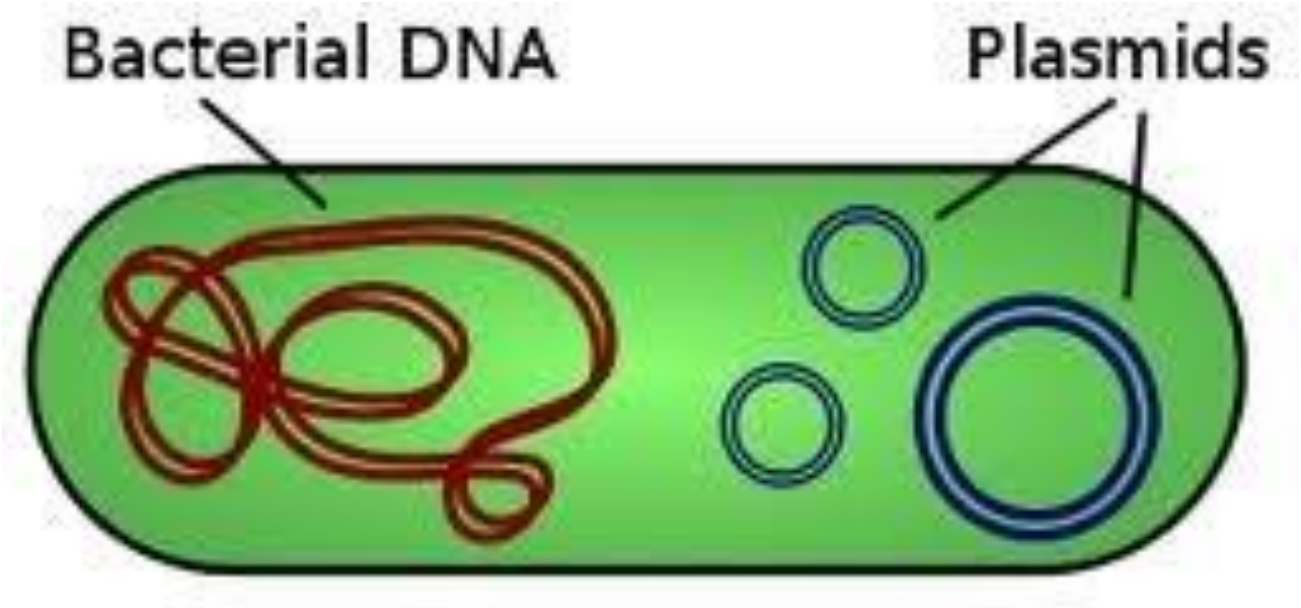
LESSON 4

Genetics of microorganisms. Basics of antimicrobial therapy. Chemical therapy drugs. Antibiotics, mechanisms of action and rules of application. Antibiotics resistancy of microorganisms. The antimicrobial susceptibility methods of microorganisms

Organization of the genetic apparatus in bacteria

- The genetic information of bacteria is stored both in DNA (chromosome) and in extrachromosomal structures - plasmids, and in migrating genetic elements.
- DNA is the material basis of heredity. All signs of an organism are stored in the form of a sequence of nucleotides of a DNA molecule.
- An exception may be RNA-containing viruses, in which the genetic information is enclosed in an RNA molecule.
- The bacterial chromosome is represented by a double helical, circular, covalently closed supercoiled DNA molecule built from two polynucleotide chains.

Organization of the genetic apparatus in bacteria



Nucleoid of bacteria

- By analogy with the nucleus of eukaryotic cells, bacterial DNA is called a nucleoid, which consists of approximately 4,000 genes. The bacterial chromosome has a haploid set of genes; its doubling is usually accompanied by cell division.
- When a bacterial cell divides, the number of chromosomes can reach 2-4, and sometimes 10-15. Usually the chromosome of a bacterial cell contains 5x106 bp. (for comparison, the total length of human chromosomal DNA is 3x109). The length of the bacterial chromosome (for example, in Escherichia coli) in the unfolded state is about 1 mm

Genes

- A gene is a unit of heredity. It is a structural unit of DNA encoding the primary structure of the corresponding polypeptide chain.
- By functionality, they distinguish:
- Structural genes - carrying information about the structure of a particular protein,
- Regulatory genes - controlling the work of structural genes

Генотип

- The totality of the genes of a bacterial cell determines its hereditary characteristics, i.e. genotype

- Genes that provide the synthesis of a substance are designated by the initials of this substance. For example, the gene for the amino acid arginine is shown as arg+ and the gene for the lactose enzyme is shown as lac+.
- Sensitivity to antibiotics and phages is indicated by the letter s (sensitive - sensitivity), and resistance - by the letter r (resistance). For example, the streptomycin susceptibility gene is shown as strs and the resistance gene as strr.

Phenotype

- The phenotype is the result of the interaction between the genotype and the environment.
- The phenotype is controlled by the genotype. The manifestation of a genotype in a phenotype is called expression.
- However, changes in the genotype are not always manifested in the phenotype, that is, expression does not always occur.
- In bacteria, the phenotype is designated in the same way as the genotype, but the name of the phenotype is written in capital letters. For example, the arg+ genotype corresponds to the Arg+ phenotype, and the lac+ genotype corresponds to the Lac+ phenotype.

Extrachromosomal genetic elements

- Extrachromosomal heredity factors of bacteria are represented by plasmids and migrating genetic elements.
- They encode functions that are not essential for the life of a microbial cell, but provide adaptation to environmental conditions.

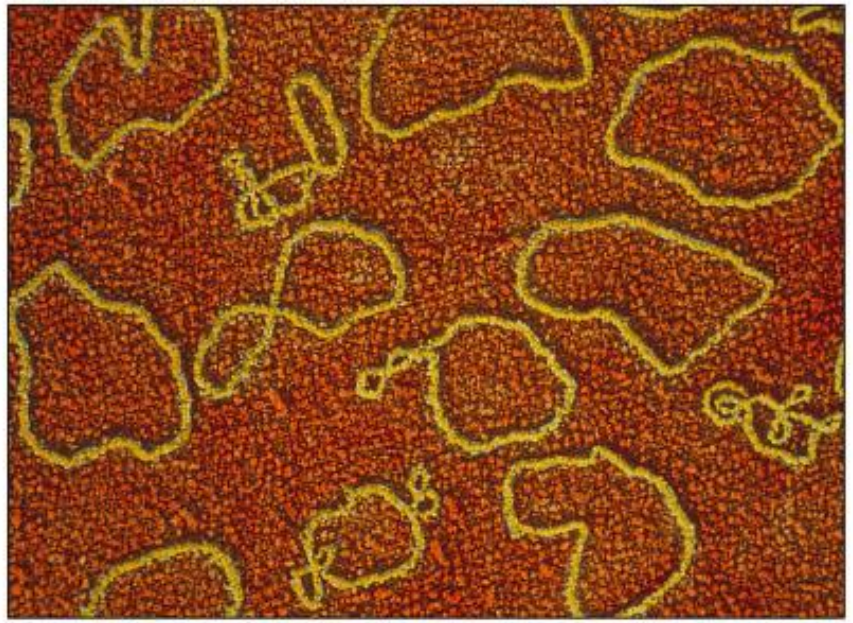
Plasmids

- Plasmids are extrachromosomal DNA molecules carrying approximately 40-50 genes. Autonomous (not associated with the chromosome) and integrative (embedded in the chromosome) plasmids are isolated.
- Plasmids have the following properties:
- Replicate independently of the chromosome
- Passed from one cell to another
- Present in circular or linear form
- Can be transmitted from cell to cell.

Plasmids

- Being extrachromosomal factors of heredity, plasmids determine the resistance of bacteria to antibiotics, the formation of colicins, the production of toxins, and other properties. In accordance with certain features encoded by plasmid genes, the following plasmids are isolated:
- F-plasmids (from English fertility - fertility) - participate in conjugation
- R-plasmids (from the English resistance - resistant) - determine the synthesis of enzymes that destroy antibacterial drugs
- tox+-plasmids - determine the synthesis of exotoxins (eg, diphtheria and botulinum toxins)
- Col+-plasmids - determine the synthesis of colicins and other bacteriocins by Escherichia coli E.coli

Plasmids



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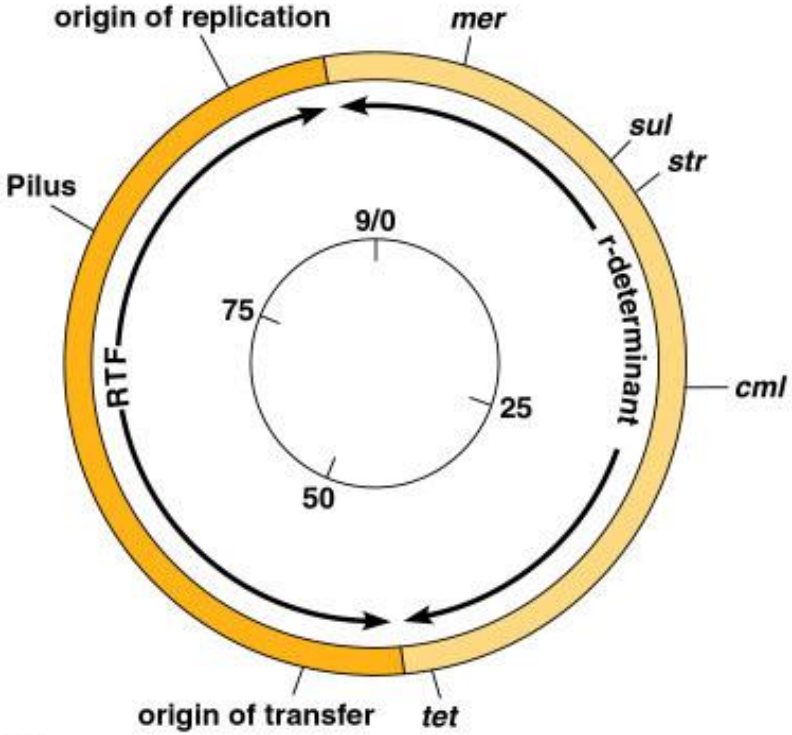

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Figure 8.29

Types of variability in bacteria

- Non-hereditary variability (modification). It is sometimes called phenotypic variability, since it does not affect the genotype, but only the phenotype of bacteria.
- Genotypic variability - variability associated with the genotype. Hereditary (genotypic) variability of microorganisms can occur as a result of mutations and genetic recombinations.

Modifications

- As a result of modifications, changes in the morphological, cultural, biochemical, and other characteristics of microorganisms occur.
- There are 2 types of modification variability:
- stable or long-term modification - persists in the offspring for several generations;

Modifications

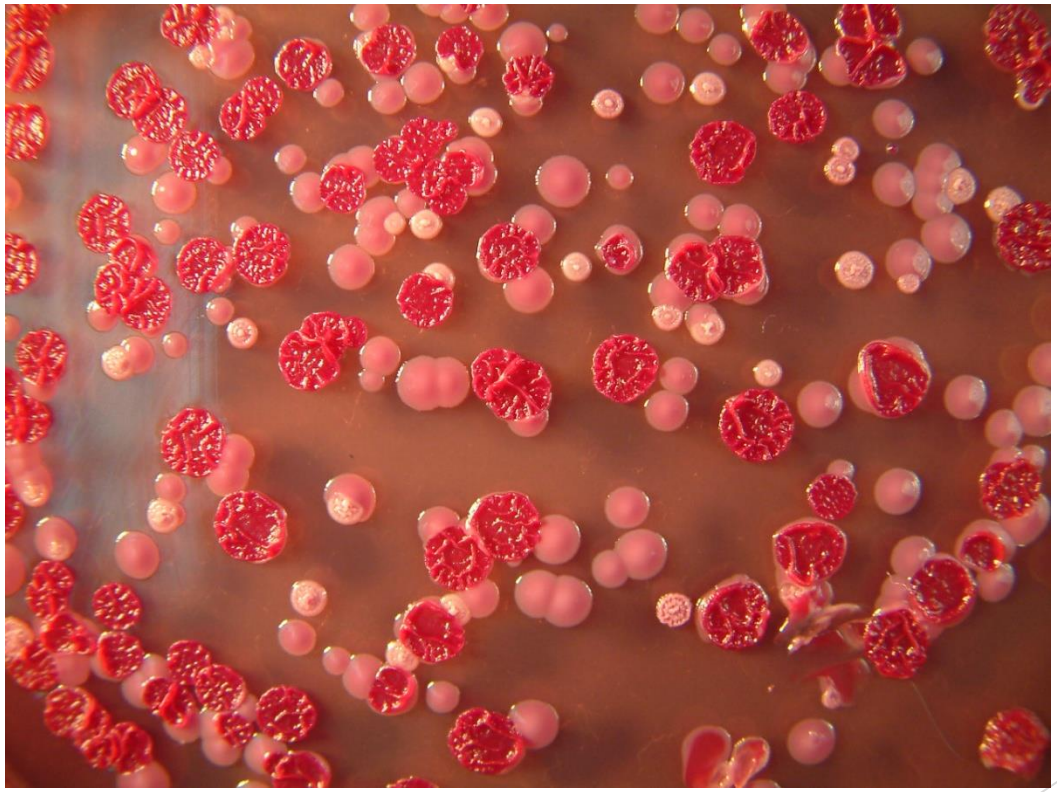
- short-term modification - with the disappearance of the acting factor, the changes also disappear.
- This variability allows microbial populations to quickly adapt to environmental factors.
- One of the manifestations of modification variability is dissociation observed in some populations of microorganisms.

Dissociation

- The essence of dissociative variability lies in the fact that some bacteria, when cultivated on dense nutrient media, form colonies of different types.
- Smooth, shiny colonies are designated as S-colonies (from the English smooth - smooth), rough colonies (from the English rough - rough) are called R-colonies.
- As a result of dissociation, intermediate forms sometimes appear - mucous M-colonies (from the English mucoid - mucous), very small D-colonies (from the English dwarf - very small, dwarf).

R - S Dissociation

- Dissociation usually proceeds in the direction from S to R through the formation of intermediate forms. The reverse transition is observed much less frequently.
- Most bacteria pathogenic to humans form S-colonies, with the exception of Mycobacterium tuberculosis, Yersinia pestis, Bacillus anthracis, etc.



Comparative characteristics of microorganisms forming S- and R-colonies

S-colonies	R-colonies
Colonies are smooth, shiny, convex	Irregular, cloudy, rough colonies
Causes cloudiness in liquid media	Form a precipitate in liquid media
May have flagella	May not have flagella
Can form a capsule	Does not form a capsule
Biochemically active	Biochemical activity low
Pathogenic species are highly virulent	Weakly virulent
They are found mainly in the acute form of the disease.	Usually found in the chronic form of the disease

Hereditary Variability

- Since hereditary variation affects the genotype, it is sometimes called genotypic variation.
- Genotypic variability in microorganisms occurs through mutations and genetic recombinations.

Mutations

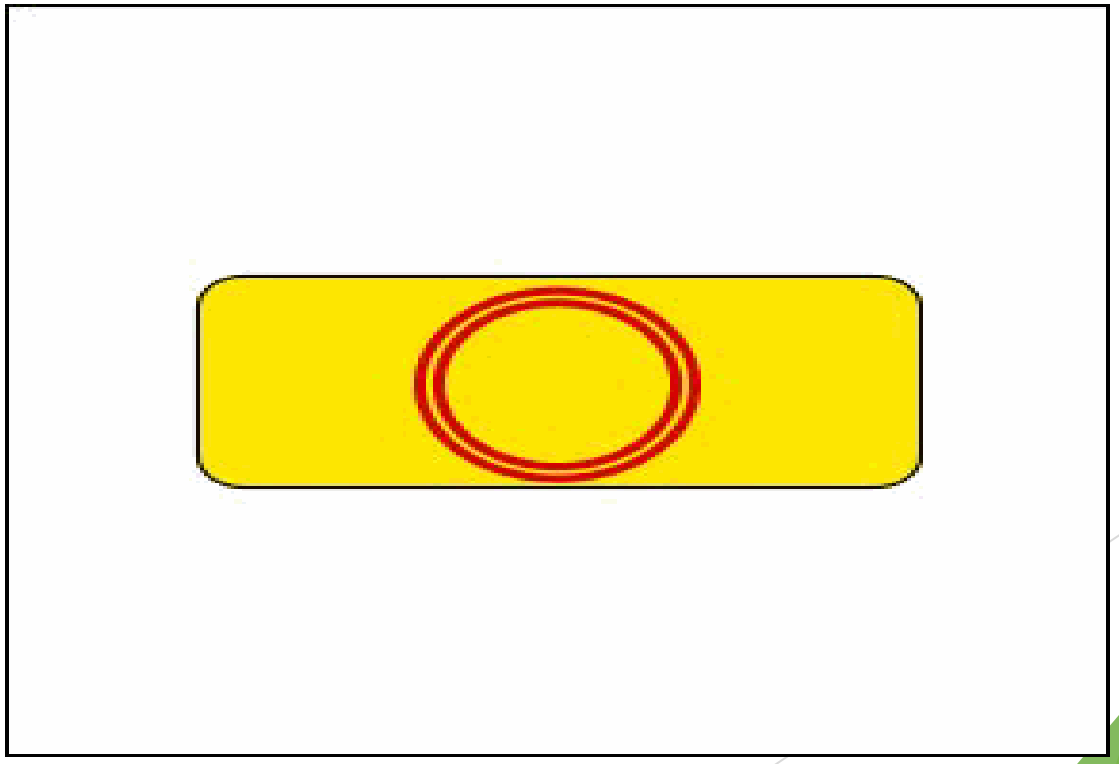
- Mutations (from Latin mutatio - change, change) are changes in the sequence of individual DNA nucleotides, manifested by the loss or change of signs. As a rule, these changes are passed on to subsequent generations.
- A strain formed as a result of mutation of a natural (wild) strain is called a mutant strain.

Mutations

- Spontaneous
- - reverse, or reversions
- induced
- - mutagens (chemical, physical, biological)
- Point (gene) mutations
- - mutation frameshift (with frameshift)
- - missense mutations (with a change in meaning)
- - nonsense mutations (antisense, meaningless)
- Chromosomal mutations (deletions, inversions, duplications)
- According to phenotypic traits - (neutral, conditionally lethal, lethal mutations)

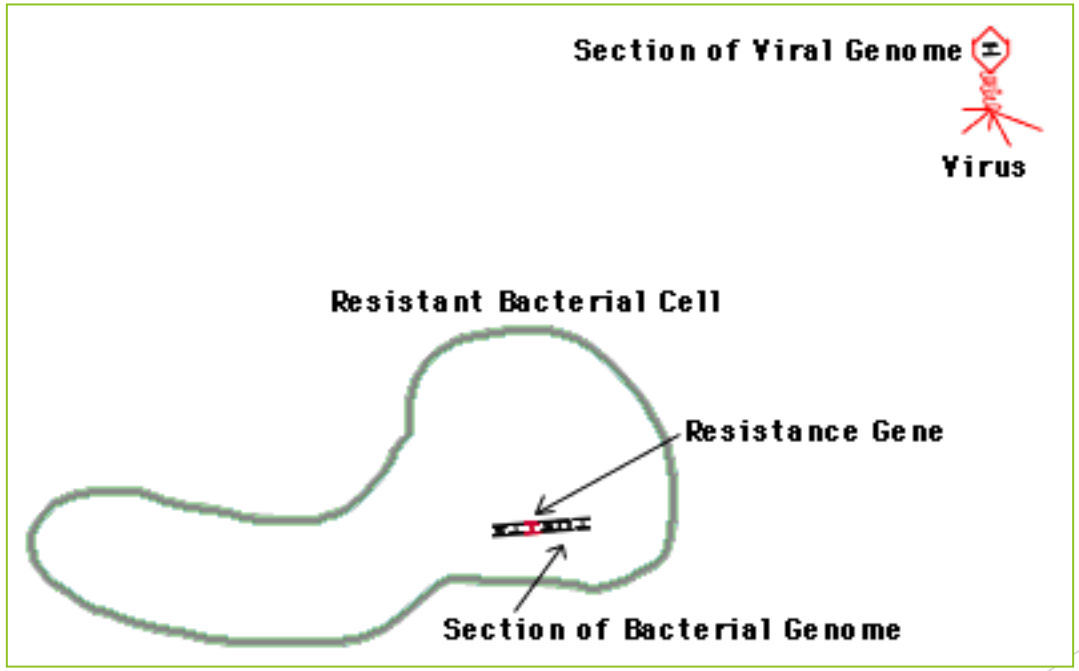
Transformation

Transformation is the direct transfer of the genetic material (highly polymerized DNA) of a donor into a recipient cell.



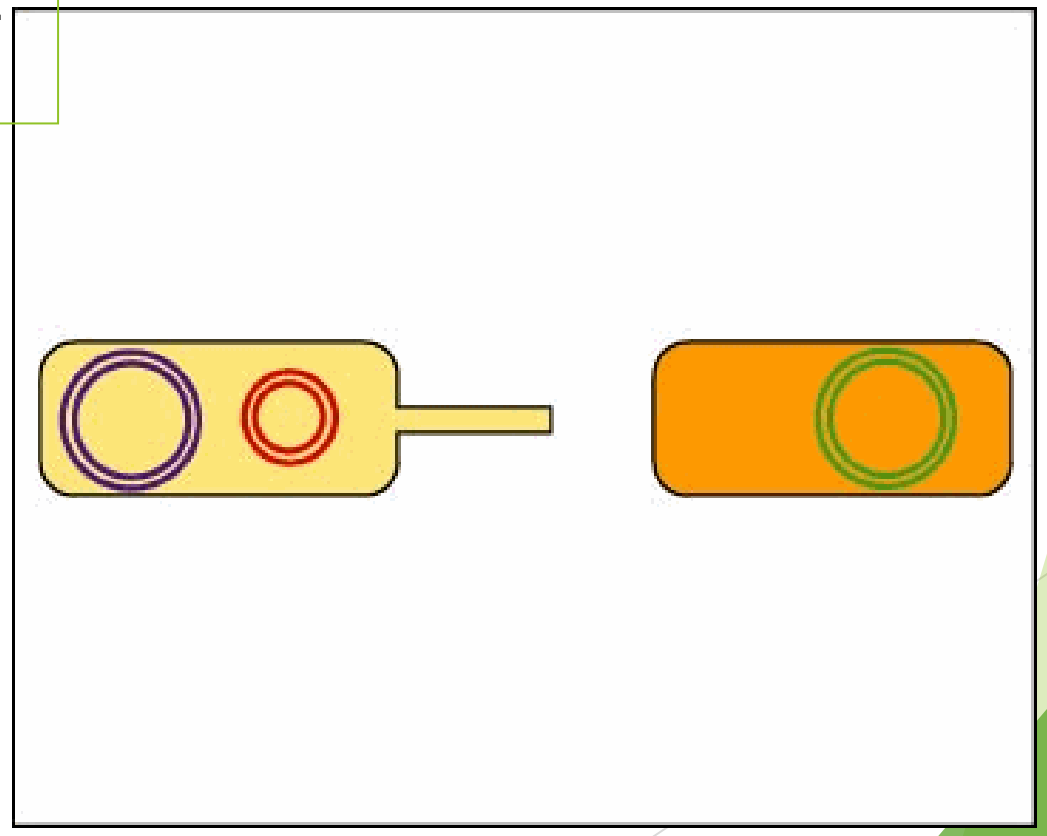
Transduction

Transduction is the transfer of bacterial DNA from a donor to a recipient via a bacteriophage.



Conjugation

Conjugation is the transfer of genetic material from a donor cell to a recipient cell by direct cell contact.



Basics of antimicrobial therapy.

Basics of chemotherapy

- Treatment of infectious diseases with chemotherapy drugs is called chemotherapy.
- Since the action of these drugs is directed against the etiological factor, and not the symptoms of the disease that it causes, they are called etiotropic drugs.

Paul Ehrlich-the founder of chemotherapy

- Receptor theory P. Erlich. In 1885, P. Ehrlich found that the effect of chemicals on pathogenic microbes is due to the presence of specific receptors in them.
- Based on the results of his own research, P. Ehrlich developed the theory of the "magic bullet" - one of the basic principles of chemotherapy - the principle of "destruction of the living in the living", without harming the host's body when the pathogen is destroyed.
- To assess the activity of drugs used in chemotherapy, the chemotherapeutic index is used - the ratio of the minimum effective dose of a drug that can kill the pathogen to the maximum dose tolerated by the body

Chemotherapeutic drugs

- Currently, many chemical compounds are known to have antimicrobial activity, but only some of them are used as chemotherapeutic drugs.
- The choice of a drug for chemotherapy determines the spectrum of its activity and the sensitivity of microorganisms to it.
- Drugs with specific activity include antibacterial, antifungal, antiprotozoal, and antiviral drugs.

The spectrum of activity

- Depending on the spectrum of activity, narrow and broad spectrum drugs are distinguished.
- Narrow-spectrum drugs are active against only a small number of either Gram-negative or Gram-positive bacteria.
- Broad-spectrum drugs are active against a sufficiently large number of bacterial species of both groups.

Action type

According to the type of action, chemotherapy drugs are divided into:

- microbicidal (bactericidal, fungicidal, etc.) - detrimental to microbes due to irreversible damage;
- microbostatic (bacteriostatics, fungistatics, etc.) - inhibiting the growth and reproduction of microbes

Ways to obtain

According to the method of preparation, there are 2 main groups of antimicrobial chemotherapeutic drugs:

- Synthetic chemotherapy drugs Synthetic analogues of antibiotics, the molecules of which are synthesized chemically.
- Antibiotics - biosynthetic (natural) and semi-synthetic antibiotics

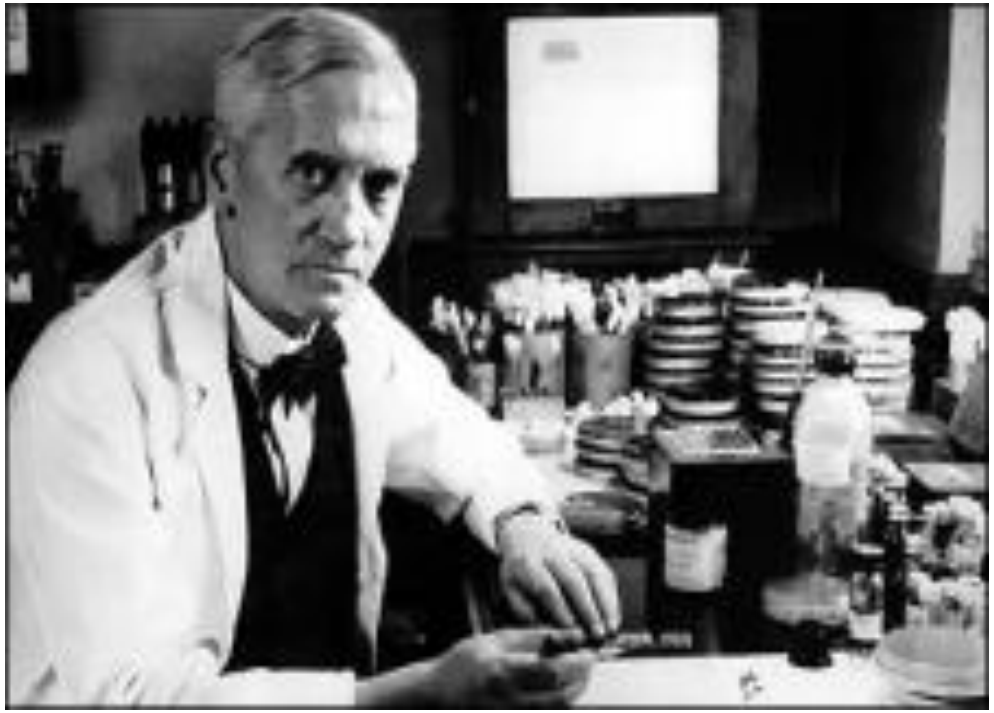
Main groups of chemotherapy drugs

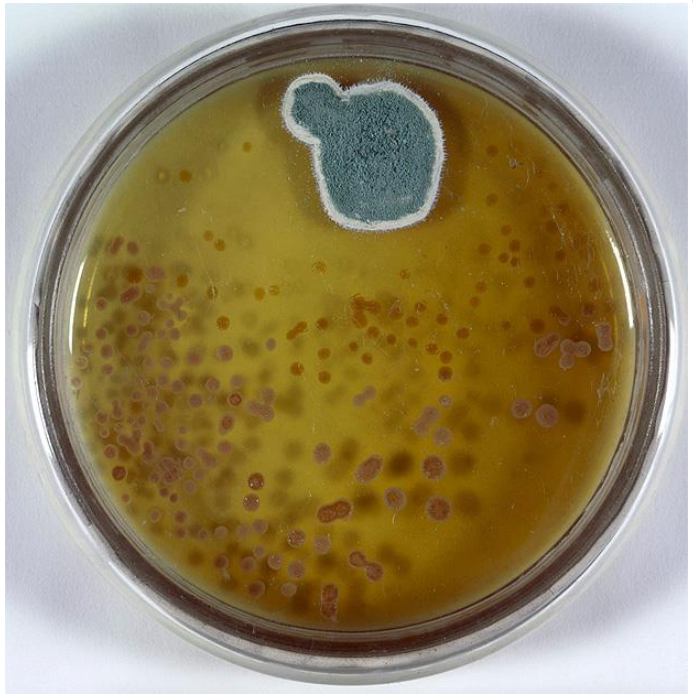
- Sulfonamides–co-trimoxazole (biseptol). streptocid, sulfadimezin, sulfadimetoksin
- Quinolones - nalidixic acid (nevigramon), ofloxacin, ciprofloxacin, norfloxacin, etc.
- Nitroimidazoles are especially active against anaerobic bacteria, as well as protozoa (metronidazole, ornidazole, etc.).
- Imidazoles and triazoles - antifungal drugs that inhibit the synthesis of CPM (clotrimazole, ketoconazole, miconazole, fluconazole)
- Nitrofurans - used as uroseptics (furozalidon, furadonin, furagin)
- Oxyquinolines - 5-nitroxoline, quinosol, intestopan, etc.)
- Antimetabolites - nicotinic acid hydrazides
- (isoniazid, ftivazid, tubazid, etc.)

Antibiotics

- One of the most common forms of antagonism is the ability of microorganisms to form antibiotics (from Greek anti, bios - against life).
- Low concentrations of these substances have a damaging or destructive effect on other microorganisms.
- The term "antibiotic" was proposed by Waksman in 1942 to refer to natural substances produced by microorganisms that help to suppress the growth or death of certain bacteria.

Discovery of antibiotics





In 1928-1929. English microbiologist A. Fleming discovered a mold strain (Penisillium notatum), which produces a toxic substance that inhibits the growth of Staphylococcus aureus

Getting antibiotics

- Under optimal cultivation conditions, microbes-producers secrete antibiotics in the course of their life activity.
- In some cases, antibiotics are obtained by semisynthesis or synthesis.
- Therefore, there are three ways to obtain antibiotics:
- biological synthesis
- Biosynthesis followed by chemical modifications
- Chemical synthesis

Classification of antibiotics by source of origin

Antibiotics of microbial origin:

- typical bacteria - eubacteria, bacilli, pseudomonads (polymyxin, gramicidin, etc.);
- actinomycetes (streptomycin, tetracycline, chloramphenicol, etc.);
- mold fungi (penicillins, cephalosporins, etc.);

Antibiotics of plant origin (phytoncides)

Antibiotics of animal origin (lysozyme, interferon, etc.)

Classification of antibiotics

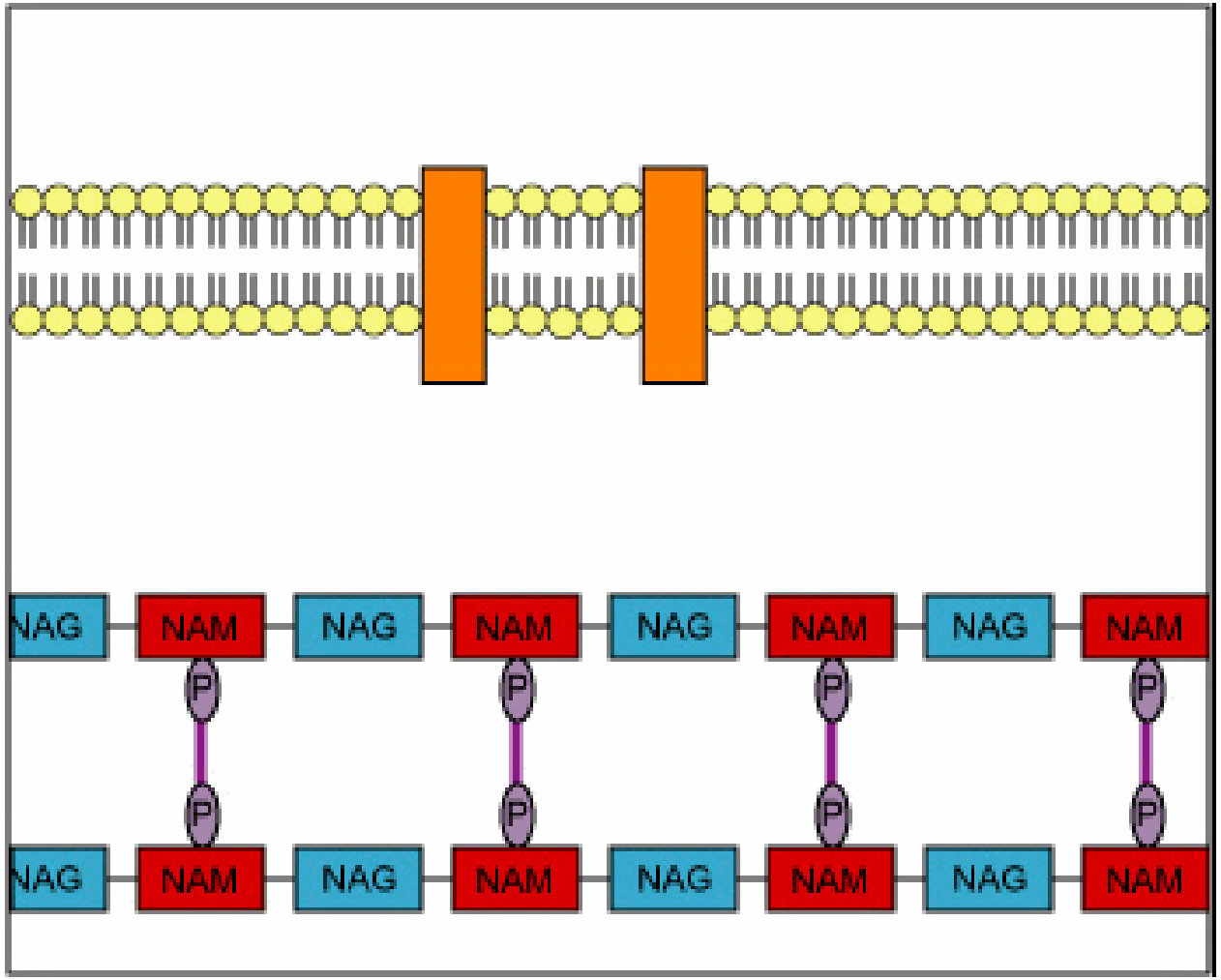
By chemical structure:

- Beta-lactam antibiotics (penicillins, cephalosporins, carbapenems, monobactams)
- Macrolides (erythromycin, spiramycin, clarithromycin)
- Azalides (azithromycin)
- Tetracyclines (tetracycline, doxycycline)
- Aminoglycosides (streptomycin, kanamycin, gentamicin)
- Levomycetin (chloramphenicol)
- Glycopeptides (vancomycin)
- Rifamycins (rifampicin)
- Cyclic polypeptides (polimiksinlər, basitrasinlər)
- Polyenes (nystatin, levorin, amphotericin B, etc.)

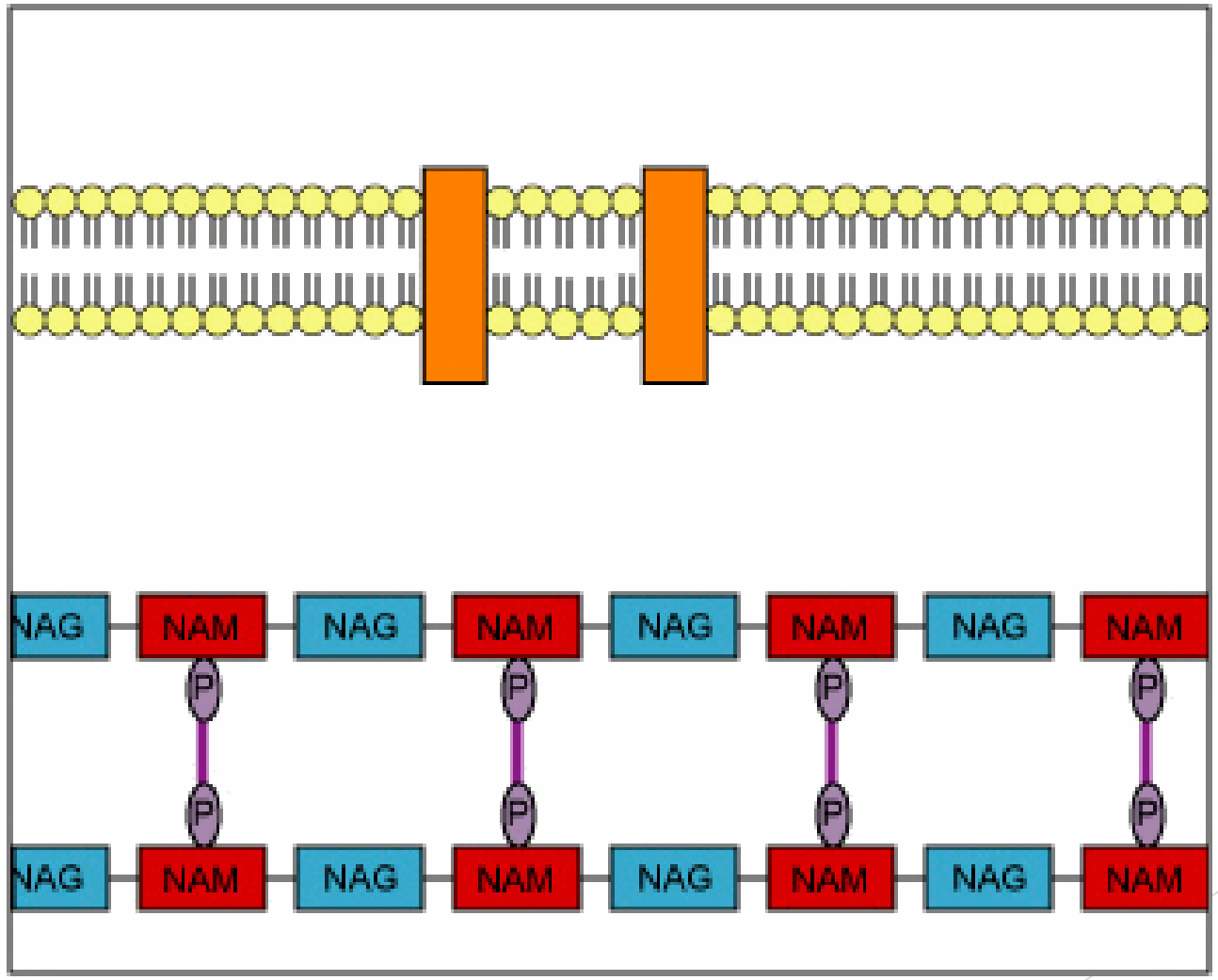
Mechanism of antimicrobial action of antibiotics

- Cell wall synthesis inhibitors - Beta-lactam antibiotics (penicillins and cephalosporins), glycopeptides (vancomycin and teicoplanin)
- Protein synthesis inhibitors aminoglycosides and tetracyclines binding to the 30S subunit of ribosomes; macrolides, chloramphenicol and lincosamides - with the 50S-ribosomal subunit, disrupt protein synthesis in the bacterial cell.
- Inhibitors of nucleic acid synthesis - rifamycins (rifampicin), binding to RNA polymerase, block mRNA synthesis
- Cytoplasmic membrane permeability inhibitors (membranotropic antibiotics) - polypeptides (polymyxins), polyene antibiotics (nystatin, levorin, amphotericin B, etc.)

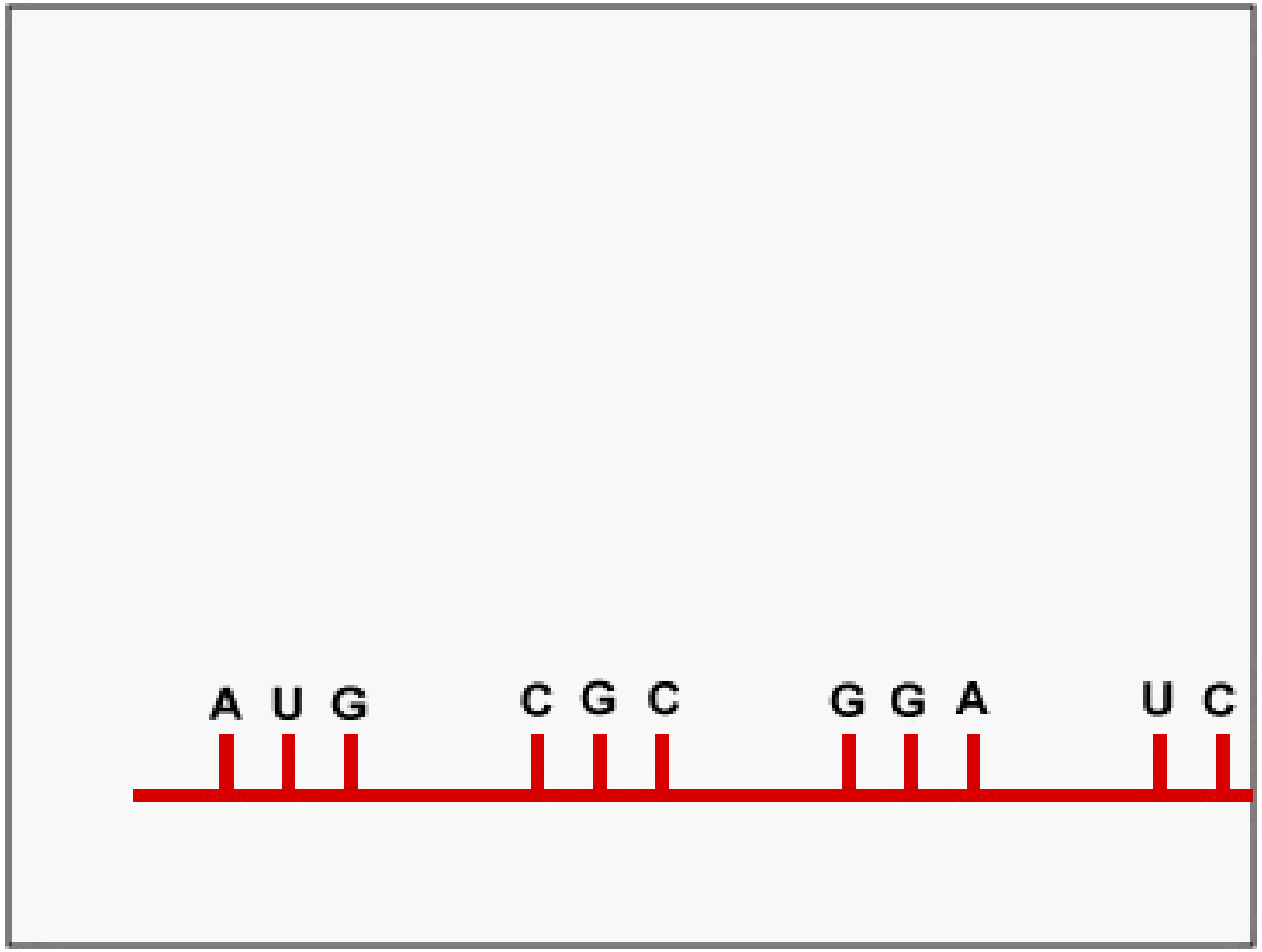
Cell wall peptidoglycan synthesis



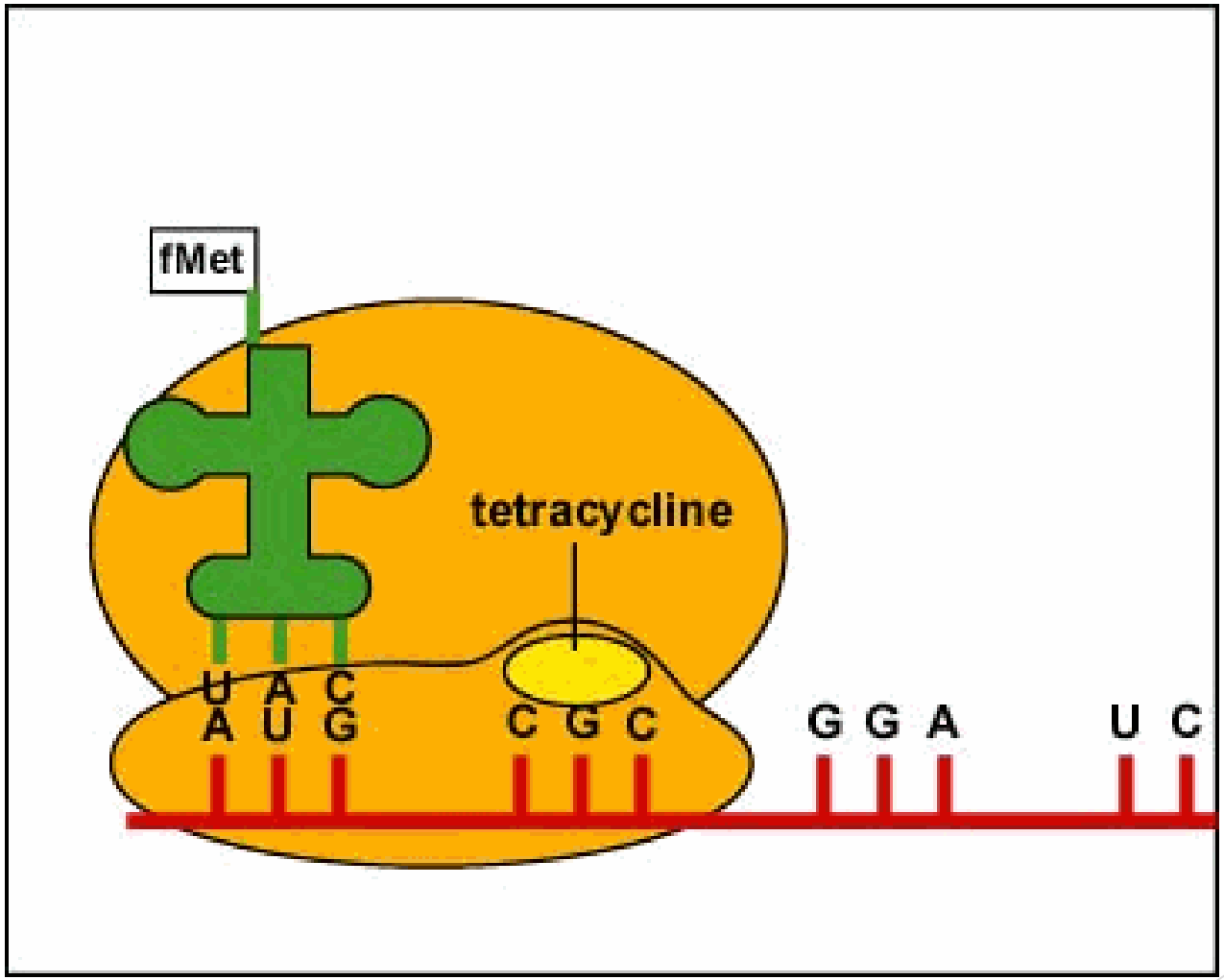
Suppression of cell wall peptidoglycan synthesis by penicillin



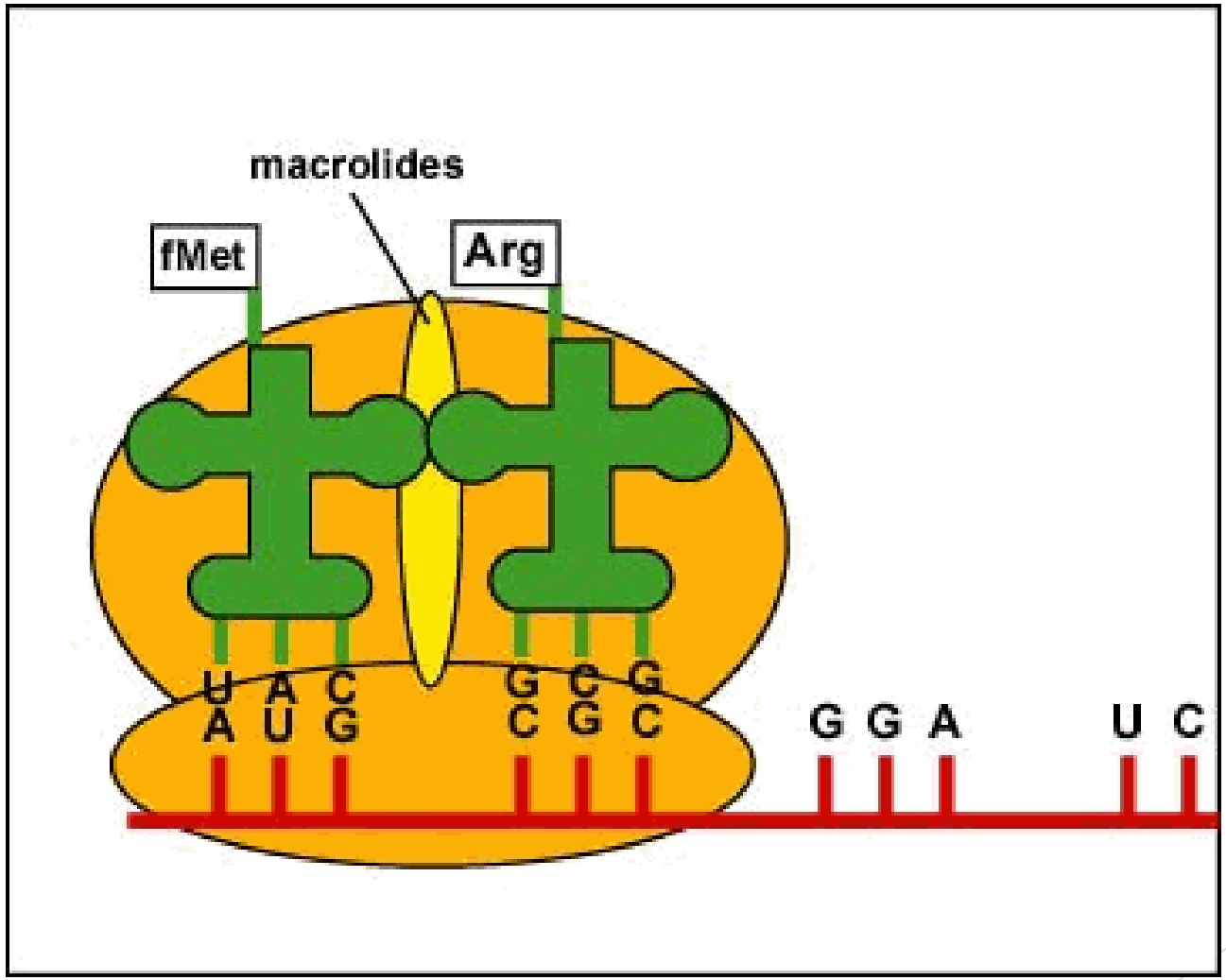
Aminoglycosides bind to the 30S subunit of the ribosome and inhibit bacterial protein synthesis



Tetracyclines bind to the 30S subunit of the ribosome and inhibit bacterial protein synthesis



Macrolides attaching to the 50S subunit of ribosomes inhibit protein synthesis in a bacterial cell



Resistance of microorganisms to antibiotics and its mechanisms

Antibiotic resistance can be natural (natural) and acquired

- The natural resistance of microorganisms is associated with the absence of targets on the cell wall, or its impermeability to certain drugs.

Acquired resistance is associated with the adaptation of microorganisms to environmental conditions and is formed under the influence of antibiotics.

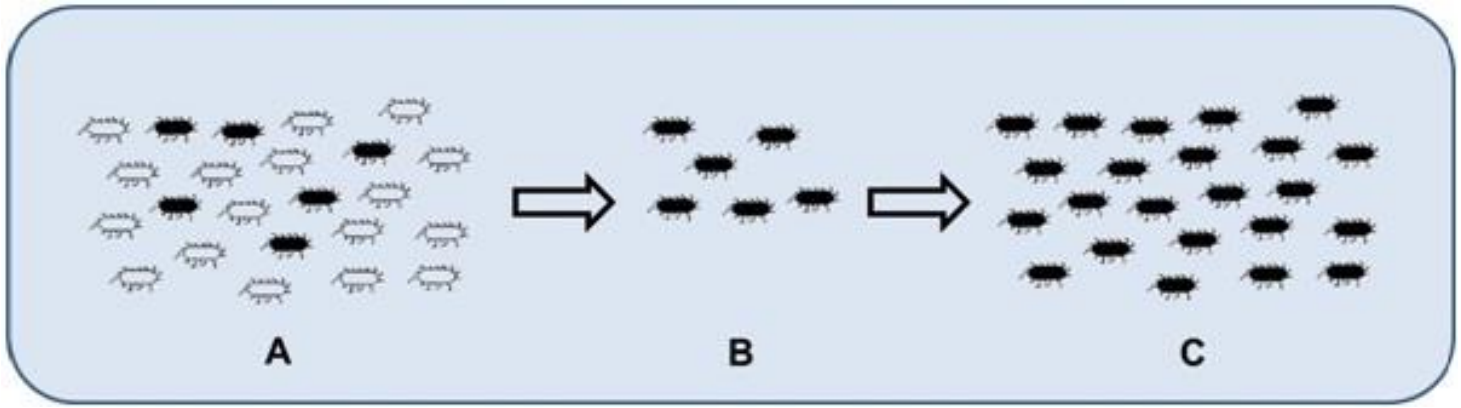
- Decreased cell wall permeability and disruption of antimicrobial drug transport to intracellular targets
- Acceleration of the exit of the antimicrobial agent from the cell
- Modification of the antimicrobial target
- Inactivation of the antimicrobial agent

Antimicrobial agent inactivation

- Refers to the main mechanisms of drug resistance
- Some bacteria are able to produce special enzymes that inactivate drugs
- H-p, beta-lactamases (penicillinases) of penicillins and cephalosporins destroy the ß-lactam ring with the formation of inactive compounds.
- Synthesis of beta-lactamases is determined by R-plasmids

Genetic basis of antibiotic resistance

- Antibiotic resistance is determined and maintained by resistance genes. Plasmids containing resistance genes are called R-plasmids or R-factors. Resistance genes primarily encode the synthesis of enzymes (beta-lactamase, etc.) that cause drug resistance.
- Antibiotics do not induce the formation of r-genes, they only contribute to the selection of microbial populations with such genes.

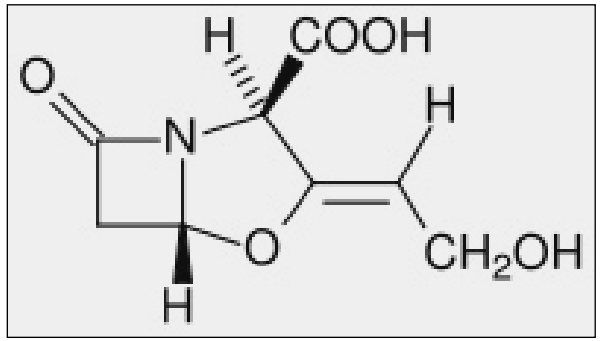


Genetic basis of antibiotic resistance

- In ensuring the resistance of microorganisms to antibiotics, a certain role belongs to mutations that occur in the microbial population.
- A number of strains of S. aureus develop resistance to methicillin due to a mutation in the genes, which encodes the synthesis of a penicillin-binding protein that is not able to bind to beta-lactam antibiotics. And therefore, all methicillin-resistant strains of Staphylococcus aureus (MRSA) are resistant to beta-lactam antibiotics.

Mechanism of overcome antibiotic resistance

- prudent use of antimicrobials
- synthesis of new antibiotics
- combination of antibiotics with beta-lactamase enzyme inhibitors (sulbactam and clavulanic acid):
- - These substances contain a beta-lactam ring and are able to bind and neutralize the beta-lactamase of bacteria, as a result, the action of this enzyme on beta-lactam antibiotics is prevented
- - In medical practice, combined preparations of ampicillin and sulbactam (ampicid, etc.), amoxicillin and clavulanic acid (augmentin, amoxiclav, etc.)



How do you overcome antibiotic resistance?

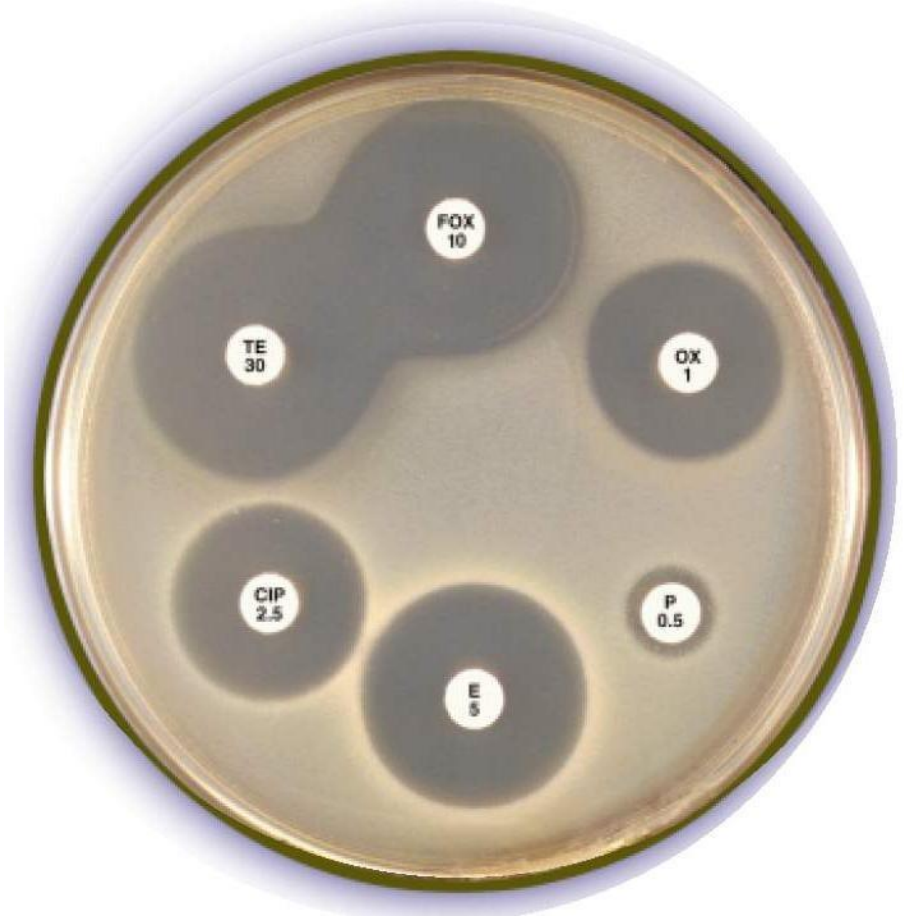
- One of the ways to prevent the development of antibiotic resistance is to determine the sensitivity of microorganisms to antibiotics, which is taken into account during therapy.
- Determination of the sensitivity of bacteria to antibiotics is carried out by a qualitative and quantitative method.
- Qualitative method - agar diffusion method (Kirby-Bauer method)
- Quantitative method - determination of the minimum inhibitory and bactericidal concentrations of antibiotics

Qualitative Method

- Disk diffusion method. The studied culture is inoculated on a dense nutrient medium, then paper discs soaked in antibiotics are introduced.
- No more than 6 paper discs are usually placed on a Petri dish with a diameter of 90 mm.
- Antibiotic susceptibility is assessed based on bacterial growth around the discs after a 24-hour specimen incubation under optimal culture conditions.
- In the presence of sensitivity to the antibiotic, the appearance of sterile zones of various diameters (lack of growth) around the discs on a nutrient medium is observed.
- The size of the sterile zone shows the degree of sensitivity of microorganisms to antibiotics.

Antibiotic-impregnated paper discs

Disc diffusion method



Standard Approaches for Antibiotic Susceptibility Testing

- There are standard approaches to determining antibiotic susceptibility.
- One of them is the EUCAST (European Committee for Antimicrobial Susceptibility Testing) standard.
- When determining antibiotic susceptibility, EUCAST principles should be followed, which include: the concentration of antibiotic on a paper disk, the composition of the culture media used, the assessment of the diameter of the sterile zone, and the need to determine antibiotic susceptibility (selective antibiogram) for each microorganism.
- *EUCAST guidelines are updated periodically

Quantitative method

A quantitative method allows you to determine the minimum inhibitory concentration (MIC) of an antibiotic

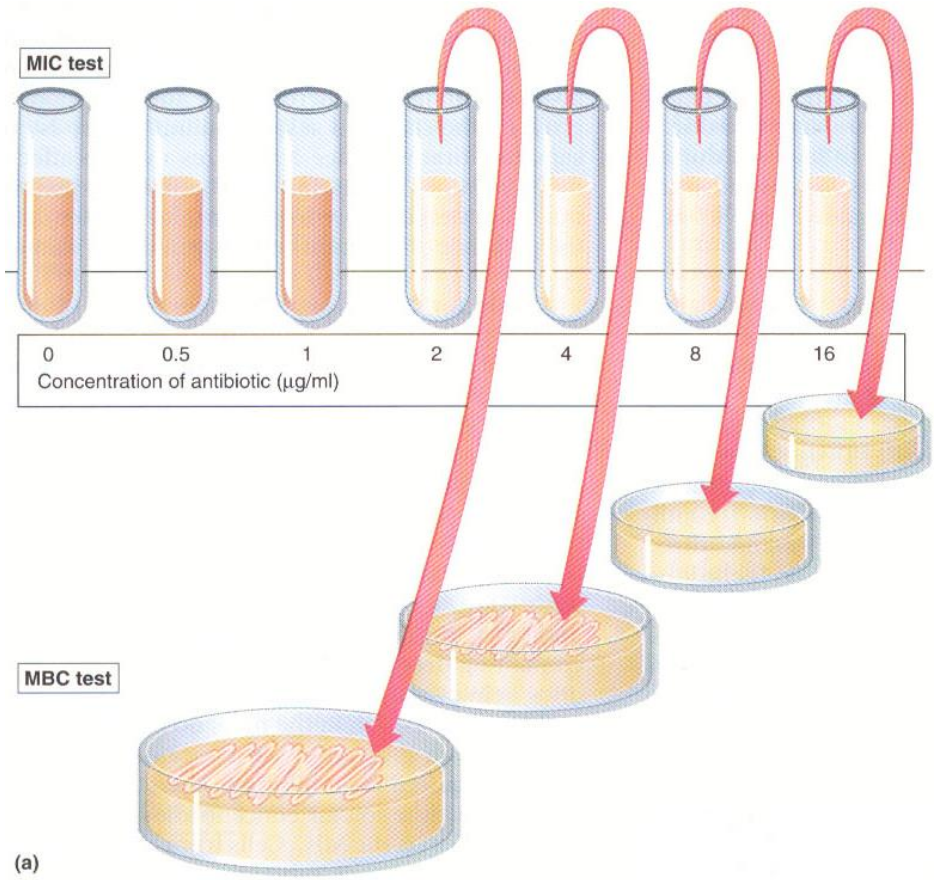
- The principle of the method is based on inhibiting the growth of microorganisms in nutrient media when certain concentrations of antibiotics are added to them.
- The criteria for the activity of drugs are the minimum inhibitory concentration (MIC) - the lowest concentration of the drug that inhibits the growth of microorganisms and the minimum bactericidal concentration (MBC) - the lowest concentration of the drug that causes the death of microorganisms.
- These values are usually expressed in micrograms (μg/mL) per ml. For some antibiotics, these values are expressed in units of action (U). The unit of action of an antibiotic is the smallest dose that stops the growth of a microorganism. For most antibiotics, 1 unit is approximately equal to 1 mcg.

Serial dilution method

- The method of serial dilutions in liquid media allows you to set the MIC of the drug for the pathogen
- For example, in order to determine the MIC of tetracycline for a culture of Staphylococcus aureus, double dilutions of bactericidal concentrations of this antibiotic are prepared in test tubes with nutrient broth.
- The concentration of the antibiotic is reduced accordingly from the 1st to the 2nd tube, etc. pouring each time 1 ml from the previous tube, the final volume of the medium in each tube is 1 ml.

Determination of MIC by the method of serial dilutions

The principle of the method is based on the inhibition of the growth of microorganisms in nutrient media with the addition of certain concentrations of the antibiotic.

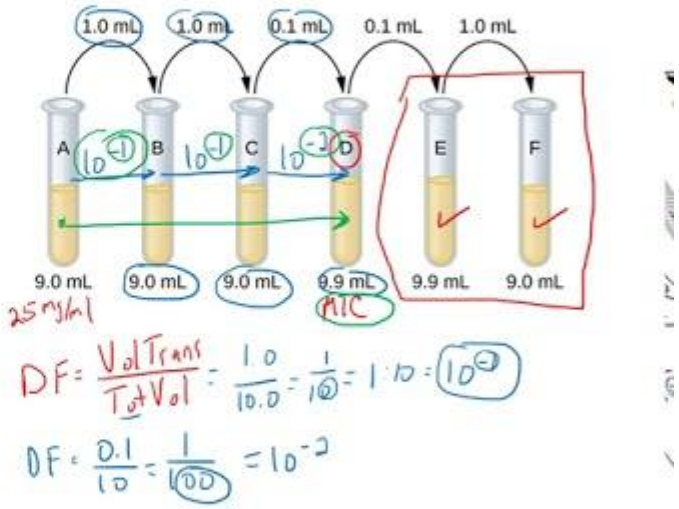


Determination of MIC by the method of serial dilutions

Determining the minimal inhibitory concentration (MIC)

- An MIC assay is shown below. Tube A contains vancomycin at a concentration of 25 mg/mL. The serial dilutions performed are shown. Then *Staphylococcus aureus* was inoculated into each tube. The tubes were then incubated for 24 hours. After 24 hours, growth was observed only in tubes E and F. From this experiment, identify which tube contains the MIC and calculate the MIC of vancomycin for *S. aureus*.

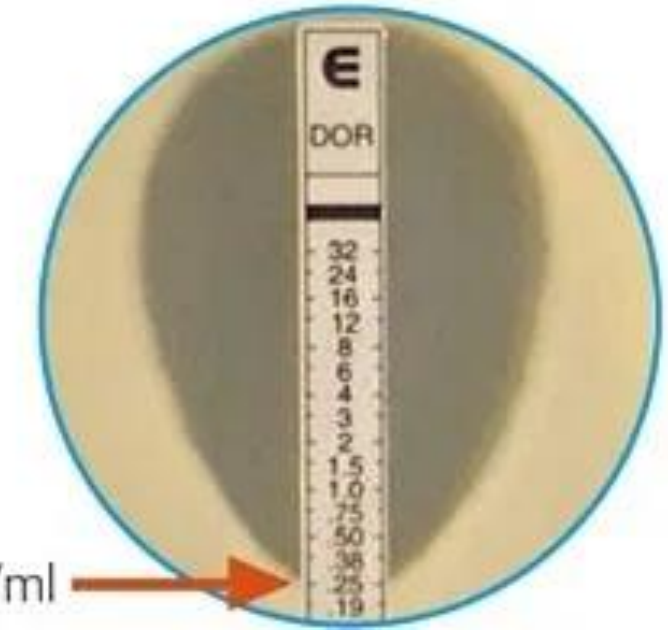
TDF = 10^{-4}



Epsilometric method (E-test)

- For the E-test, a filter paper strip impregnated with various concentrations of the drug (128, 64, 32, 16, 8, 4, ..., μg / ml) is used.
- E-test strips are applied to a Petri dish seeded with a test culture so that the area with the lowest concentration is located closer to the center, and the area with the highest concentration is closer to the periphery.
- After incubation, an elliptical sterile zone is formed around the strip, the shape of which is due to the action of different concentrations of the drug on sensitive bacteria at once. MIC corresponds to the section of the strip where it crosses the border of the growth retardation zone.

E-test



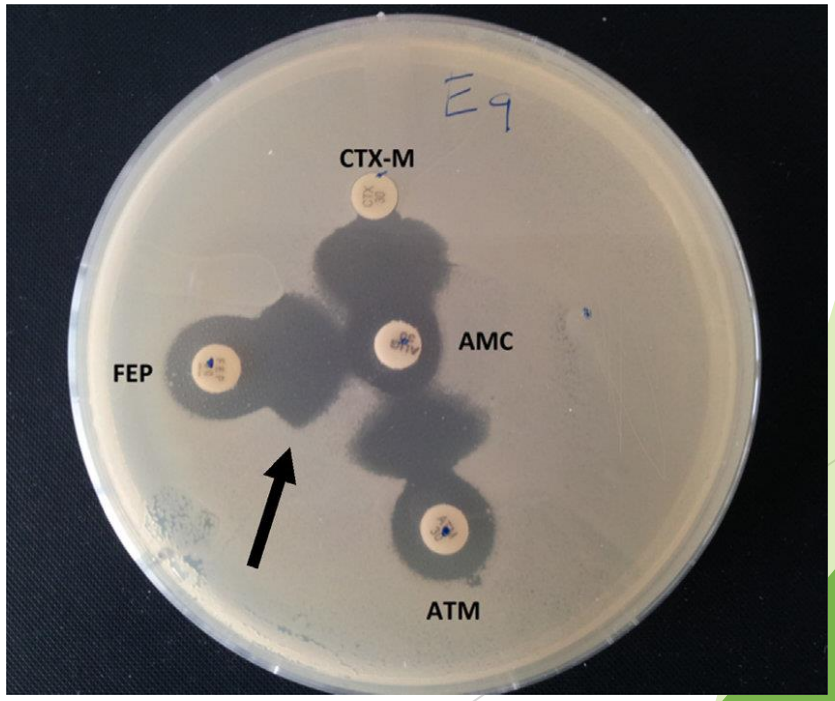
MIC 0.25 µg/ml

Mechanisms of antibiotic resistance

- One of the mechanisms for the formation of resistance of microorganisms to antimicrobial drugs is the production of enzymes that inactivate the antibiotic molecule.
- These enzymes include beta-lactamase, which destroys the beta-lactam ring of antibiotics.
- The production of such enzymes is encoded by the genes of plasmids of microorganisms.
- Recently, there has been an increase in the number of microorganisms producing broad-spectrum beta-lactamases (BLSS), which cause the destruction of antibiotics that are resistant to conventional beta-lactamases.

Determination of Extended-Spectrum beta-lactamase synthesis (ESBL) (phenotypic test)

- A disk containing a beta-lactam antibiotic (eg, cefepime) and a disk containing a beta-lactamase inhibitor and an antibiotic (eg, amoxicillin + clavulanic acid) are applied to the surface of the inoculated solid medium.
- The evaluation of the results is carried out after a daily incubation of the dish with the medium
- In the case of the synthesis of ESBL by bacteria, the sterile zone around the disc with cefepime spreads towards the disc with amoxiclav and clavulanic acid.



Complications of antimicrobial therapy and ways to prevent them

allergic reactions

- prescribing drugs in accordance with the individual sensitivity of the patient

Dysbiosis and dysbacteriosis

- combination of treatment of the underlying disease with antifungal therapy.

- the use of probiotics,

- use narrow-spectrum drugs whenever possible

Toxic action

- take into account contraindications and side effects