Microbiology in Bioengineering
Course 3: Antibiotics

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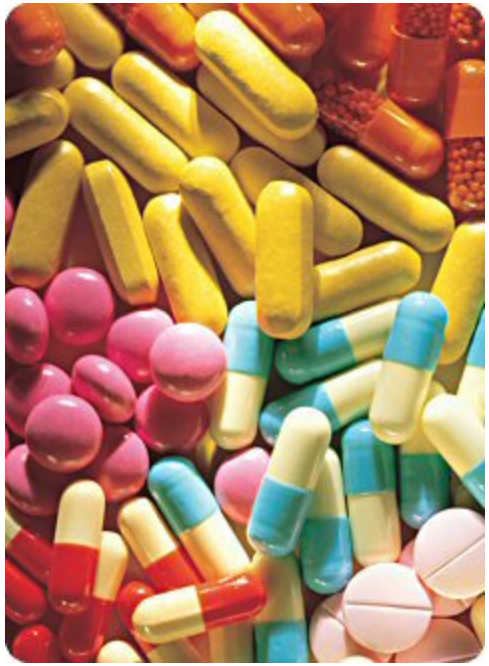
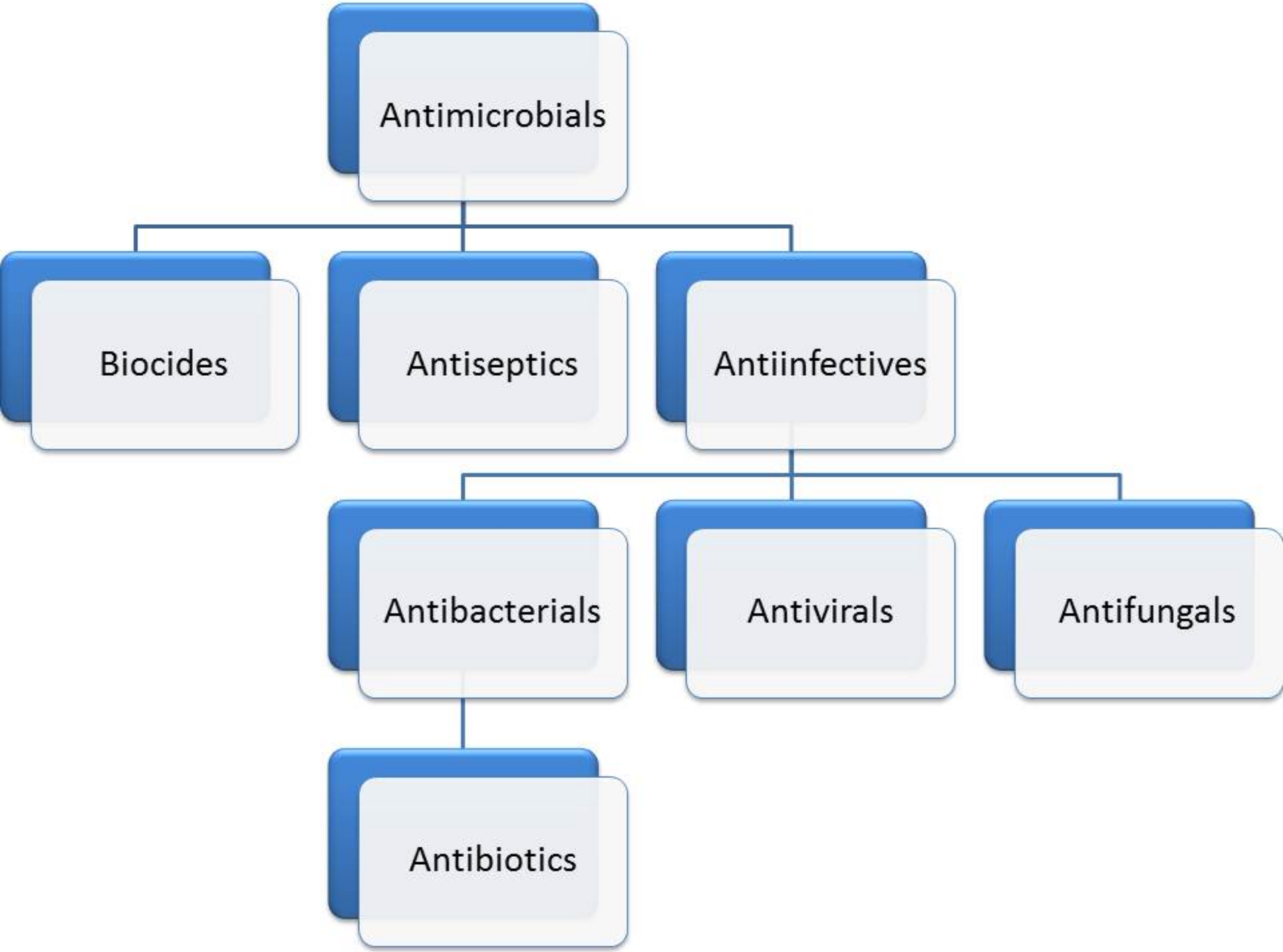
Antimicrobial Drugs: Effect Mechanisms



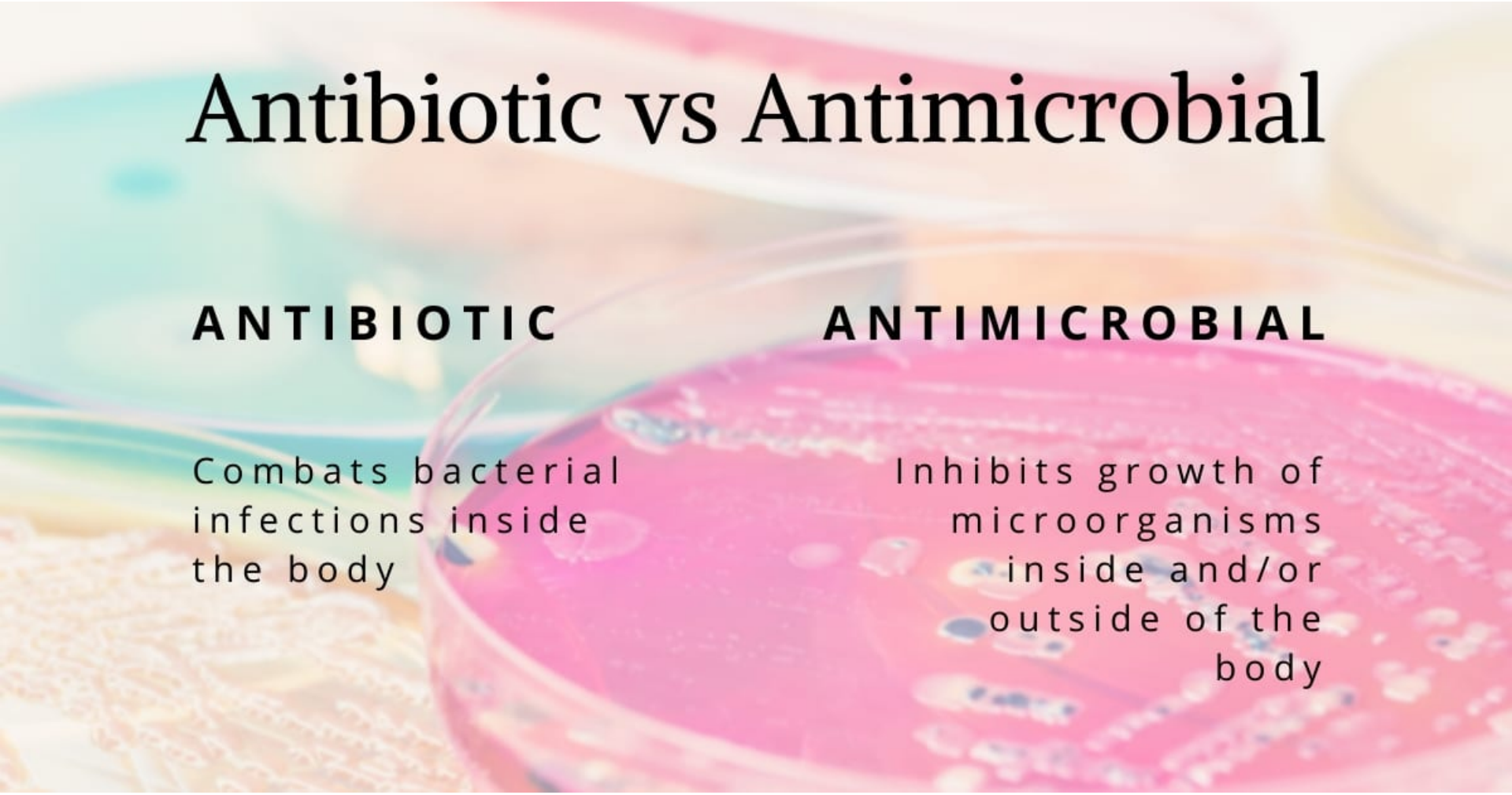
- The basis of antimicrobial therapy is to investigate selective toxicity, that is, to stop the growth of microorganisms without damaging the host

- Selective toxicity is to reveal the differences between microorganism and human metabolism.

- For example, penicillins and cephalosporins inhibit peptidoglycan synthesis found in bacteria but not human cells.


Antimicrobials
Biocides
Antiseptics
Antiinfectives
Antibacterials
Antivirals
Antifungals
Antibiotics

Antibiotic vs Antimicrobial



ANTIBIOTIC

Combats bacterial infections inside the body

ANTIMICROBIAL

Inhibits growth of microorganisms inside and/or outside of the body

What are antibiotics?

- **Antibiotics** are medicines used to kill or stop the growth of microbial life in the body
  - *In general conversation however, the term 'antibiotic' usually refers to medication for a bacterial infection*

- The term **antimicrobials** is accepted as a broader definition, and includes medicines used for:

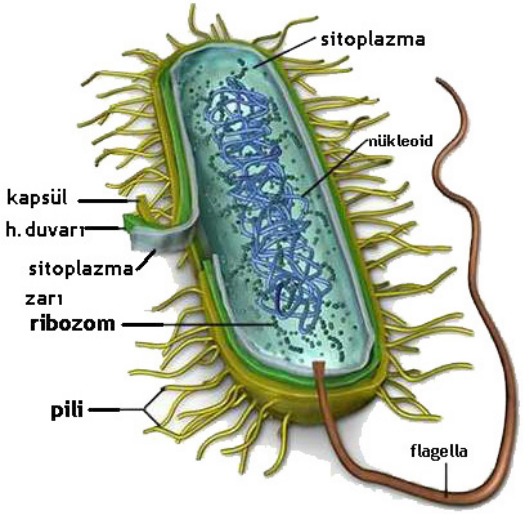
bacterial
viral
fungal
parasitic
} infections

CLINICAL EXCELLENCE COMMISSION

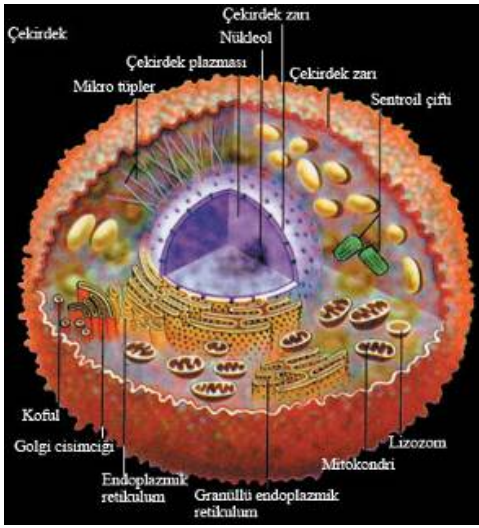
Main targets of Antibacterial Drugs

- There are four important differences that distinguish the bacterial cell from the human cell.
- These constitute the main targets of clinically effective drugs.

- These differences are;
  - Cell wall
  - Ribosomes
  - Nucleic acids
  - Cell membrane



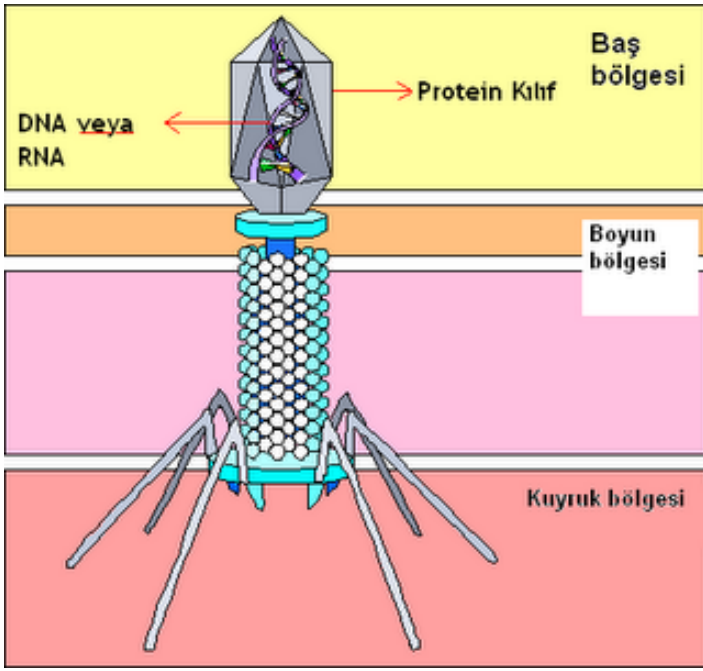
BACTERIA



HUMAN CELL

Why Antiviral Drugs are Less?

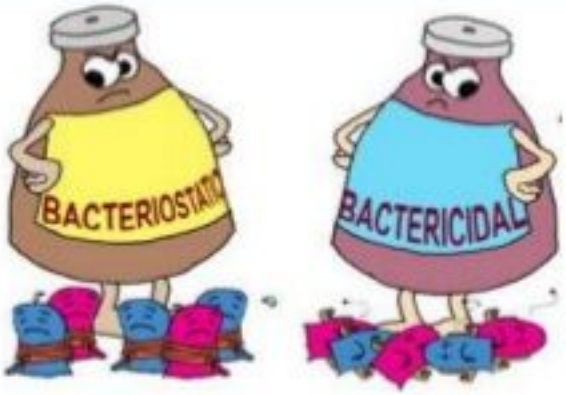
- There are much more antibacterial drugs than antiviral drugs.
- **Because viruses use many normal cellular functions of the host for reproduction, it is not easy to develop a drug that specifically inhibits viruses without damaging host cells.**



Classification of Antibiotics

Based on mode of Action

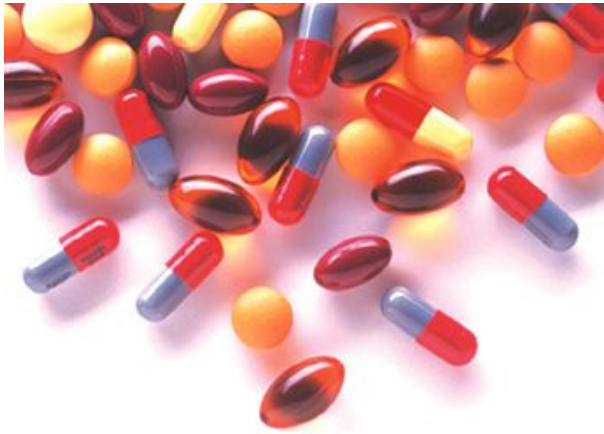
- Bacteriostatic
- Bactericidal



Based on their spectrum of action

- Broad-spectrum
- Narrow Spectrum

Antibiotics



- **Broad spectrum antibiotics**
  - are effective against many microorganisms.
- For example; tetracyclines shows activity against many gram-negative rods, chlamydia, mycoplasmas and rickettsia
- **Narrow-spectrum antibiotics**
  - on the other hand, are only effective against a few types.

Antibiotic activity

- **Bactericidal**
  - Kills the organism
  - Examples : B lactams , Vancomycin, Fluroquinolones, Aminoglycosides, Daptomycin, metronidazole
- **Bacteriostatic**
  - Inhibits the growth
  - Requires aid of host defenses
  - Relapses can occur after discontinuation of drug
  - Examples: Macrolides, Clindamycin, Sulfonamides, Linezolid, chloramphenicol

EFFECT MECHANISMS OF ANTIBACTERIAL DRUGS

1. INHIBITION OF CELL WALL SYNTHESIS
2. INHIBITION OF PROTEIN SYNTHESIS
3. INHIBITION OF NUCLEIC ACID SYNTHESIS
4. CHANGES IN CELLULAR MEMBRANE

Cell Wall (peptidoglycan synthesis)

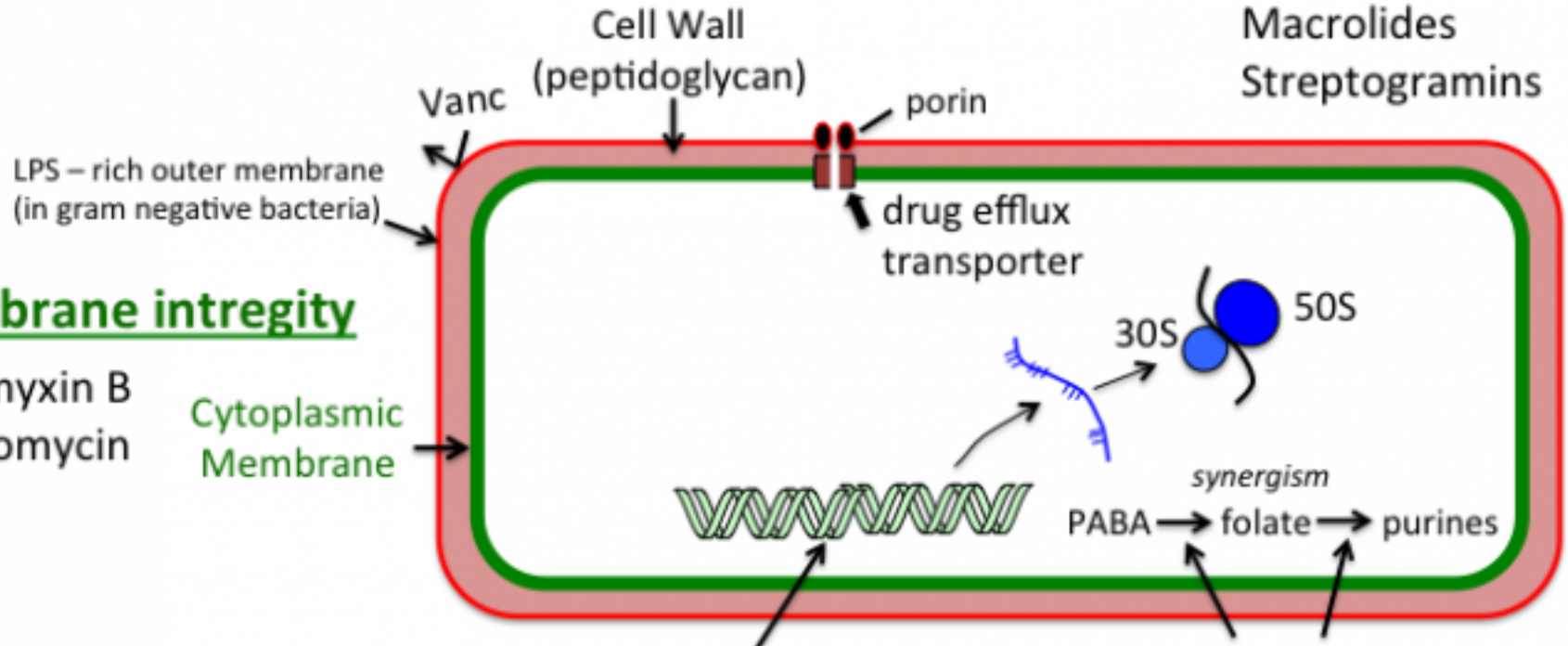
β-lactams
- Penicillins
- Cephalosporins
- Carbapenems
- Monobactams

Bacitracin
Glycopeptides

Protein synthesis

30S inhibitors
Aminoglycosides
Tetracyclines
Tigecycline

50S inhibitors
Chloramphenicol
Clindamycin
Linezolid
Macrolides
Streptogramins



Membrane intregity

Polymyxin B
Daptomycin

Nucleic acid synthesis

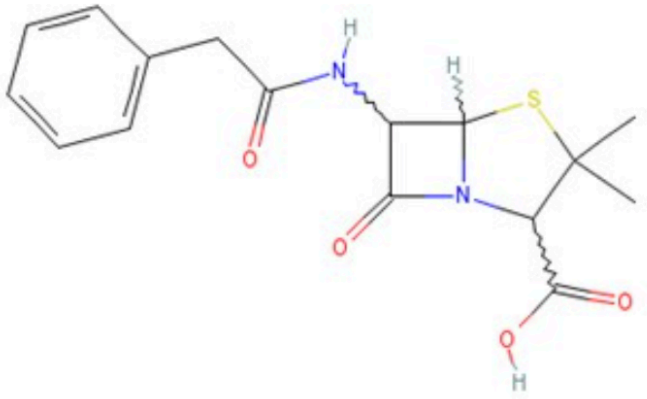
Fluoroquinolones
Metronidazole
Rifamycins

Metabolic pathways (folate)

Sulfonamides
Trimethoprim

1. Inhibition of Cell Wall Synthesis

Inhibition of cell wall synthesis
B-lactam antibiotics.



- **The basic structure of penicillin – B-lactamring**
- **Penicillin have a five – membered ring.**

10. MECHANISM OF ACTION OF PENICILLINS

1.Penicillin-binding proteins:

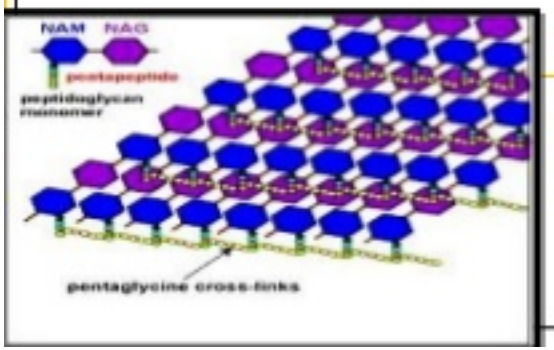
Penicillins inactivate numerous proteins on the bacterial cell membrane. These penicillin-binding proteins (PBPs) are bacterial enzymes involved in the synthesis of the cell wall and in the maintenance of the morphologic features of the bacterium.

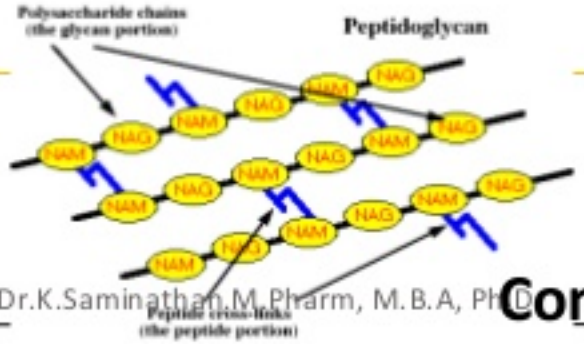
2.Inhibition of transpeptidase:

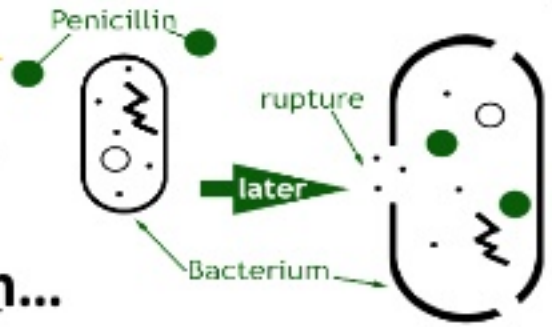
Penicillins inhibit this transpeptidase-catalyzed reaction, thus hindering the formation of cross-links essential for cell wall integrity. As a result of this blockade of cell wall synthesis.

3.Production of autolysins:

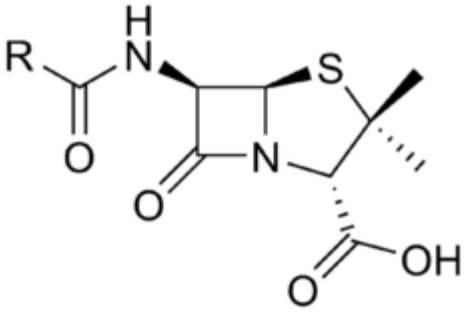
Many bacteria, particularly the gram-positive cocci, produce degradative enzymes (autolysins) that participate in the normal remodeling of the bacterial cell wall.

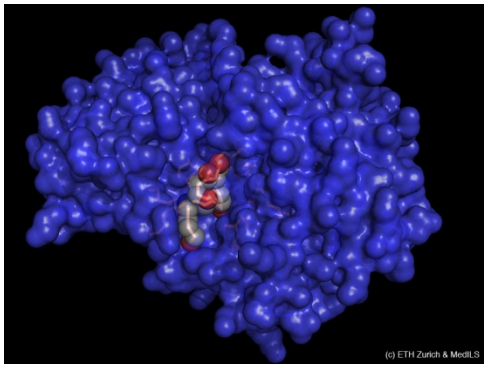






Con...





- Penicillins are called beta-lactam drugs because they contain beta-lactam ring.
- The beta lactam ring forms the basis of penicillin's antibacterial activity.
- **Penicillinases and beta-lactamases found in bacteria destroy the beta lactam ring and neutralize penicillin.**

- **There are three main forms of Penicillin G:**

1. **Aqueous penicillin G is the most rapidly metabolized form.**

2. **Procaine penicillin G, where penicillin G is conjugated with procaine.**

3. **Benzathine penicillin G, where penicillin G is conjugated with benzathine.**

- Benzylpenicillin is one of the most widely used and effective antibiotics.

- Disadvantages of benzylpenicillin:

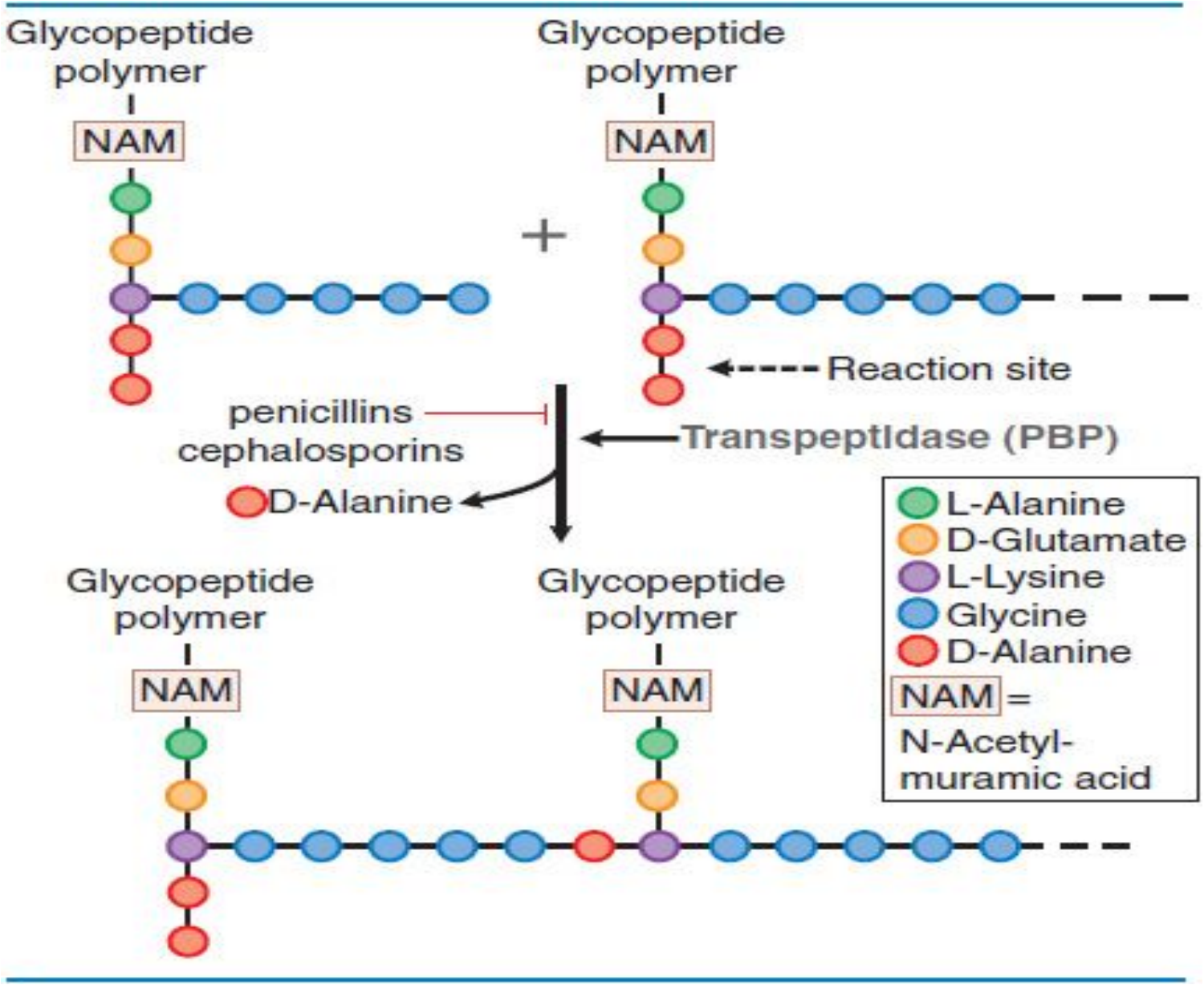
- Limited efficacy against many gram-negative sticks
- It cannot be used orally because it is hydrolyzed by stomach acids.
- Inactivation by beta-lactamases
- Hypersensitivity and especially anaphylaxis are seen in some people who use the drug.

- The most negative side of penicillins is hypersensitivity seen in 1-10% of cases.
- Hypersensitivity reactions;
  - anaphylaxis,
  - skin rashes,
  - hemolytic anemia,
  - nephritis and
  - drug fever
- Anaphylaxis occurs in 0.5% of cases.
- death due to anaphylaxis; seen in 0.002% of cases.

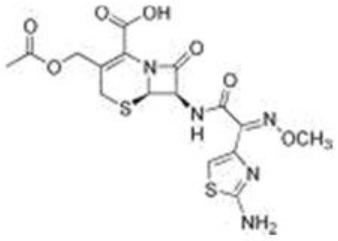
Cephalosporines

- They are beta-lactam drugs that act in the same way as penicillins; that is, bactericidal agents that **inhibit the crosslinks of peptidoglycan.**

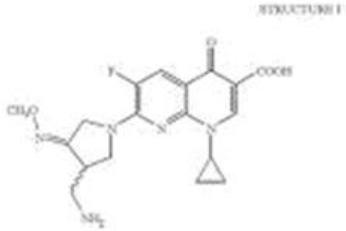
- The first generation of cephalosporins mainly acts on gram-positive cocci.
- Similar to penicillins, new cephalosporins with wider efficiency have been synthesized targeting gram-negative bacteria.


Glycopeptide polymer
NAM
Glycopeptide polymer
NAM
Reaction site
penicillins
cephalosporins
Transpeptidase (PBP)
D-Alanine
Glycopeptide polymer
NAM
Glycopeptide polymer
NAM
L-Alanine
D-Glutamate
L-Lysine
Glycine
D-Alanine
NAM = N-Acetyl-muramic acid

- Cephalosporins
  - affects a wide range of microorganisms,
  - generally well tolerated and
  - Causes fewer hypersensitivity reactions than penicillins.



Struktur Sefotaxim



Struktur Desacetylcefotaxime

Monobactams

- Monobactams are also beta-lactam drugs that differ in structure from penicillin and cephalosporins.

- Aztreonam, the most useful monobactam used today
  - Shows excellent activity against many gram-negative rods such as Enterobacteriaceae and Pseudomonas
  - It is ineffective against gram-positive and anaerobic bacteria.

- It is resistant to most **beta-lactamases.** Beta-lactamases break down the beta-lactam ring, rendering the drug ineffective

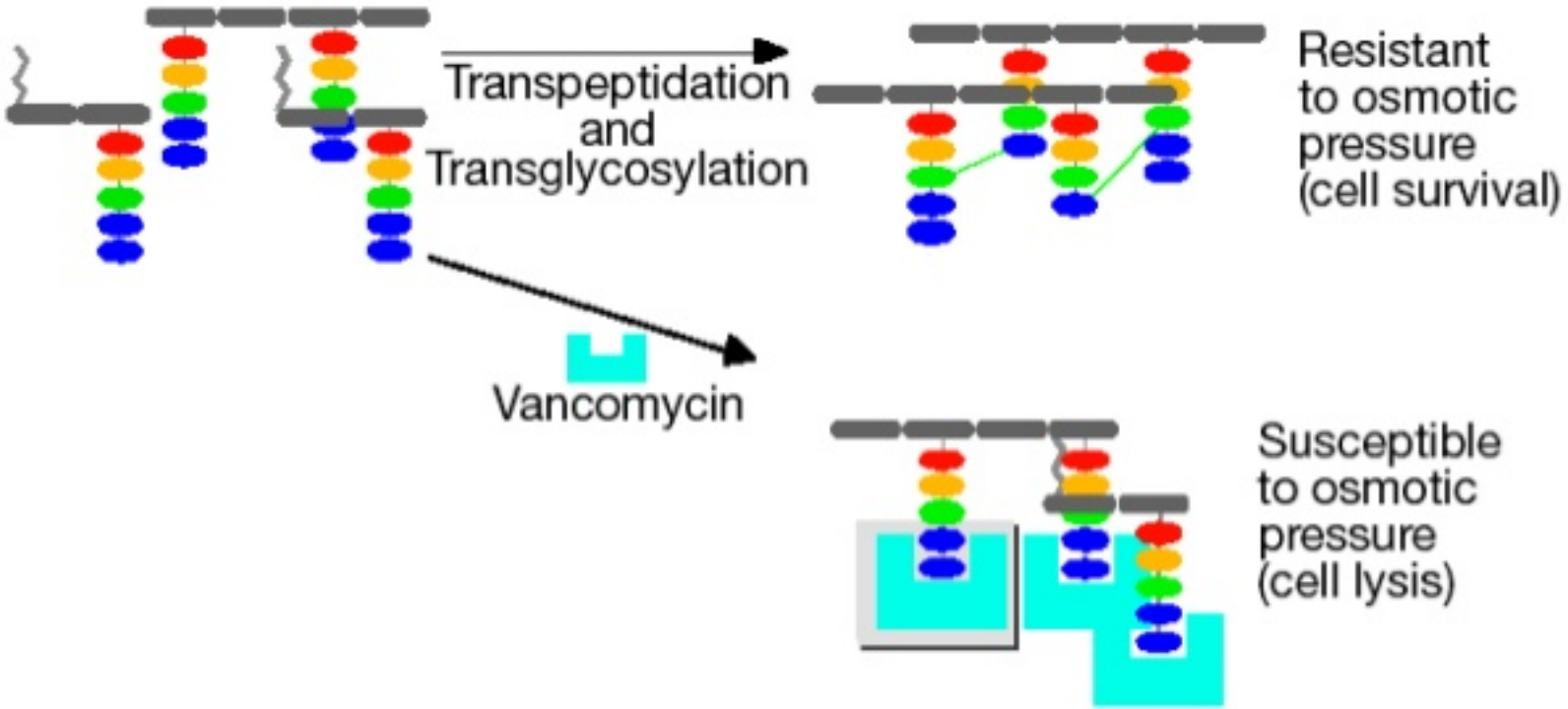
Vancomycin

- Vancomycin is a glycopeptide that inhibits cell wall synthesis by blocking transpeptidation by a mechanism different from beta-lactam drugs.
- Vancomycin shows activity against some gram-positive bacteria

Vancomycin: Mechanism of Action

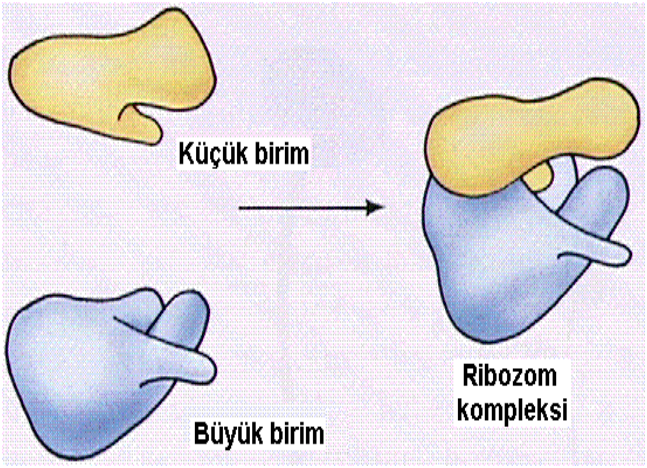
Vancomycin, the crucial "drug of last resort," inhibits PG synth by binding **directly** to the D-Ala—D-Ala end of the peptide

- forms a **cap** over the end of the chain; blocks cross-linking



2. Inhibition of Protein Synthesis

Many drugs inhibit protein synthesis in bacteria without damaging the protein synthesis in human cells.



- Ribosomes where protein synthesis takes place have two subunits:
- **Small subunit and large subunit**
- **During protein synthesis mRNAs bind to small subunit Then, the small and large subunits combine to form the ribosome complex and protein synthesis begins.**

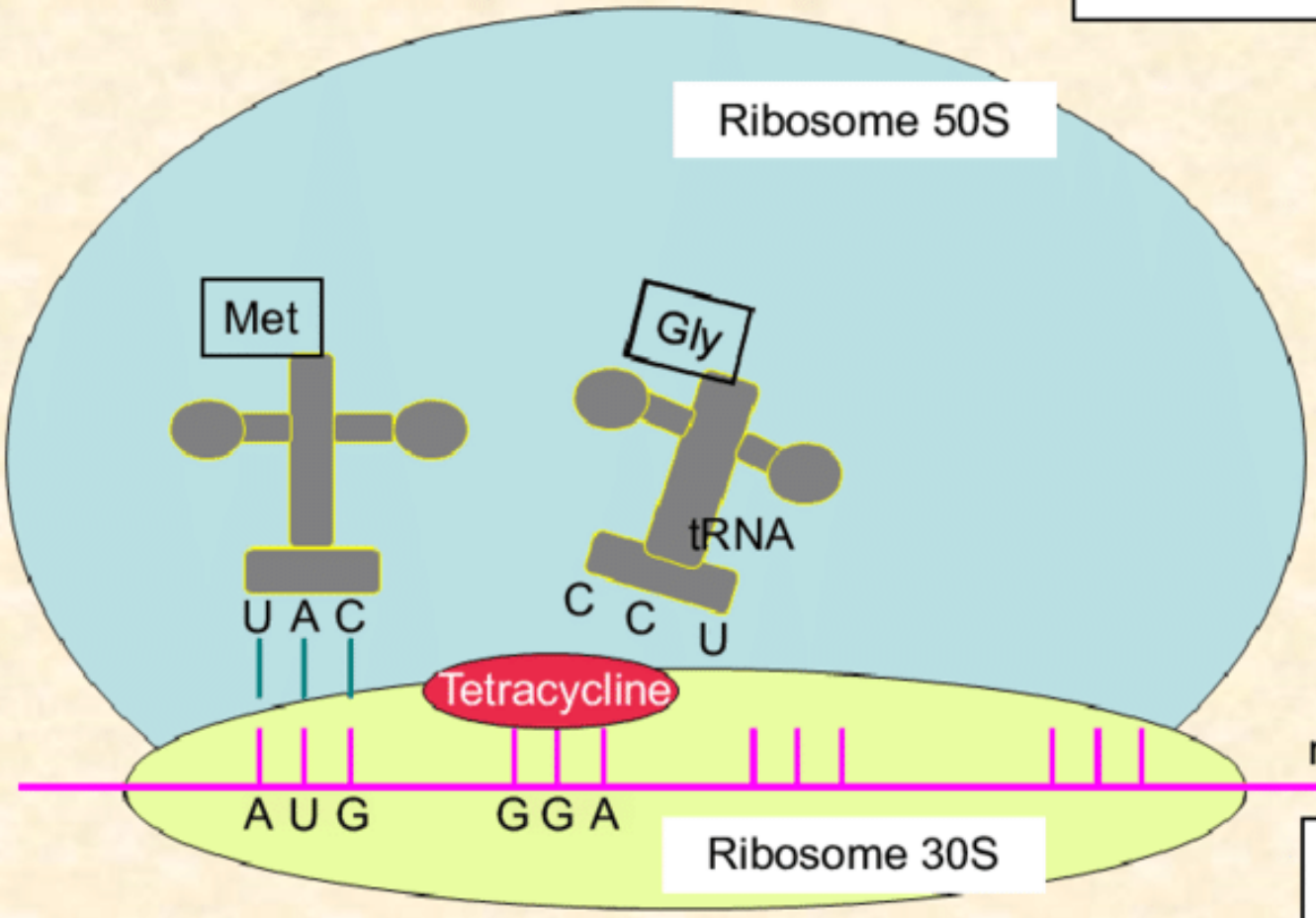
- The sizes of small and large subunits differ in prokaryotic and eukaryotic cells.
- **While bacteria have subunits of 50S and 30S size; human cells have 60S and 40S size subunits**
- In addition, the enzymes used by prokaryotic and eukaryotic cells in protein synthesis are also different from each other.
- For these reasons, while antimicrobial drugs inhibit the protein synthesis of bacteria, they cannot be effective on protein synthesis in humans.

1. Drugs Affecting 30S Subunits

Tetracyclines

- Tetracyclines against a variety of gram-positive and gram-negative bacteria, mycoplasmas, chlamydia and bacteriostatic effect against rickettsia
- These inhibit protein synthesis by binding to the 30S subunit and blocking the entry of aminoacyl transfer RNA (tRNA) into the ribosome.

Tetracycline block of ribosomal protein synthesis in bacteria



Human mitocondrial ribosomes (55S) are 39S and 28S. The inhibition by tetracycline is similar

Decreased cytochrome C synthesis

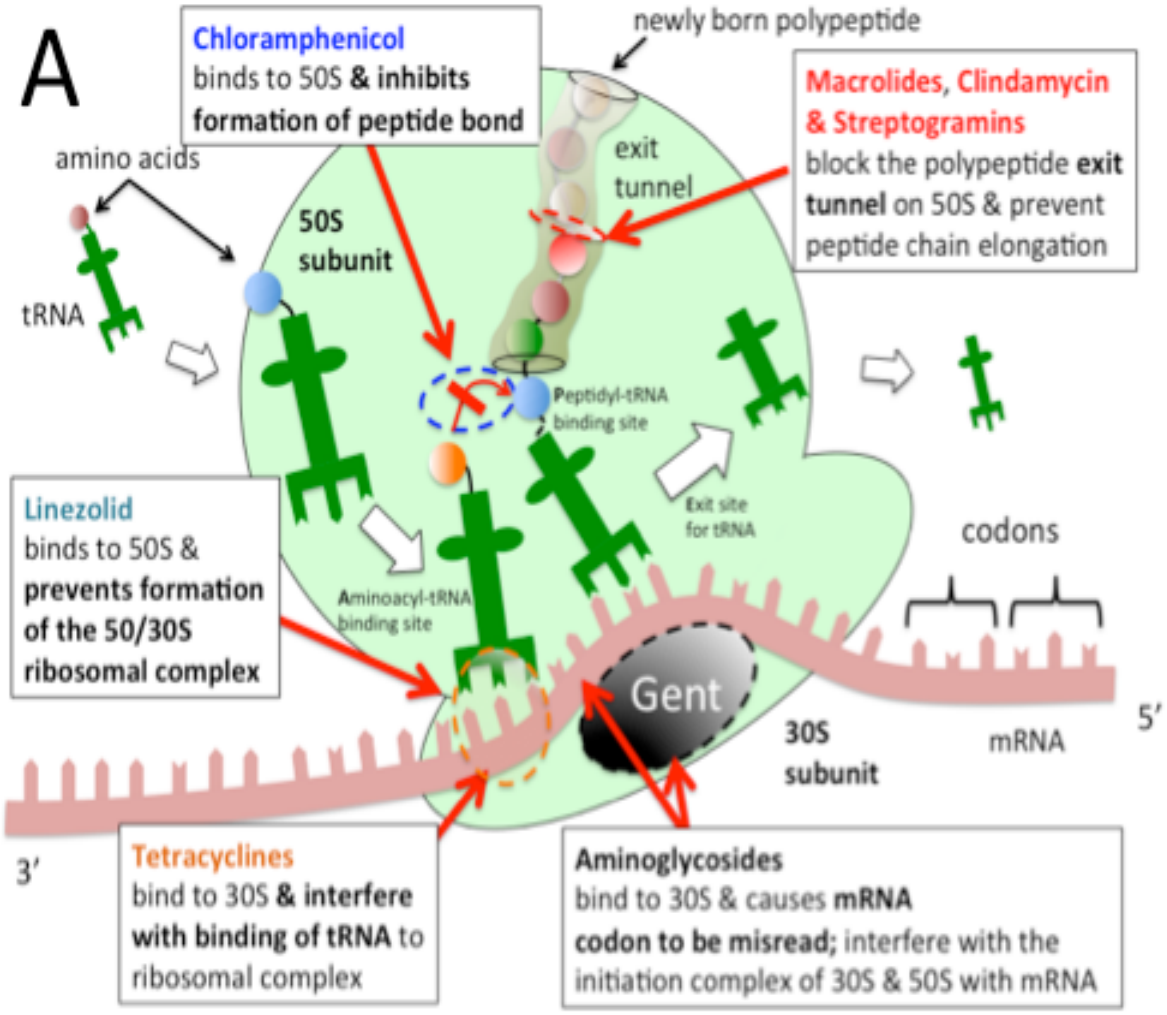
Decreased OXPHOS

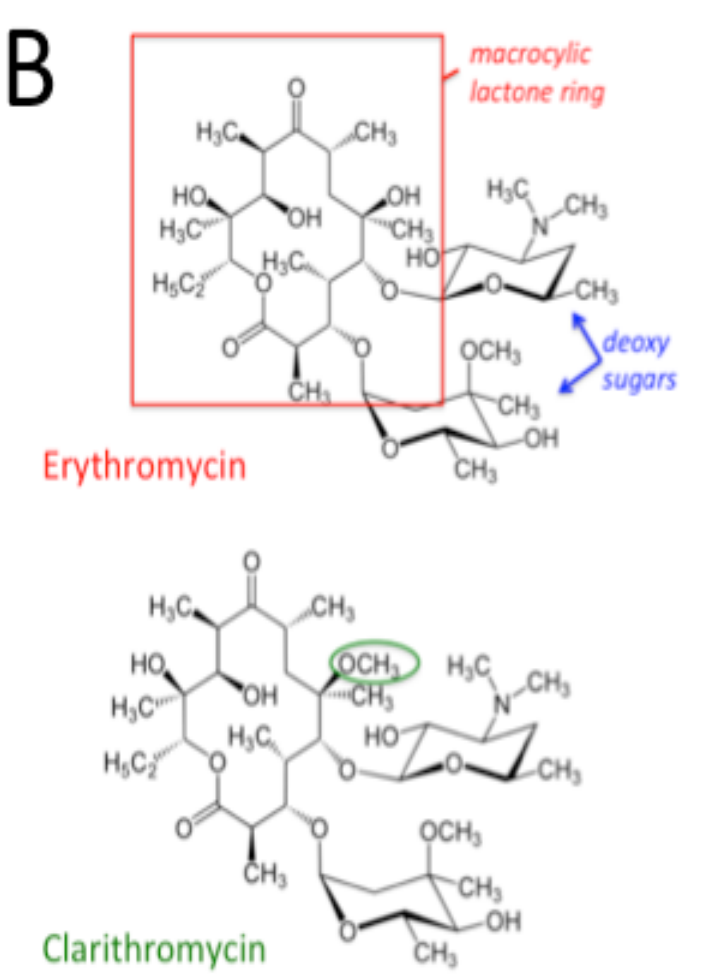
Increased aerobic glycolysis and lactic acid

2. Drugs Affecting 50S Subunits

Chloramphenicol

- Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and blocking the peptidyltransferase action; this phenomenon prevents the formation of new peptide bonds.
- While this drug does not affect transferase in the 60S human ribosomal subunit, the 50S selectively inhibits bacterial protein synthesis due to its binding to the catalytic point of the transferase in the bacterial ribosomal subunit.


A
Chloramphenicol
binds to 50S & inhibits formation of peptide bond
newly born polypeptide
Macrolides, Clindamycin & Streptogramins
block the polypeptide exit tunnel on 50S & prevent peptide chain elongation
amino acids
exit tunnel
50S subunit
tRNA
Peptidyl-tRNA binding site
Exit site for tRNA
Linezolid
binds to 50S & prevents formation of the 50/30S ribosomal complex
Aminoacyl-tRNA binding site
codons
Gent
30S subunit
mRNA
5'
3'
Tetracyclines
bind to 30S & interfere with binding of tRNA to ribosomal complex
Aminoglycosides
bind to 30S & causes mRNA codon to be misread; interfere with the initiation complex of 30S & 50S with mRNA


B
macrocylic lactone ring
deoxy sugars
Erythromycin
Clarithromycin

Erythromycin

- Erythromycin is a bacteriostatic drug with a broad spectrum of activity.
- **Erythromycin binds to the 50S subunit and blocks the binding of subunits to each other**.

Linezolid

Linezolid ;

- vancomycin-resistant enterococci,
- methicillin-resistant S.aureus and S.epidermidis and
- It is useful in the treatment of penicillin-resistant pneumococci.

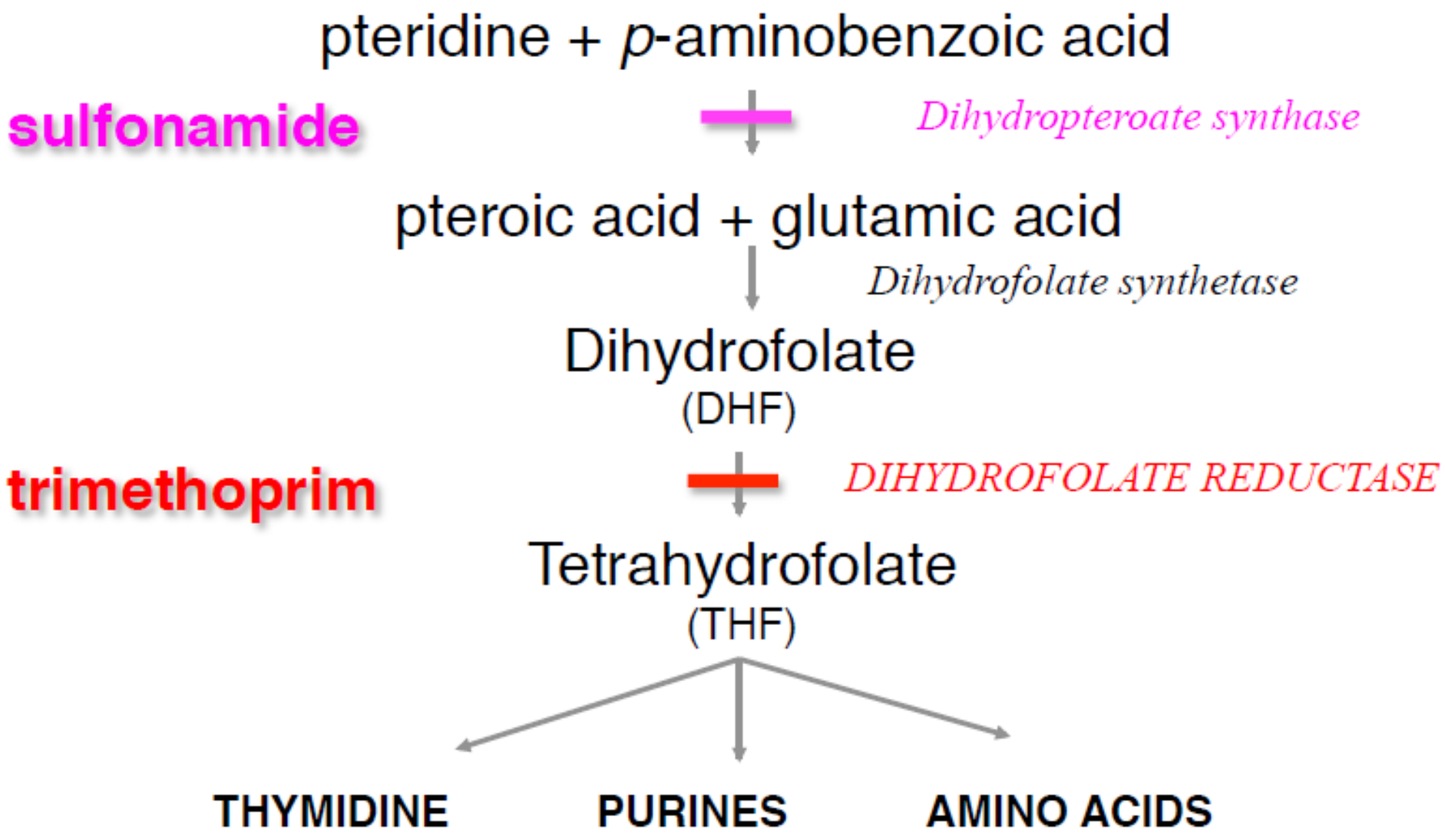
It is bacteriostatic for enterococci and staphylococci, while bactericidal for pneumococci.

Linezolid binds to 23S ribosomal RNA in the 5OS subunit and inhibits protein synthesis.

3. Inhibition of Nucleic Acid Synthesis

- **Sulfonamides** block the synthesis of tetrahydrofolic acid, which acts as a methyl donor in the production of adenine, guanine and thymine.
- Sulfonamides are also structural analogs of p-amino-benzoic acid (PABA). For this reason, bacteria may mistakenly use sulfonamide instead of PABA in their metabolism.

Mechanism of Action



- The basis of the selective effect of sulfonamides on bacteria is that **human cells take folic acid as an external nutrient, although many bacteria synthesize folic acid from precursors containing PABA.**

4. Changes in Functions of Cellular Membrane

- Because of the structural and chemical similarities between bacteria and human cell membranes, there are only a few antimicrobial compounds that act on the cell membrane.
- Polymyxins are a family of polypeptide antibiotics. Effective against Gram-negative rods and especially P. aeruginosa.
- The positively charged free amino groups act like a cationic detergent that breaks down the phospholipid structure of the cell membrane.

Antibiotic Resistance

Defined as micro-organisms that are not inhibited by usually achievable systemic concentration of an antimicrobial agent with normal dosage schedule and / or fall in the minimum inhibitory concentration (MIC) range.

Antibiotic Resistance (DR)
= MIC / MCC > Toxic Plasma Concentration

Antibiotic Resistance

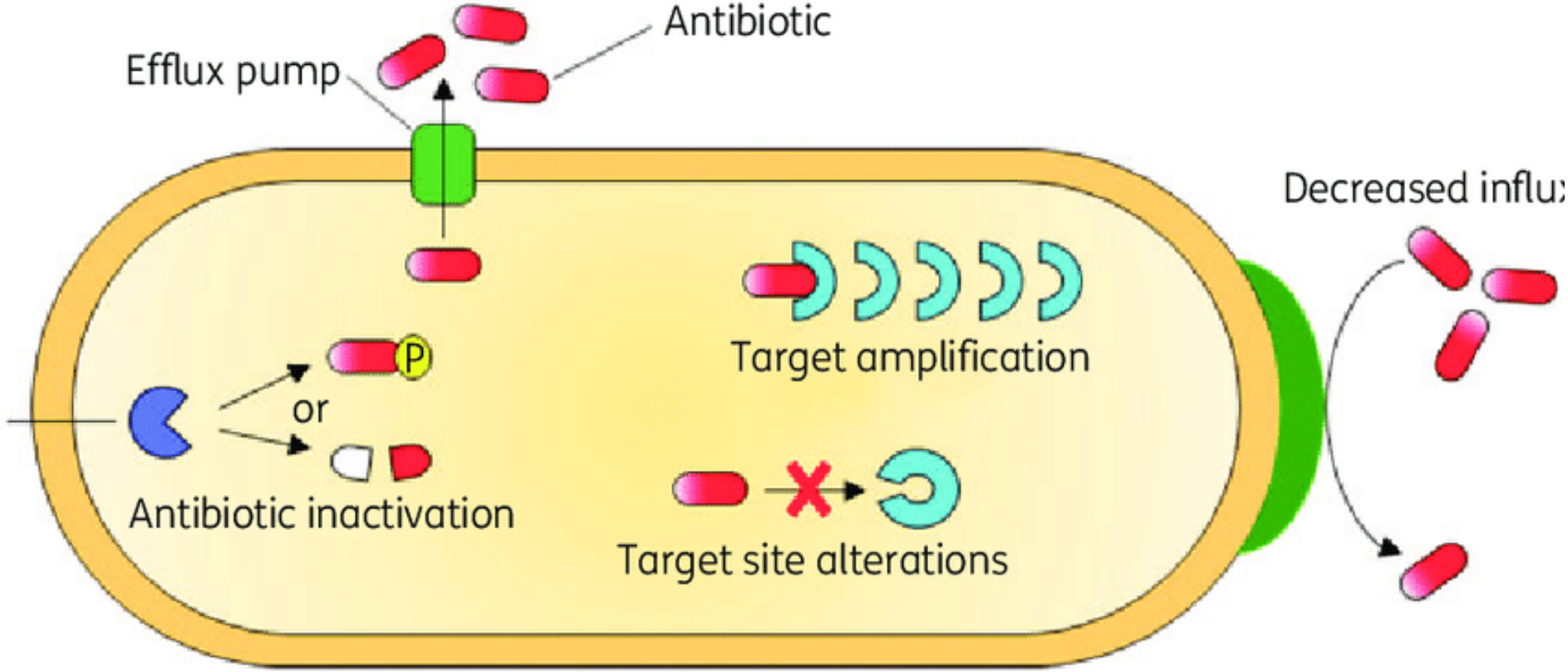
- There are 4 main mechanisms.

1-Bacteria produce enzymes that inactivate the drug;

2-Bacteria synthesize altered targets for which the drug is not effective;

3-Bacteria reduce their permeability so that effective intracellular concentration of the drug cannot be obtained;

4-Bacteria efficiently expel drugs using a "drug resistance pump" (MDR)


Antibiotic
Efflux pump
Decreased influ
Target amplification
P
or
Antibiotic inactivation
Target site alterations

Mechanism Antibiotic Resistance

Intrinsic (Natural)

Acquired

Genetic Methods

Chromosomal Methods

Mutations

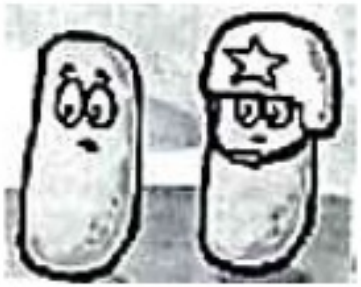
Extra chromosomal Methods

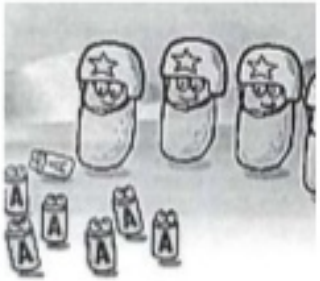
Plasmids

Acquired resistance

Mutations

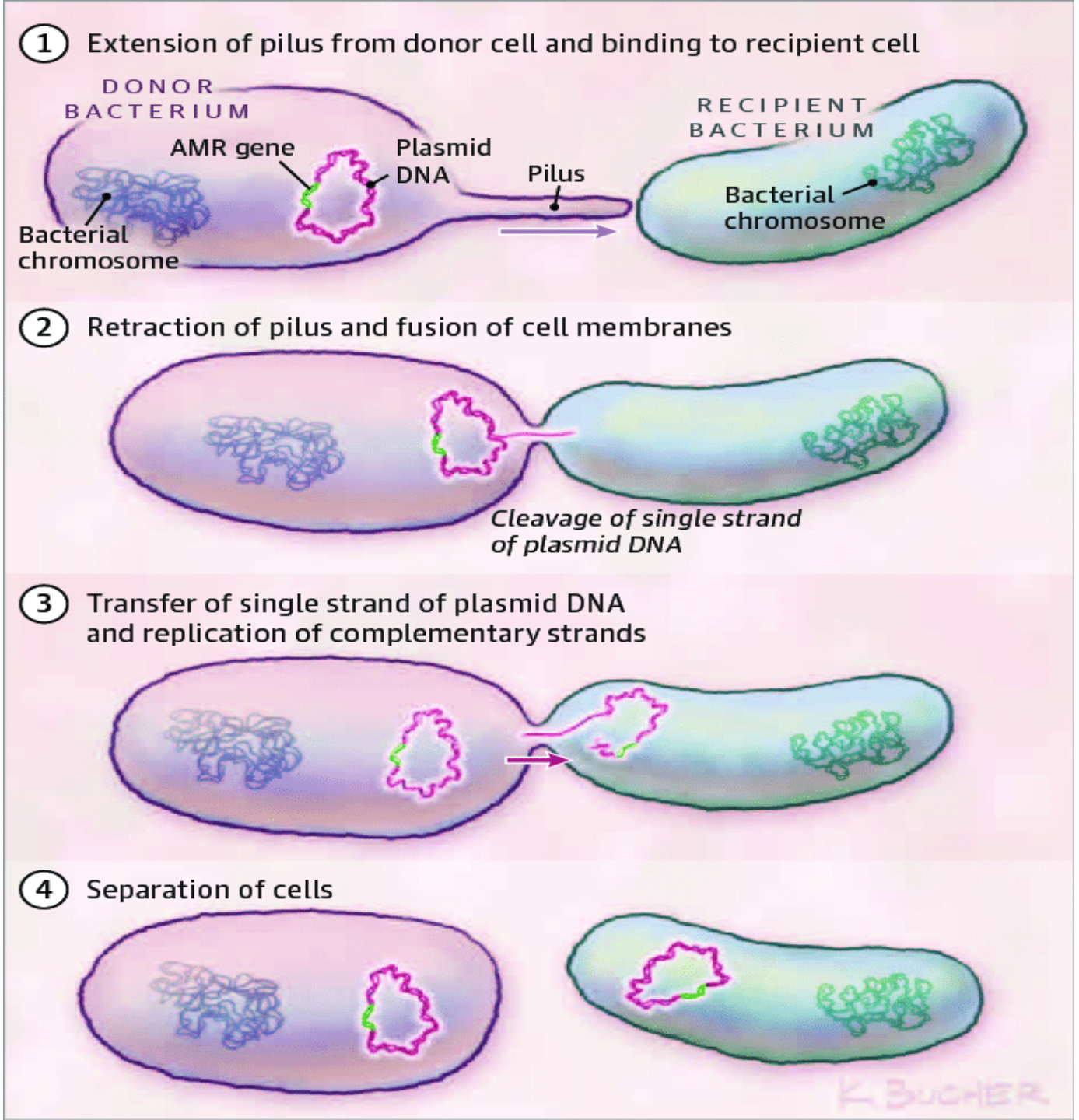
- It refers to the change in DNA structure of the gene.
- Occurs at a frequency of one per ten million cells.
- Eg.Mycobacterium tuberculosis,Mycobacterium lepra , MRSA.
- Often mutants have reduced susceptibility





Plasmids

- Extra chromosomal genetic elements can replicate independently and freely in cytoplasm.
- Plasmids which carry genes resistant ***(r-genes***) are called **R-plasmids**.
- These *r-genes* can be readily transferred from one R-plasmid to another plasmid or to chromosome.
- Much of the drug resistance encountered in clinical practice is plasmid mediated


1 Extension of pilus from donor cell and binding to recipient cell
DONOR BACTERIUM
AMR gene
Plasmid DNA
Pilus
RECIPIENT BACTERIUM
Bacterial chromosome
Bacterial chromosome
2 Retraction of pilus and fusion of cell membranes
Cleavage of single strand of plasmid DNA
3 Transfer of single strand of plasmid DNA and replication of complementary strands
4 Separation of cells
K. BUCHER

Mechanisms of Resistance Gene Transfer

- **Transfer of r-genes from one bacterium to another**
  - Conjugation
  - Transduction
  - Transformation
- **Transfer of r-genes between plasmids within the bacterium**
  - By transposons
  - By Integrons

Transfer of r-genes from one bacterium to another

- **Conjugation** : Main mechanism for spread of resistance

  The conjugative plasmids make a connecting tube between the 2 bacteria through which plasmid itself can pass.

- **Transduction :** Less common method

  The plasmid DNA enclosed in a **bacteriophage** is transferred to another bacterium of same species.
  Seen in Staphylococci , Streptococci

- **Transformation** : least clinical problem.

  Free DNA is picked up from the environment (i.e.. From a cell belonging to closely related or same strain.

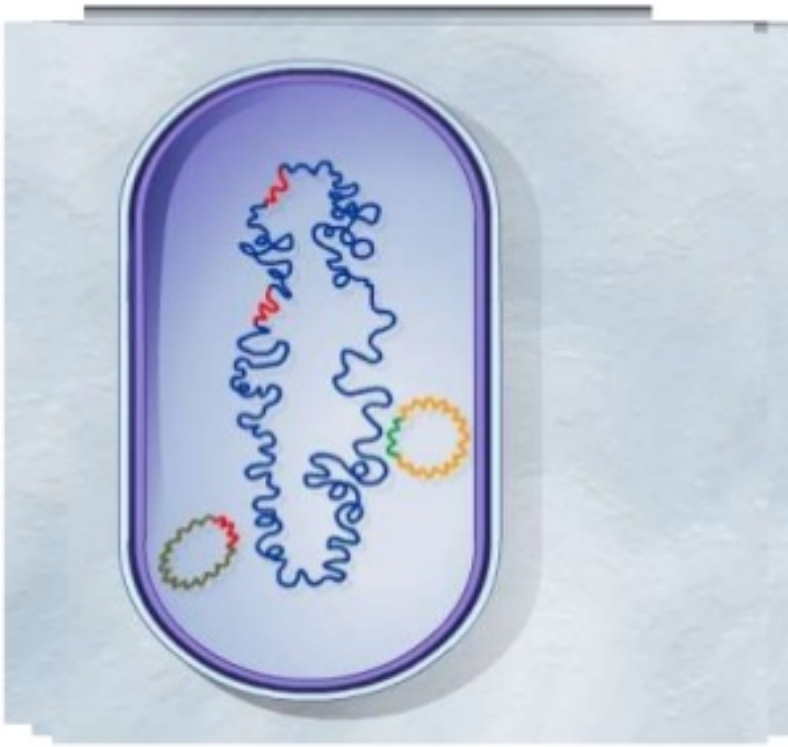
Mechanisms of Resistance Gene Transfer

Transposons

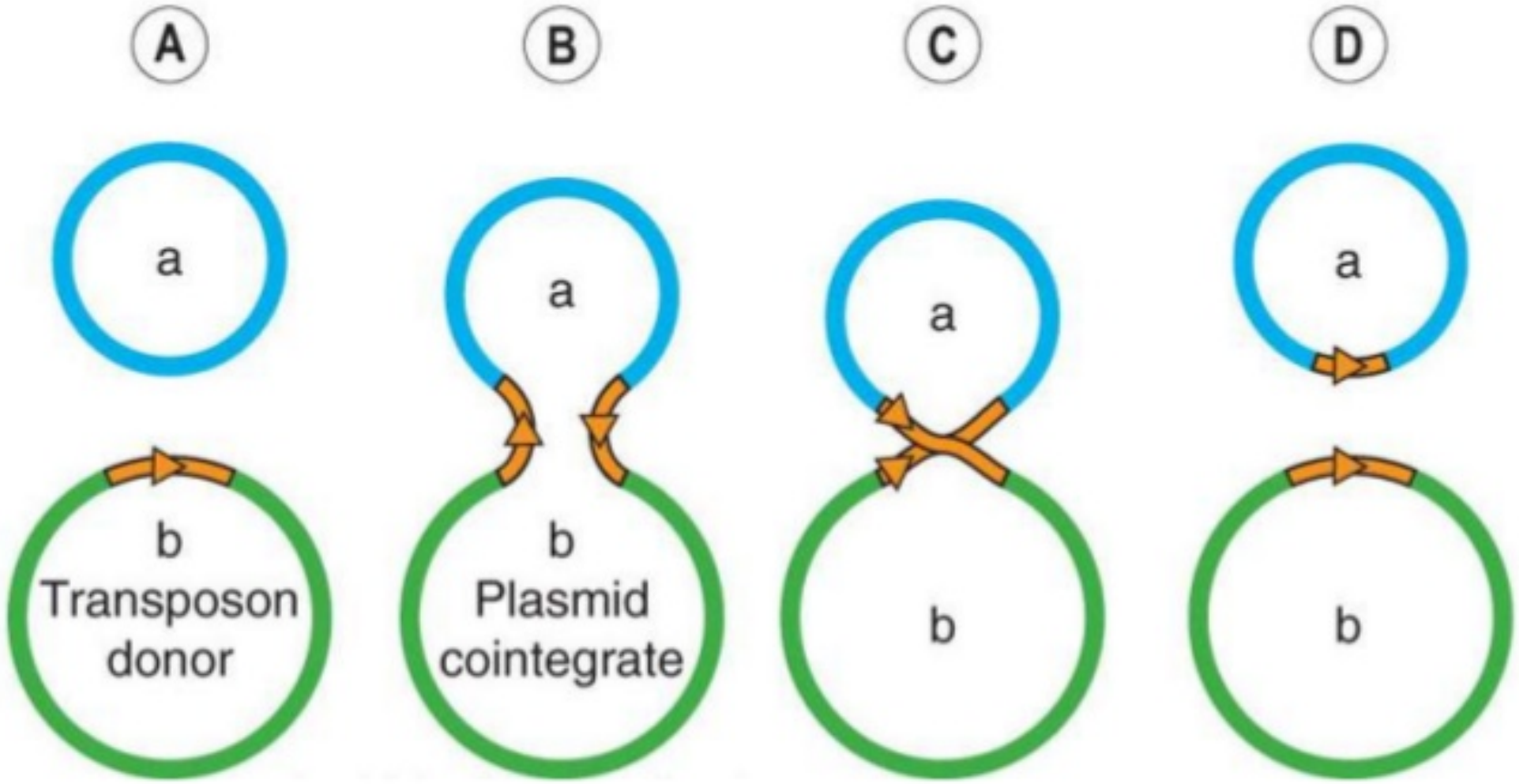
- Transposons are sequences of DNA that can move around different positions within the genome of single cell.

- The donor plasmid containing the Transposons, co-integrate with acceptor plasmid. They can replicate during cointegration

- Both plasmids then separate and each contains the r-gene carrying the transposon.



- Eg ; Staphylococci,Enterococci


A
B
C
D
a
b
Transposon
donor
a
b
Plasmid
cointegrate
a
b
a
b

ANTIBIOTIC RESISTANCE
HOW IT SPREADS

HANDLE ANTIBIOTICS WITH CARE

Antibiotics are given to patients, which can result in drug-resistant bacteria developing in the gut

Patient attends hospital or clinic

Drug-resistant bacteria spreads to other patients through poor hygiene and unclean facilities

Drug-resistant bacteria spreads to the general public

Drug-resistant bacteria reaches humans through food, the environment (water, soil, air) or by direct human-animal contact

Animals develop drug-resistant bacteria in their gut

Antibiotics are given to food producing animals and crops

Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.

www.who.int/drugresistance

#AntibioticResistance

World Health Organization

Rise of the superbugs

How antibiotic-resistant bacteria evolve and how they can infect people.

WHAT THEY ARE

Usually, only some bacteria are **naturally resistant** to drugs. But in the **absence of antibiotics**, these germs typically are at a disadvantage.

But when **antibiotics** kill non-resistant bacteria…

These **drug- resistant bacteria** can then grow and take over.

HOW THEY SPREAD

Chickens receive antibiotics routinely, which can kill off weaker bacteria and promote antibiotic resistant bacteria. Resistant bacteria can leave the farm through:

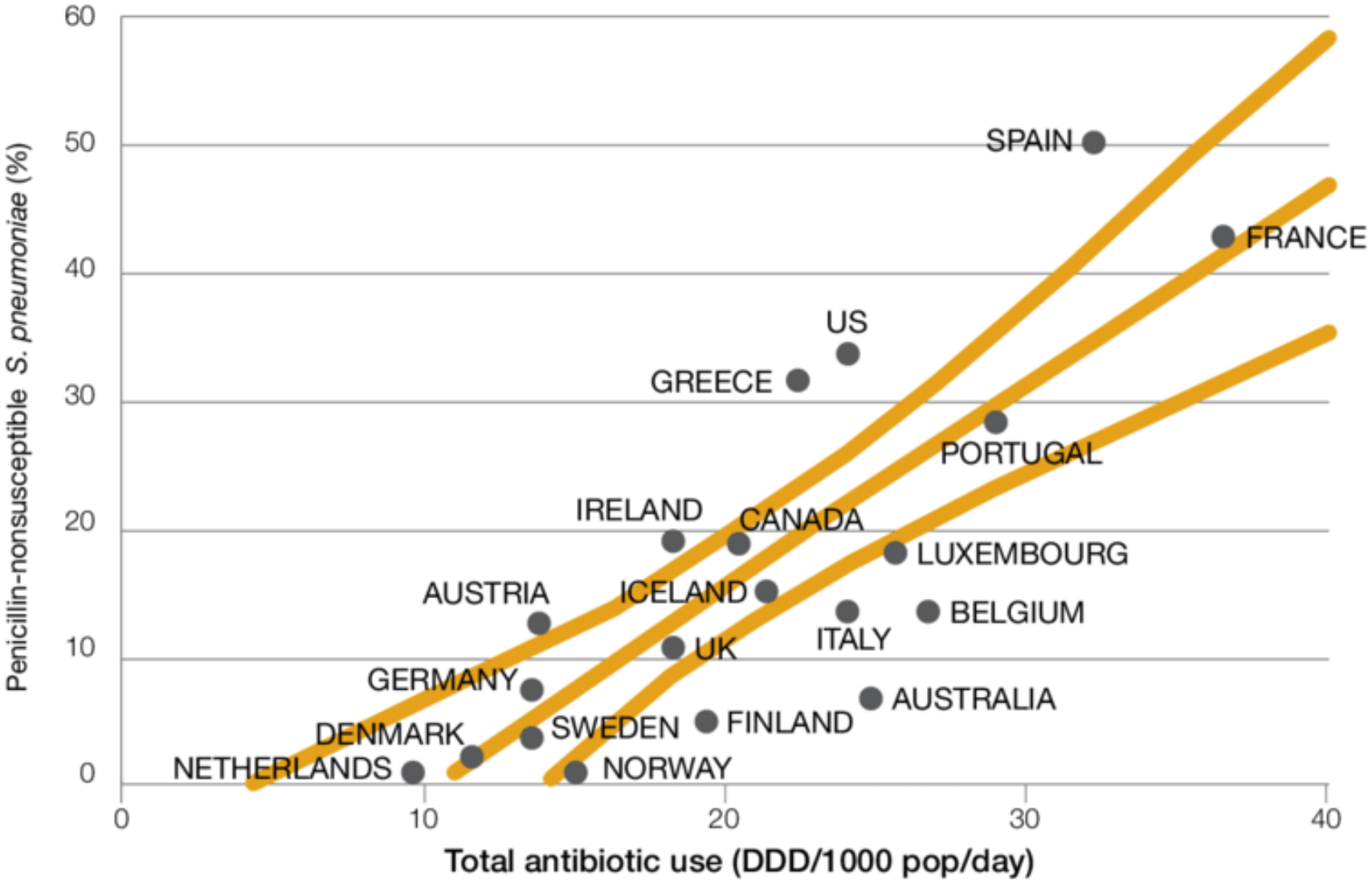
Contact with animals

The spreading of manure contaminated with bacteria onto crops as fertilizer

Human mishandling of contaminated meat.

Sources: Centers for Disease Control and Prevention;
Keeve E. Nachman, PhD, MHS, Johns Hopkins Center for a Livable Future

Staff, 15/09/2014



DDD/1000 pop/day = defined daily dose per 1000 population per day

Minimum Inhibitory Concentration (MIC)

- MIC is the lowest concentration of an antibiotic that inhibits visible growth (broth turbidity) upon in vitro testing of a particular organism.
- MIC **breakpoint**is a discriminating concentration used in the interpretation of results to define isolates as susceptible, intermediate or resistant.

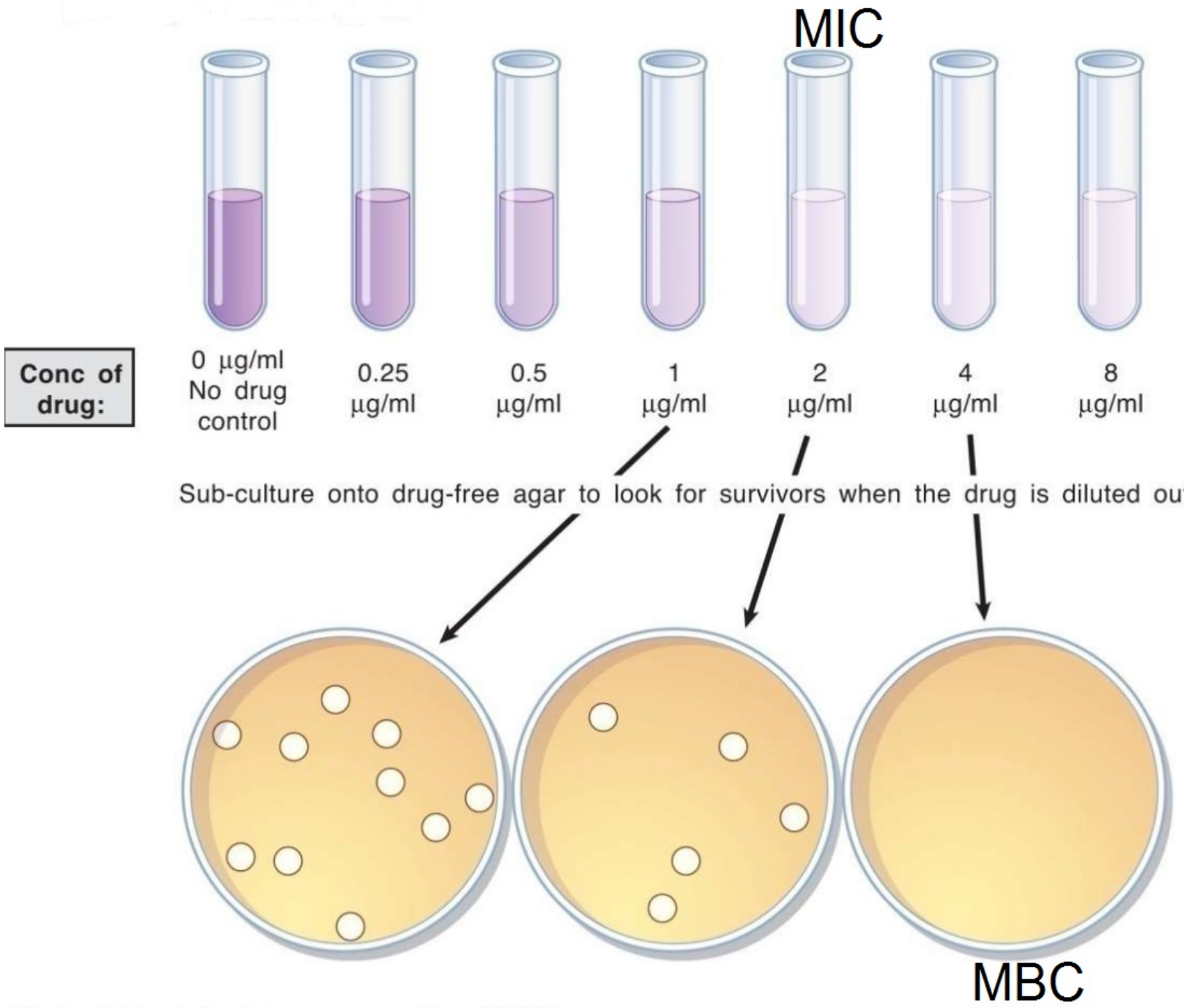
MacGowan et al. *Antimicrob Agents Chemother.* 2009 Dec;53(12):5181-4.
Hessen and Kaye. *Infect Dis Clin North Am.* 2004 Sep;18(3):435-50

MIC:

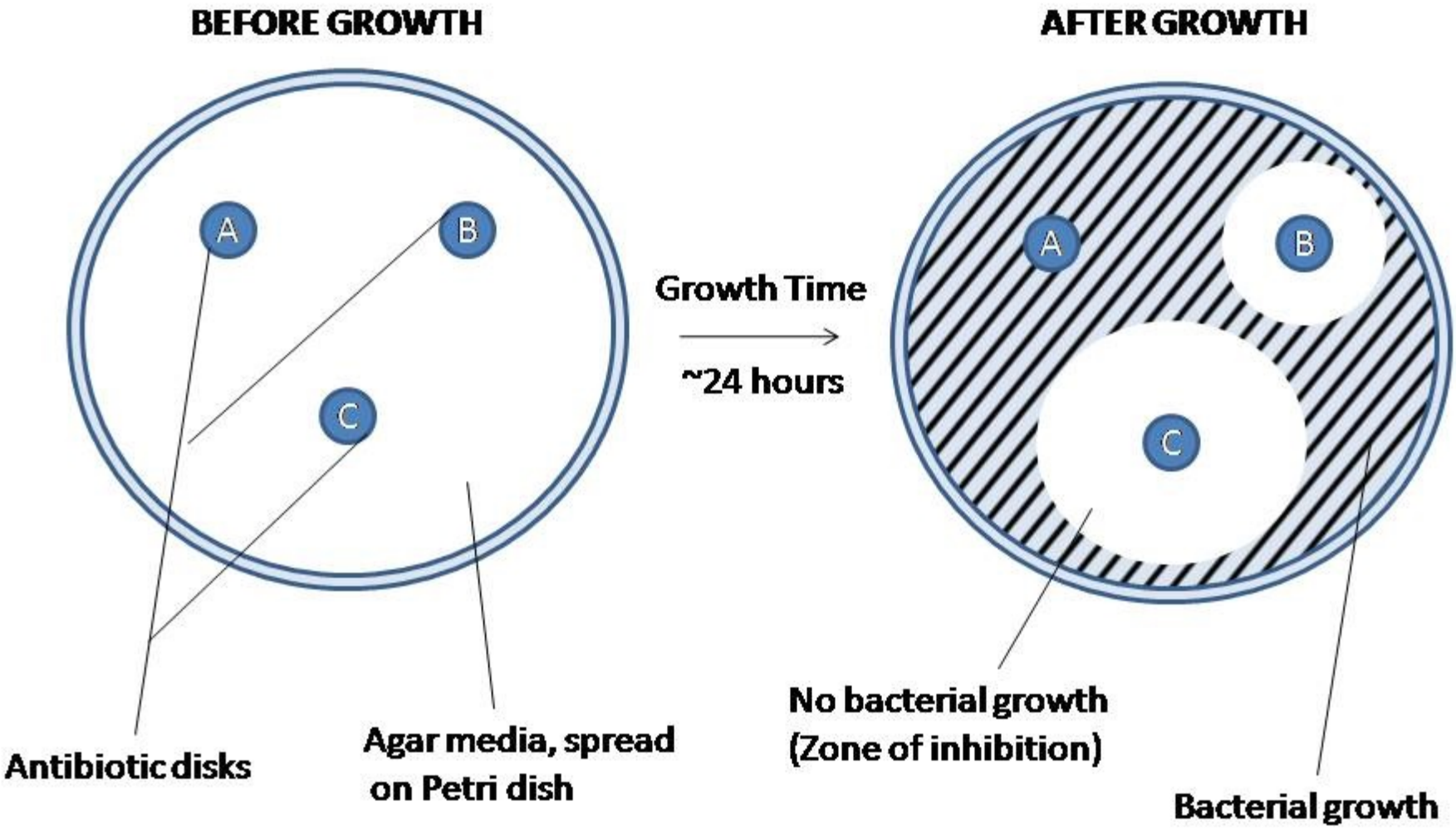
It is the lowest concentration of the antimicrobial agent that inhibits the growth of the test organism but not necessarily kills it.

MBC(minimum bactericidal conc.):

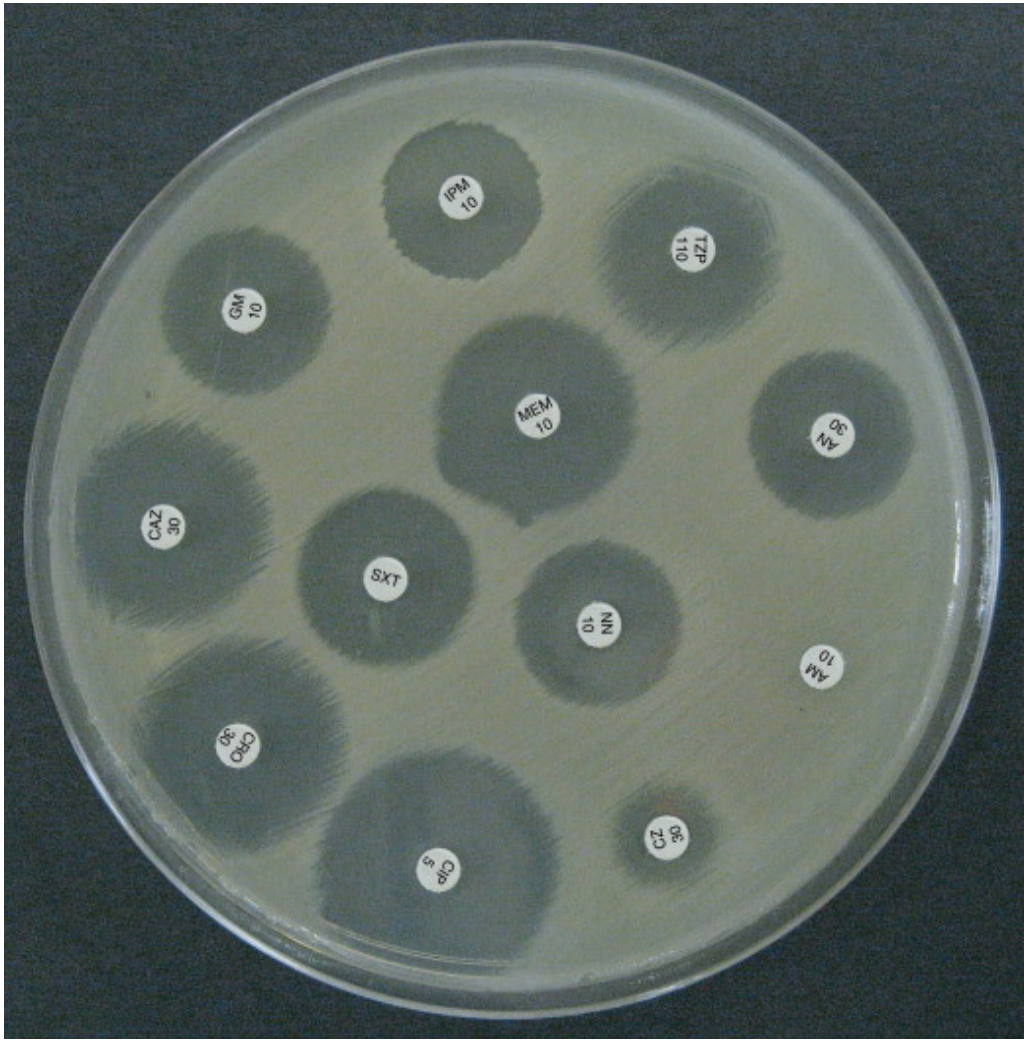
It is the lowest concentration of the antimicrobial agent that kills the test organism.


MIC
Conc of drug:
0 µg/ml No drug control
0.25 µg/ml
0.5 µg/ml
1 µg/ml
2 µg/ml
4 µg/ml
8 µg/ml
Sub-culture onto drug-free agar to look for survivors when the drug is diluted out.
MBC

Disk Diffusion Technique



Disk Diffusion Technique



CO-USE OF ANTIBIOTICS

- In some cases, two or more drugs are used together:

1. To treat severe infections before the diagnosis of the microorganism is determined;
2. To achieve a synergistic inhibitory effect against some microorganisms
3. To prevent the emergence of resistant microorganisms

- Two drugs can interact with each other in three different ways:

- **additive interaction**
- **synergistic interaction**
- **antagonist interaction**

- In additive interaction, the effect of drugs on microorganisms cannot be distinguished from each other,
- In synergistic interaction, the effect of two drugs together is more than the sum of their effects alone.
- The effect observed in the antagonist interaction is lower than the sum of the efficacy observed when these drugs are used alone.

Bacterial Vaccines

- Bacterial diseases can be prevented using immunization that stimulates active or passive immunity.
- Effective immunization is provided by vaccines prepared from bacteria or their products.
- Passive immunization is achieved by using preformed antibody in preparations called immunoglobulins.
- Passive-active immunization involves the administration of immediate protective immune globulins together with a long-term protection vaccine.

Active Immunization

- Bacterial vaccines

A. CAPSULE POLYSACHARIDE VACCINES
B. TOXOID VACCINES
C. TREATED PROTEIN VACCINES
D. LIVE, ATTENUENT BACTERIAL VACCINES
E. KILLED BACTERIA VACCINES

- **A. CAPSULE POLYSACHARIDE VACCINES**
- They are polysaccharide vaccines formed by purification of capsule polysaccharides.
- **B. TOXOID VACCINES**
- They are prepared using bacterial toxins as vaccines.

C. PURIFIED PROTEIN VACCINES

- They are vaccines obtained by purification of bacterial proteins.

- There is a *B. pertussis* vaccine:
  - dead bacteria
  - proteins purified from the microorganism.
- The vaccine against Lyme disease contains a purified outer surface protein (OspA) of *Borrelia burgdorferi* as an immunogen.

- *Bacillus anthracis* vaccine contains "protective antigen" purified from the microorganism. It is applied to people who are at risk of anthrax due to their duties.

D. LIVE, ATTENUENT BACTERIAL VACCINES

- Vaccines are created by making bacteria incapable of causing disease.

- The vaccine against tuberculosis is prepared against a live, attenuated strain of *Mycobacterium bovis* called BCG.

- A vaccine against typhoid uses live, attenuated *Salmonella typhi.*

E. KILLED BACTERIA VACCINES

- These are vaccines obtained by using dead bacteria.
- Vibrio cholerae vaccine contains dead microorganisms and is administered to travelers traveling to areas where cholera is endemic.
- Yersinia pestis vaccine contains dead microorganisms and is indicated for individuals at high risk of contracting plague.
- The vaccine against typhus contains killed Rickettsia microorganisms and is mainly used to vaccinate members of the armed forces.