria
- Tetracyclines are effective in treatment of *Mycoplasma pneumoniae* infections, cholera, rickettsial disease, brucellosis, chlamydial urethritis, as well as gonorrhea, uncomplicated UTI
- **Resistance**- is primarily due to increased efflux of the antibiotic from the cell. The gene encoding for this mechanism is on a transferable plasmid.
- **Adverse effects**
  - ✓ **Destruction of normal intestinal flora;**
  - ✓ **staining and impairment of the structure of bone and teeth.**

2. Antimicrobials that Bind to the 50S Ribosomal Subunit

•Chloramphenicol

- **Mode of action -** These antimicrobial bind to the 50S ribosome and inhibit peptidyl transferase activity.
- **Spectrum of activity** has a broad antibacterial spectrum similar to that of tetracycline but is considered the drug of choice only for treatment of typhoid fever. The reason is that, chloramphenicol disrupts protein synthesis in human bone marrow cells and can produce blood dyscrasias such as aplastic anemia (1 case per 24,000 treated patients). Chloramphenicol **exerts its effect on bacterial protein synthesis by binding to the 5OS subunit and blocking peptide bond formation.**
- **Resistance -** in bacteria producing chloramphenicol acetyltransferase, which catalyzes acetylation of the β-hydroxy group of chloramphenicol.
- **Adverse effects - is toxic (bone marrow suppression) but is used in the treatment of bacterial meningitis.**

•Lincosamides: Lincomycin, Clindamycin (bacteriostatic)

•**Clindamycin**, like chloramphenicol and the macrolides, blocks protein synthesis by binding to the 5OS ribosome. It inhibits peptidyl transferase by interfering with binding of the amino acid-acyl-tRNA complex.

•Clindamycin is active against staphylococci and anaerobic gram-negative bacilli but generally inactive against aerobic gram-negative bacteria

•Bacterial resistance is mediated by induction of an enzyme that methylates the 5OS ribosomal RNA. Because both erythromycin and clindamycin can induce this enzymatic resistance (also plasmid-mediated), cross-resistance between these two classes of antibiotics is observed.

•Macrolides (bacteriostatic)

erythromycin, clarithromycin, azithromycin, spiramycin

- **Mode of action -** The macrolides inhibit translocation. The antibiotic disrupts protein synthesis by binding to the 5OS ribosomal subunit.
- **Spectrum of activity -** Gram-positive bacteria,
- Erythromycin, a macrolide antibiotic, is a bacteriostatic organic base used mainly to treat pulmonary infections caused by *Mycoplasma*, *Legionella*, *Chlamydia*, *Campylobacter*, and gram-positive organisms **in patients allergic to penicillin.**
- **Resistance -** develops by modification of ribosomal RNA, which prevents binding by the antibiotic.
- Modification of the macrolide structure has led to the development of newer agents including **azithromycin and clarithromycin**. These macrolides have better pharmacological properties, as well as improved antibacterial activity.

- **Antimicrobials that Interfere with Elongation Factors**

<u>Fusidic acid</u> (bacteriostatic)

Mode of action - binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

S**pectrum of activity -** Gram-positive cocci

IV. Inhibitors of Nucleic Acid Synthesis

1. Inhibitors of RNA Synthesis

Selectivity due to differences between prokaryotic and eukaryotic RNA polymerase

•Rifampin, Rifamycin, Rifampicin, Rifabutin (bactericidal)

- **Mode of action -** These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of mRNA synthesis.
- Rifampin, a semisynthetic derivative of rifamycin B produced by *Streptomyces mediterranei*,
- **Spectrum of activity -** Wide spectrum but is used most commonly in the treatment of tuberculosis- is bactericidal for *Mycobacterium tuberculosis* and is very active against aerobic gram-positive cocci, including staphylococci (including MRSA) and streptococci.
- **Resistance -** can develop rapidly. Alteration of the polymerase leads to rifampin resistance
- **Combination therapy -** Since resistance is common, rifampin is usually used in combination therapy.

2. Inhibitors of DNA Synthesis

Selectivity due to differences between prokaryotic and eukaryotic enzymes

•Quinolones (bactericidal)

nalidixic acid, **ciprofloxacin**, ofloxacin, norfloxacin, levofloxacin, lomefloxacin, sparfloxacin

- **Mode of action -** are synthetic chemoterapeutic agents that inhibit bacterial DNA gyrases, which is required to supercoil strands of bacterial DNA into the bacterial cell.
- **Spectrum of activity -** GPC and urinary tract infections
- Nalidixic acid was used to treat urinary tract infections caused by a variety of gram-negative bacteria, but resistance to the drug developed rapidly. This drug has now been replaced by newer, more active quinolones such as norfloxacin, ciprofloxacin, and ofloxacin.
- **Resistance -** Common for nalidixic acid; developing for ciprofloxacin
  - DNA gyrase consists of alpha and beta subunits, with binding of the quinolones to the alpha subunit. Alteration of this subunit is the principal mechanism of bacterial resistance, although decreased drug uptake has also been observed. Decreased uptake is mediated by changes in porin proteins on the bacterial surface. Both resistance mechanisms are chromosomally mediated.

•Metronidazole:

-Metronidazole was originally introduced as an oral agent for treatment of *Trichomonas vaginalis*.

- It is also effective in treatment of amebiasis, giardiasis, and serious anaerobic bacterial infections (including *Bacteriodes fragilis*) but has no significant activity against aerobic or facultatively anaerobic bacteria.

- The antimicrobial properties of metronidazole appear to be mediated by a partially reduced intermediate, which results in DNA breakage. A decreased rate of reduction of metronidazole to its active form has been observed in resistant strains of bacteria (e.g., *Bacteroides fragilis*).

V. Antimetabolite Antimicrobials

The final mechanism of antibiotic activity is illustrated by the sulfonamides, trimethoprim, and the antileprosy drug, dapsone.

-The sulfonamides **compete with p-aminobenzoic acid, preventing synthesis of folic acid that is required by certain microorganisms.**

-Because mammalian organisms do not synthesize folic acid (required as a vitamin), sulfonamides do not interfere with mammalian cell metabolism.

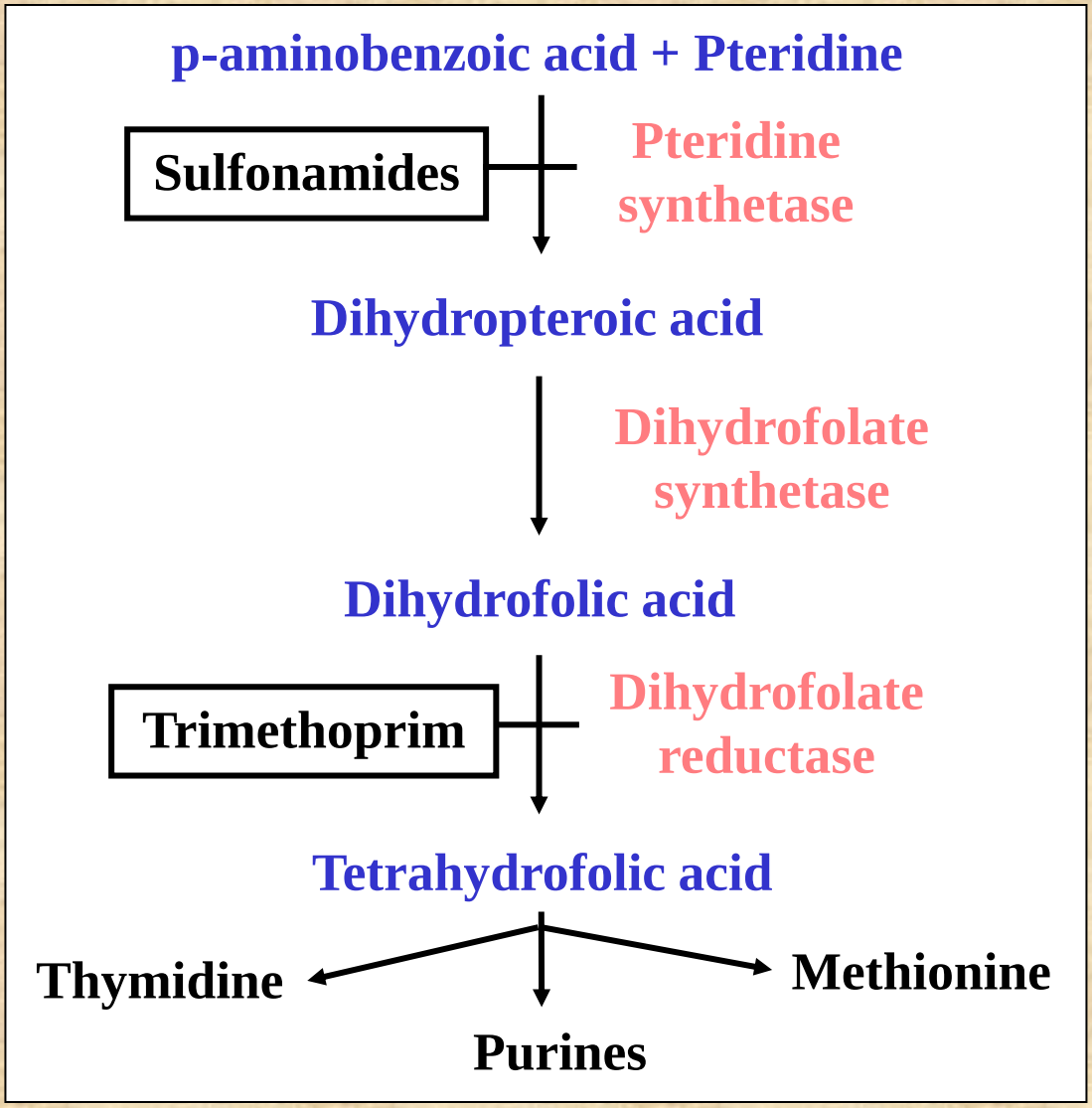
-Dapsone's activity is at the same site as the sulfonamides.

- **Trimethoprim** has a high affinity for dihydrofolate reductase, and competitively prevents conversion of dihydrofolate to tetrahydro-folate. This blocks the formation of thymidine, some purines, methionine, and glycine. Trimethoprim is commonly combined with sulfamethoxazole to produce a synergistic combination active at two steps in the synthesis of folic acid.

-Sulfonamides are effective against a broad range of gram-positive and gram-negative organisms, such as *Nocardia*, *Chlamydia*, and some protozoa.

Inhibitors of Folic Acid Synthesis

- Basis of Selectivity
- Review of Folic Acid Metabolism



Sulfonamides, Sulfones (bacteriostatic)

- **Mode of action** - These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteroic acid.
- **Spectrum of activity** - Broad range activity against gram-positive and gram-negative bacteria; used primarily in urinary tract and *Nocardia* infections.
- **Resistance** - Common
- **Combination therapy** - The sulfonamides are used in combination with trimethoprim; this combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

Trimethoprim, Methotrexate, Pyrimethamine (bacteriostatic)

- **Mode of action -** These antimicrobials binds to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.
- **Spectrum of activity -** Broad range activity against gram-positive and gram-negative bacteria; used primarily in **urinary tract and *Nocardia* infections**. Is effective against a large variety of gram-positive and gram-negative microorganisms and is the drug of choice for acute and chronic urinary tract infections. The combination is active in infections caused by ***Pneumocystis carinii***, bacterial infections of the lower respiratory tract, otitis media, and uncomplicated gonorrhea.
- **Resistance -** Common
- **Combination therapy -** These antimicrobials are used in combination with the sulfonamides; this combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

VI.Anti-Mycobacterial Antibiotics

Para-aminosalicylic acid (PSA)

(bacteriostatic)

- **Mode of action -** Similar to sulfonamides

- **Spectrum of activity -** Specific for *Mycobacterium tuberculosis*

Dapsone (bacteriostatic)

- **Mode of action -** Similar to sulfonamides

- **Spectrum of activity -** Used in treatment of leprosy (*Mycobacterium leprae*)

Isoniazid (INH) (bacteriostatic)

- **Mode of action -** Isoniazid inhibits synthesis of mycolic acids.
- **Spectrum of activity -** Used in treatment of tuberculosis
- **Resistance -** Has developed

- Combination therapy
- The activity of an antibiotic combination can differ depending on the individual components concerned.
  - ***Indifference*** occurs when the activity of the combination is equal to that of the more active component.
  - ***Addition*** takes place when the activity of the combination is equal to the sum of activities of the individual components.
  - ***Synergism*** *(potentiation)* is observed when the activity of the combination is significantly higher than the sum of activities of the individual components
  - ***Antagonism*** occurs when the activity of the combination is lower than that of the more active component.
  - The activity of an antibiotic combination is largely dependent on the ***bactericidal*** **or** ***bacteriostatic*** properties of the individual components. Antibiotics may thus be classified into four groups:
- **Prevent emergence of resistant strains**
- **Temporary treatment until diagnosis is made**
- Antibiotic synergism: Penicillins and aminoglycosides
- CAUTION: Antibiotic antagonism:Penicillins and bacteriostatic antibiotics

Antimicrobial Drug Resistance Mechanisms

•The phenomenon of bacterial resistance was detected by P. Ehrlich at the beginning of the chemotherapy era in 1909. When the microorganisms continue to proliferate at a therapeutically achievable antibiotic concentration, i.e. when the minimal inhibitory concentration (MIC) is higher in vitro than in vivo attainable serum or tissue concentration, bacterial resistance is present.

•This is caused by the lack of penetration of the antibiotic into the bacterial cell (permeability barrier), by modification of the "target" in the cell so that it is insensitive to the antibiotic, or, very importantly, by inactivation of the antibiotic due to the action of a bacterial enzyme.

•Not only the various species of bacteria but also the bacterial strains of a species differ with respect to their antibiotic susceptibility.

•Even within a bacterial population there are variants with different susceptibility. Susceptible and resistant microorganisms can readily be distinguished by means of the disc test, the antibiotic concentration normally attainable in the blood of the patient being regarded as the susceptibility limit.

- **Altered permeability**
  - Altered influx
    - Gram negative bacteria
  - Altered efflux
    - tetracycline
- **Inactivation**
  - β-lactamase
  - Chloramphenicol acetyl transferase
- **Altered target site**
  - Penicillin binding proteins (penicillins)
  - RNA polymerase (rifampin)
  - 30S ribosome (streptomycin)
- **Replacement of a sensitive pathway**-Acquisition of a resistant enzyme (sulfonamides, trimethoprim)

The following types of resistance are recognized:

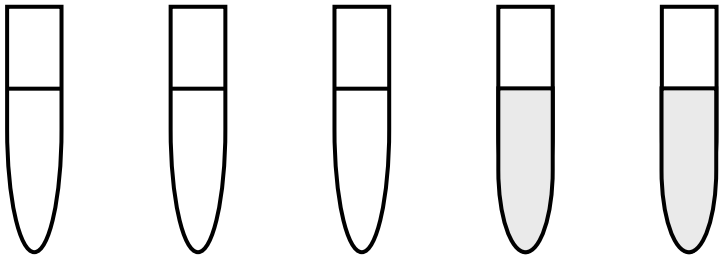
- Natural resistance which is due to a permanent genetically determined insensitivity of a bacterial species towards a certain antibiotic (example: all *Pseudomonas aeruginosa* strains are resistant to penicillin G).
- Primary resistance in which some of the existing strains of a bacterial species are resistant, while others are susceptible (example: 30 – 60% of all *E. coli* strains are resistant to tetracycline).
- Secondary resistance which is due to mutation or transfer of resistance in the case of individual organisms of an antibiotic-susceptible population. **In the development of resistant variants, antibiotics function as selecting agents: only the resistant organisms and their descendants survive under the "selection pressure" of an antibiotic so that the infection is finally maintained by a resistant population. Secondary resistance may develop at different rates.**

•A distinction is made between a rapid increase of resistance (one-step mutation) as, for example, with streptomycin, and a slow increase (multi-step mutation) as, for example, with penicillin. With the latter, several consecutive mutation steps are required for the emergence of resistance.

•Transferable resistance which is due to the transfer of genetic material, of either chromosomal or extrachromosomal origin from one bacterial cell to another. Three types of genetic transfer mechanisms have been found: ***transformation***, involving transfer of "naked" DNA; ***transduction***, in which the DNA from the donor is carried to the recipient inside a phage; and ***conjugation***, which requires contact of donor and recipient cells in which the genetic material is transferred through a channel between the two mating cells. The genes for drug resistance are carried not only on the chromosome but also on extrachromosomal elements or plasmids, which are called R factors.

Antibiotic Susceptibility Testing

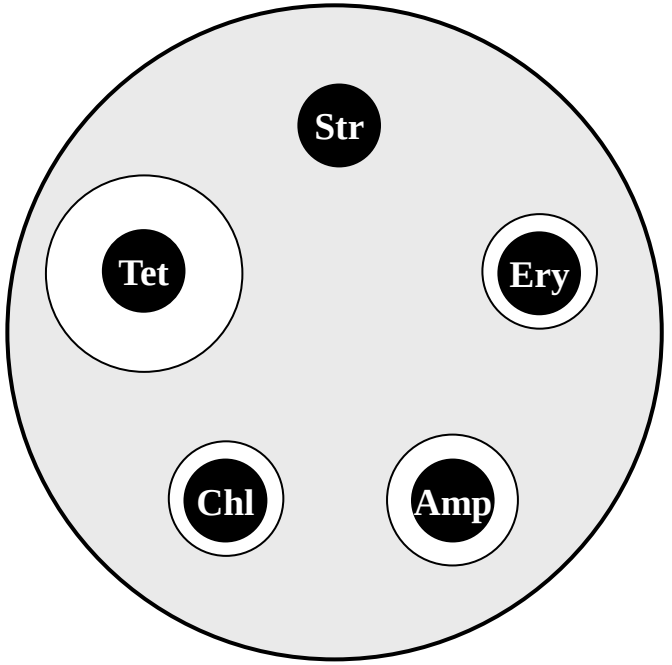
Determination of MIC



Tetracycline (□g/ml)

MIC = 2 □g/ml

Disk Diffusion Test



Zone Diameter Standards for Disk Diffusion Tests

Antimicrobial agent (amt. per disk) and organism	Zone diameter (mm)			Approx. MIC (μg/ml) for:	
	R	I	S	R	S
Ampicillin (10 μg)					
Enerobacteriacae	<11	12-13	>14	>32	<8
Haemophilus spp.	<19		>20	>4	<2
Enterococci	<16		>17	>16	
Tetracycline (30 μg)	<14	15-18	>19	>16	<4