AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 15.

Basic principles of antimicrobial therapy. Chemotherapeutic drugs. Antibiotics, obtaining and classification. Antibiotic sensitivity tests

FACULTY: General Medicine
SUBJECT: Medical microbiology - 1

Discussed questions:

1. Basic principles of antimicrobial chemotherapy.
2. Synthetic chemotherapeutic drugs (sulfonamides, quinolones, nitroimidazole, 8-oxyquinoline, nitrofuran, imidazole, thiazoles, etc.).
3. Antibiotics and their discovery.
4. Sources of antibiotics (microorganisms, animals and plants).
5. Chemical composition of antibiotics.
6. Effect mechanisms of antibiotics.
7. Effect spectrum of antibiotics (broad-spectrum antibiotics, extended-spectrum antibiotics and narrow-spectrum) and caracters (bactericidal and bacteriostatic).
8. Determination of antibacterial effects unit of antibiotics (TVT).
9. The mechanism of resistance to antibiotics and the formation of resistance in microorganisms, ways to eliminate them.
10. Determination of antibiotic susceptibility of bacteria by disk-diffusion method.
11. Determination of antibiotic susceptibility of bacteria by dilution.
12. Determination of antibiotic susceptibility of bacteria by epsilometer test (E-test).
13. Complications of antibiotic therapy and to prevent them.
14. The chemical therapy principles of viral infections.
15. Antivirus chemical drugs.

Purpose of the lesson:

To inform students about the basic principles of chemotherapy, antimicrobial drugs (chemotherapeutic drugs and antibiotics). They are taught the classification of antibiotics (according to their origin, chemical composition, spectrum of activity, spectrum of action, type and mechanism), possible complications during antibiotic therapy and their prevention, the emergence of antibiotic resistance in microorganisms and their elimination, correct selection of antibiotics for treatment, explain the importance (especially in the case of resistance to antibiotics).

Fundamentals of chemical therapy

- Treatment of infectious diseases with chemical therapeutic drugs is called **chemical therapy**
- These drugs do not treat symptoms. They have effect on etiological pathogens. Thus, they are called **etiotropic drugs.**

Paul Ehrlich is the founder of chemotherapy

- **P. Ehrlich's receptor theory**. In1885 P.Ehrlich discovered that the impact of chemical compounds on pathogens is related to specific receptors in microorganisms.
- **“Magic bullet”** idea of P.Ehrlich was one of the main principles of chemical therapy. It proposed killing microbe without impact on body tissues.
- **Chemical therapeutic index** – ratio of minimal therapeutic dose killing pathogen to the highest dose which organism can resist. This index is used for evaluation of therapeutic drugs.

Chemotherapeutic drugs

- Nowadays, thousands of chemical compounds with antimicrobial activity is known. However, only some of them are used as chemotherapeutic drugs.
- Activity spectrum of therapeutic drugs is determined by microorganism groups on which they have effect.
- Based on type of affected microorganism **antibacterial, antifungal, antiprotozoan, antiviral** etc., antimicrobial drugs exist.

Activity spectrum

- *Depending on **activity spectrum** narrow and broad spectrum drugs distinguished.*
- ***Narrow spectrum** drugs act on limited number of species, either gram negative or gram positive bacteria.*
- ***Broad spectrum** drugs both on gram negative and gram positive bacteria species.*

Action type

Based on **action type**:

- ***Microbocide*** (bactericide, fungicide etc.,) and
- ***microbostatic*** (bacteriostatic, fungistatic etc.,) drugs distinguished.
- The first group drugs kill bacteria, while representatives of the second group inhibit microbial growth.

Source

Based on method used to obtain antimicrobial drugs they are divided to:

- **synthetic** – commonly obtained by chemical synthesis;
- **antibiotics**- commonly of natural origin, sometimes obtained by syntetic or semisynthetic methods.

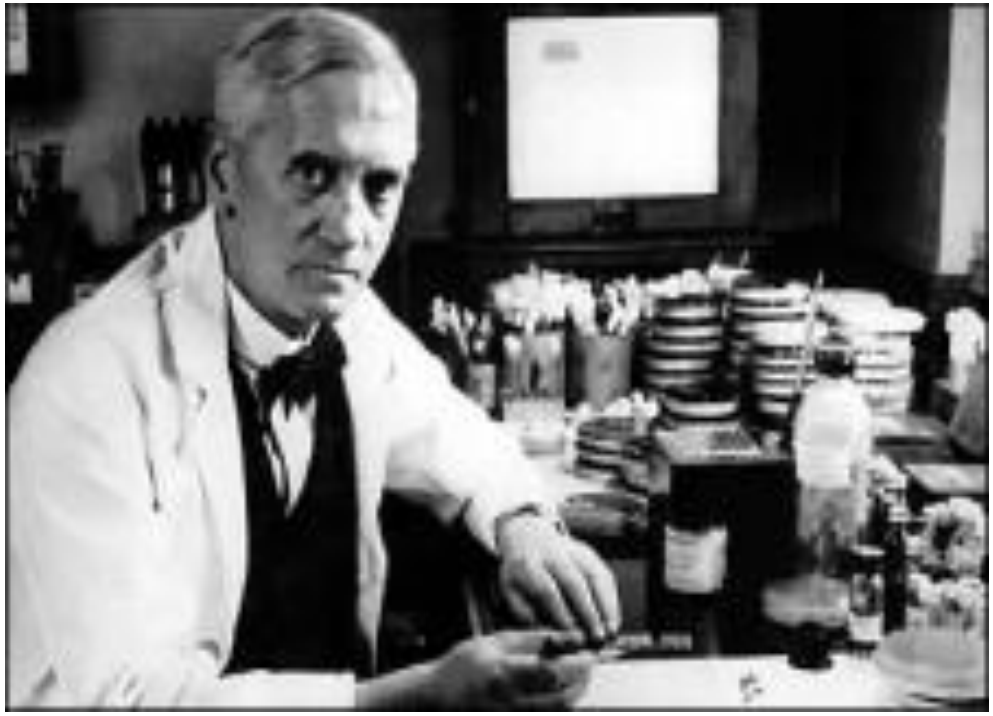
The main groups of synthetic chemotherapeutic drugs

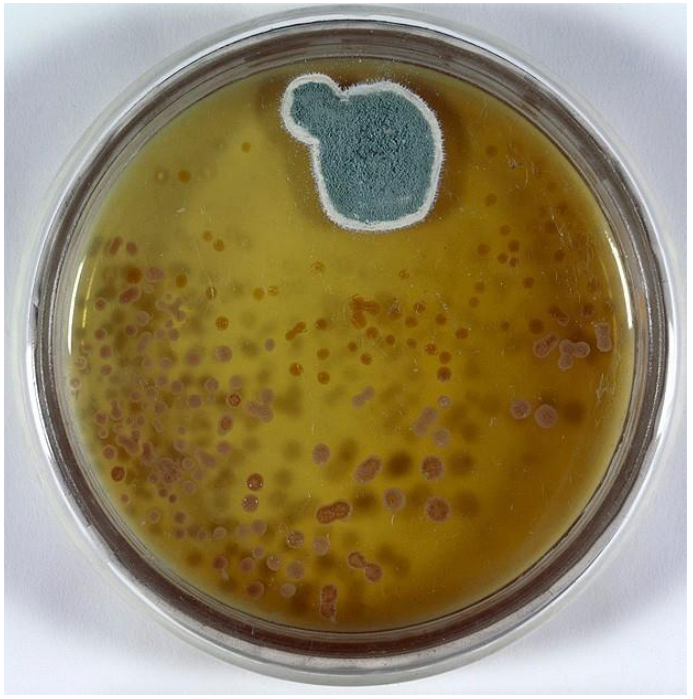
- **Sulfanilamide** (streptocide, sulfadimidine, sulfadimetoxin etc.)
- **Antimetabolites –** nicotinic acid derivatives (isoniazid, ftivazid, tubazid etc.)
- **Quinolons -** nalidixic acid(nevigramon), ofloxacin, ciprofloxacin, norfloxacin etc.,
- **Nitroimidazoles** (metronidazole, ornidazole etc.)
- **8- oxyquinoline** (5-nitroксolin, xinozol, intestopan etc.)
- **Nitrofurantoin derivatives** (furacillin, furazolidone, furagin etc.)
- **Imidazoles** (кетоконazole, miконazole, clotrimazole etc.)
- **Triazoles**(flukонazole)

Antibiotics

- Production of antibiotics (greek anti – against, bios – life) by microorganisms is the common type of antagonism.
- Small concentrations of these compounds stop growth of other microorganisms
- **The term "antibiotic" was proposed by S. Vaxman in 1942.** According to him antibiotics are compounds produced by microorganisms and stop the growth of certain bacteria or cause their destruction.

Development of antibiotics





In 1929 Alexander Fleming noted a lysis of Staphylococcus aureus colonies surrounding the culture of mould (*Penisillium notatum*) occasionally contaminated the Petri dish.

Obtaining of antibiotics

- They are excreted by microorganisms in nutrition media during growth of microorgansims and separated from media chemically.
- Some antibiotics are obtained by semisynthetic and synthetic methods. Thus, 3 main methods of antibiotic obtaining exist:
- ***Biosynthetic***
- ***semisynthetic***
- ***Chemical synthes***

Classification of antibiotics

Origin:

- ***Antibiotics of microbial origin***:

  - Bacteria(polymixin, qramicidin etc.)

  - Actynomicetes(streptomycin, tetracycline, choramphenicol etc.);

  - fungi synthesized antibiotics (penicillins, scfalosporins etc.);

- ***Plant*** (phytoncides)

- ***Animal***(lisozyme, interferone və s.)

Classification of antibiotics

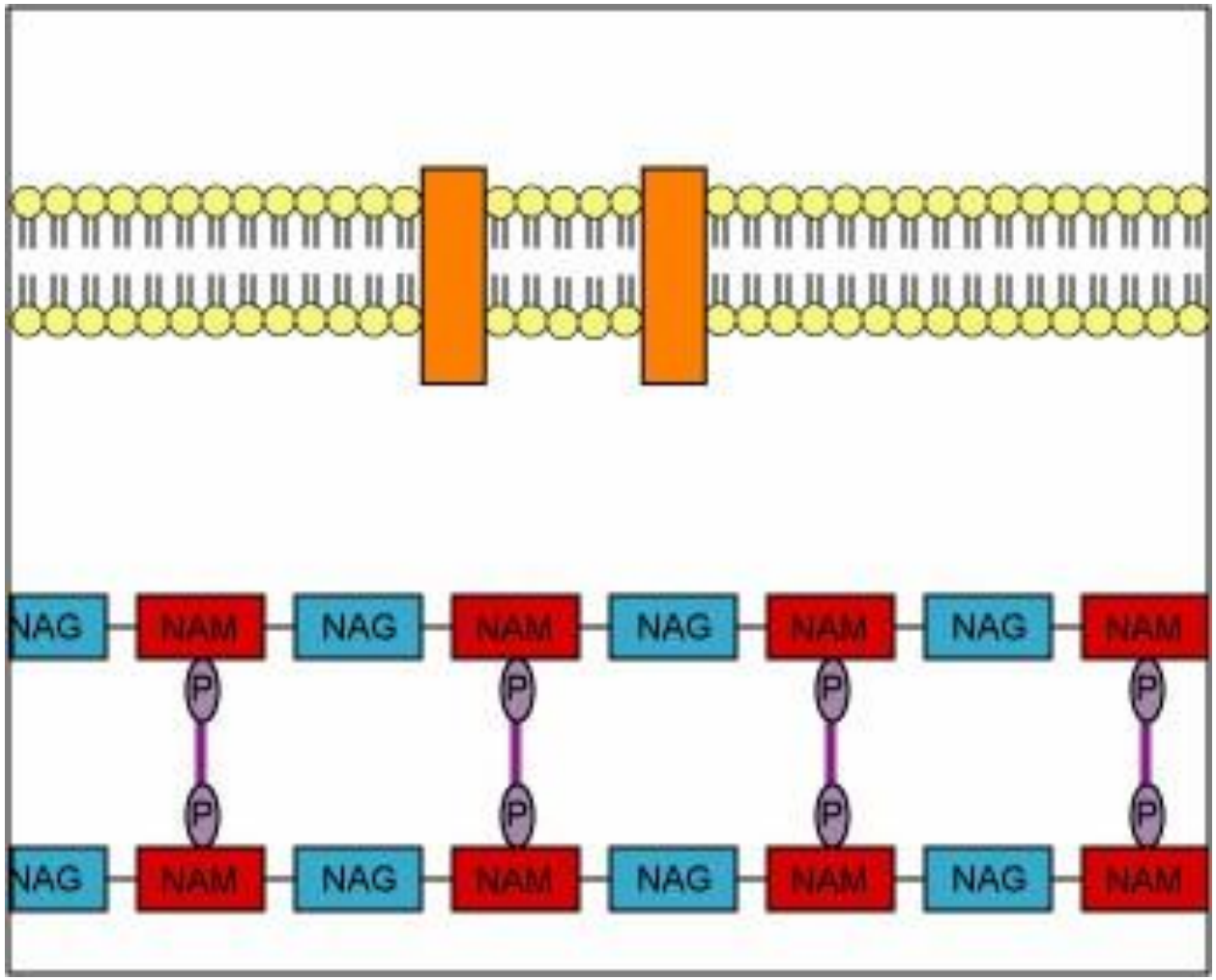
Chemical structure

- **beta-lactams** (penicillins, cefalosporins, carbapenems, monobactams)
- **macrolides** (erythromycin, spiramycin, klaritromycin etc.)
- **azalides** (azitromycin)
- **tetracyclines** (tetracycline, doxycycline)
- **aminoglicosides**(streptomycin, kanamycin, gentamicin)
- **levomycetin**(chloramphenicol)
- **glikopeptides** (vankomycin)
- **Rifamycins** (rifampin)
- **cyclic polipeptides**(polymixins, bacitracins)
- **polyenes**(nistatin, levorin, amphotericin B etc.)

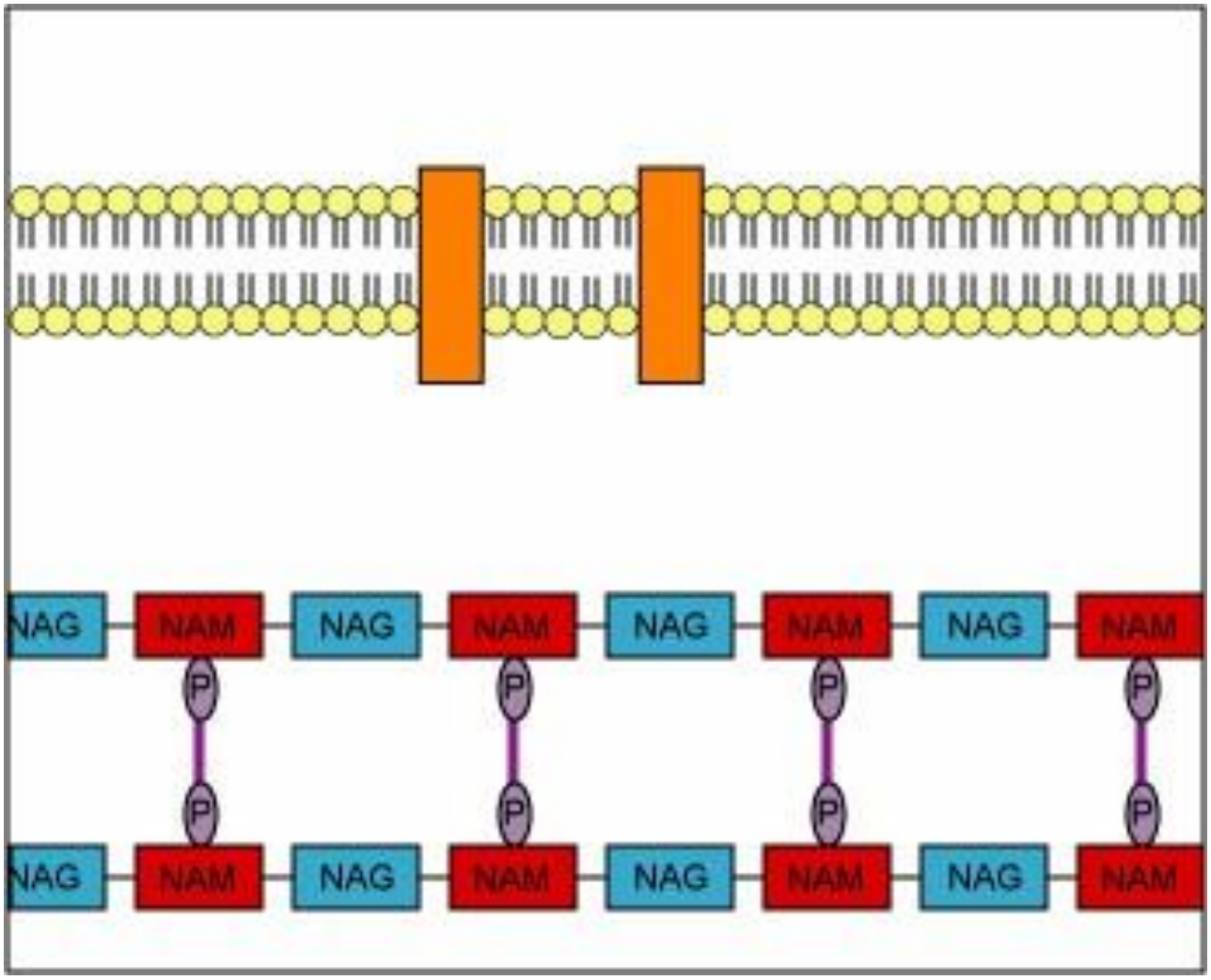
Mechanism of action of antibiotics

- **Inhibitors of cell wall synthesis (antipeptidoglycan antibiotics)**. Beta-lactams(penicillins and cefalosporins), glycopeptides (vanкomycin and teicoplanin)
- **Inhibitors of protein synthesis (antiribosomal antibiotics)** **Aminoglycosides** and **tetracyclines** act on 30S-subunit, macrolides, chloramphenicol və lincosamids – on 50S-subunit of ribosoms, resulting in stop of protein synthesis.
- **Inhibitors of nucleic acid synthesis -** rifamycins (rifampicin) bind to RNA-polimeraze and block transcription – mRNA synthesis.
- Anribiotics altering cytoplasmic membrane permeability (membranotrope antibiotics) - polipeptides (polymixins), polyenes (nistatin, levorine, amphotericin B etc.)

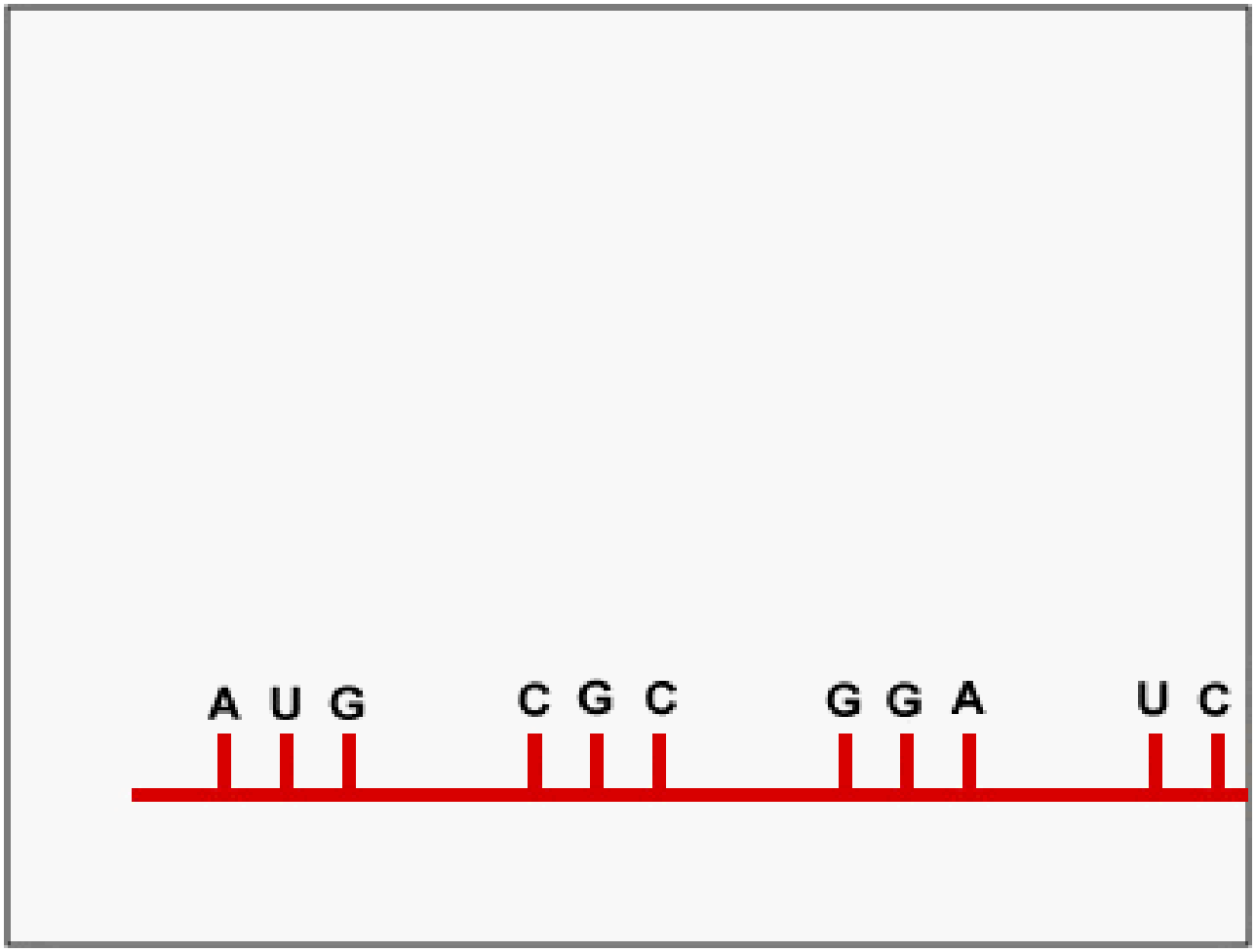
Synthesis of cell wall peptidoglycan



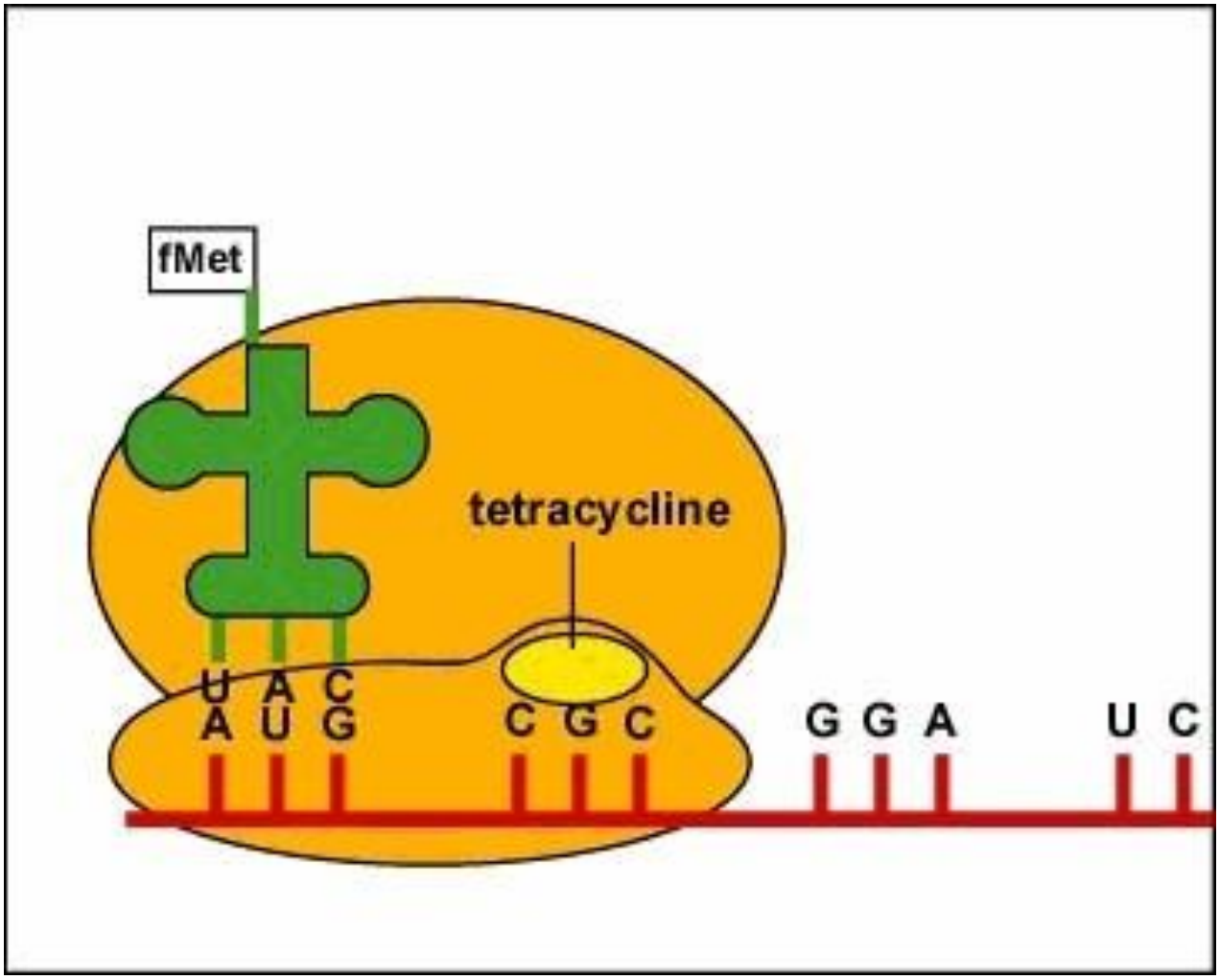
Inhibition of peptidoglycan synthesis by penicillin



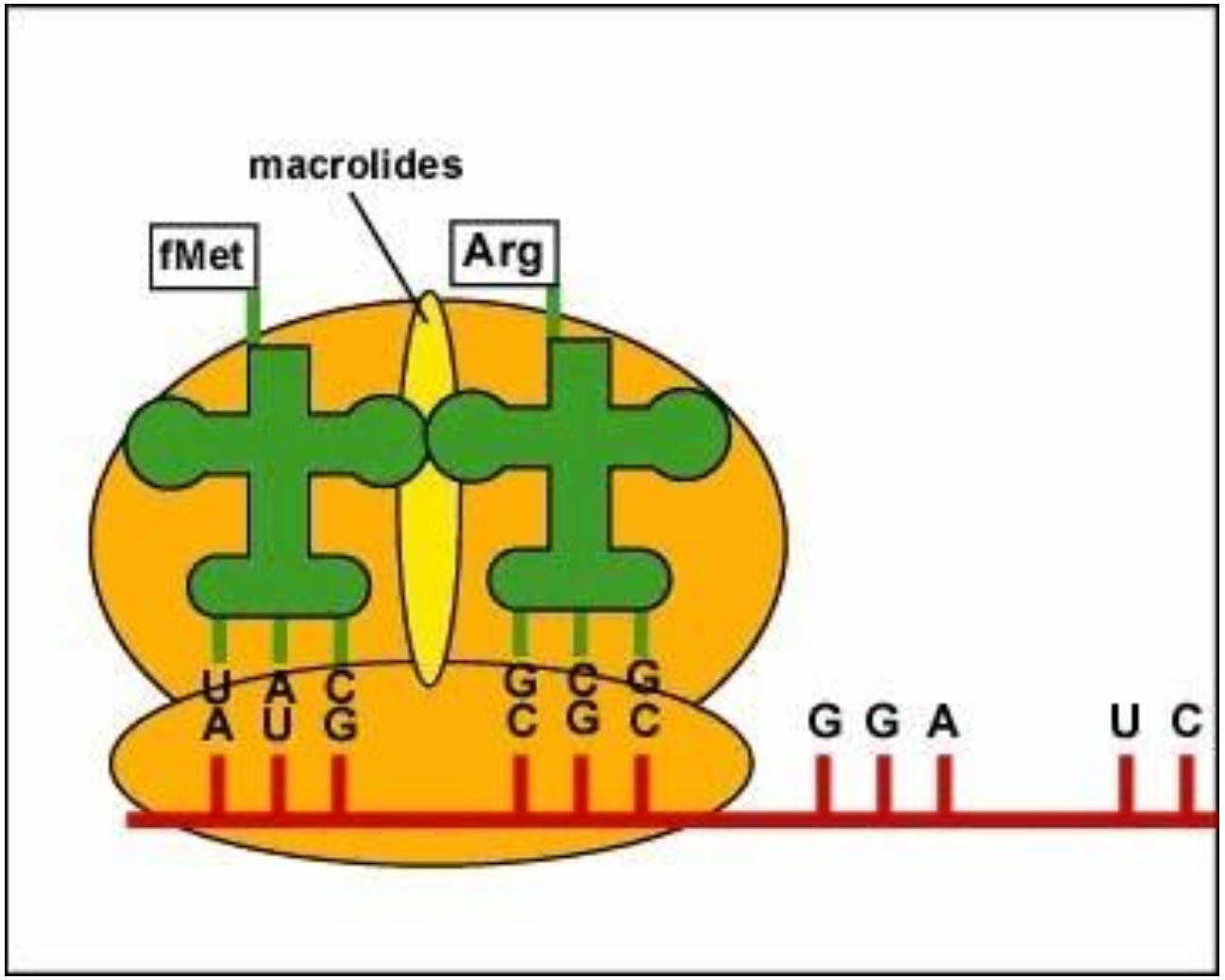
Aminoglycosides act on 30S-subunit of ribosomes and stop protein synthesis



Tetracyclines act on 30S-subunit of ribosomes and stop protein synthesis



Macrolides act on 50S-subunit of ribosomes and stop protein synthesis



Antibiotic resistance of microorganisms and its mechanisms

Intrinsic and acquired resistance.

- **Intrinsic resistance** is related to structural and biological features of microorganisms.
- **Acquired resistance** is related to adaptation of microorganisms to environmental conditions and develops after antibiotic impact.
- - ***Lack of cell wall permeability to antimicrobial agents and alteration of their intracellular transport***
- - ***Increase of antimicrobial agent removal from the microorganism cell***.

  - ***Modification of antimicrobials targets***

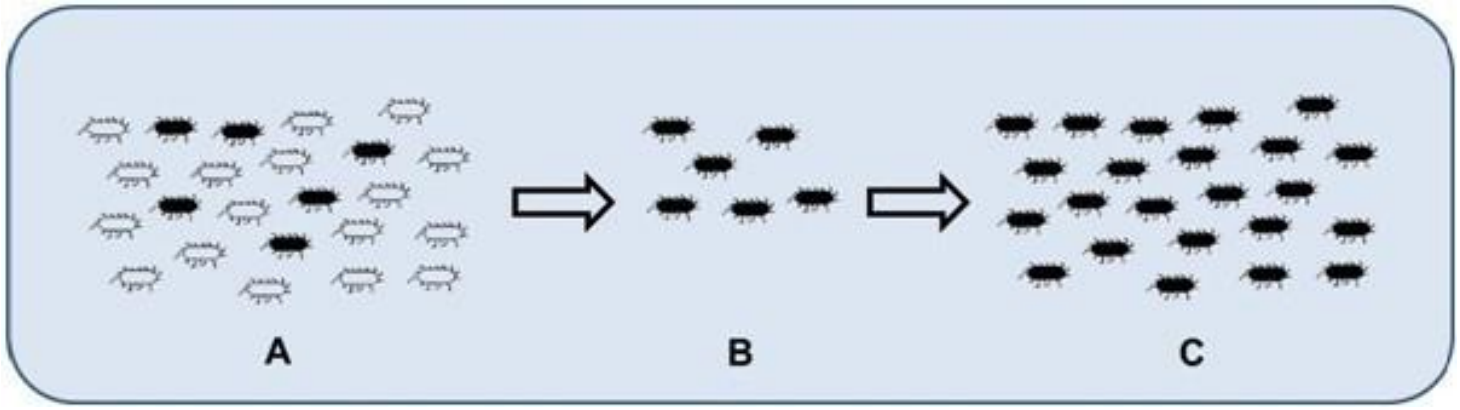
  - ***Inactivation of antimicrobial agents***

Inactivation of antimicrobial agent

- One of the main mechanisms of antimicrobial resistance.
- Some bacteria are able to synthesize enzymes inactivating antibiotics
- Beta-lactamase is a well studied enzyme breaking down beta-lactame ring of penicillins and cefalosporins
- Beta-lactamase synthesis is encoded by R-plasmid

Genetic basis of antibiotic resistance

- Resistance genes (r-genes) are responsible for antibiotic resistance .
- Plasmids containing resistance genes are called **R-plasmids** or **R-factors**. These genes encode synthesis of enzymes responsible for antibiotic resistance (exp. Beta-lactamase).
- Antibiotics do not induce r-genes transcription, they **select microbe population** which possess resistance genes.



Genetic basis of antibiotic resistance

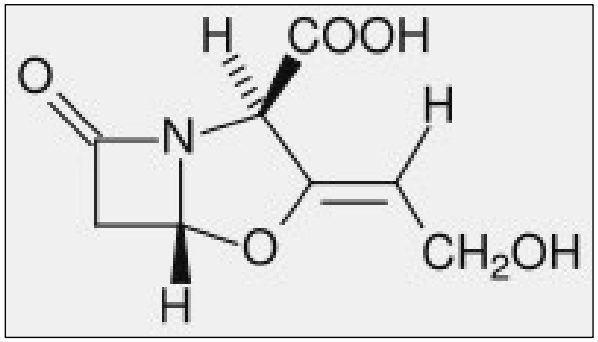
- **Mutations** have role in development of resistance in microbe population as well.
- For example, methicillin resistance in some *S.aureus* strains is due to mutation causing synthesis of defective penicillin binding protein which is unable to bind to beta-lactame antibiotics. Thus, ***methicillin resistant S.aureus (MRSA)*** strains are resistant to all beta-lactame antibiotics.

Prevention of antimicrobial resistance

- Rational use of antimicrobials
- Development of new antibiotics
- Combination of some antibiotics with beta-lactamase inhibitors ***(sulbactam and clavulanic acid)***:

  -beta-lactame ring of these compounds binds to beta-lactamase thus neutralizing it and preventing this enzyme action on beta-lactame antibiotics.

  -combinations of ampicillin with sulbactam (amoicid) and amoxicillin with clavulanic acid (augmentin, amoxiclav) are widely used in medical practice.



Prevention of antimicrobial resistance

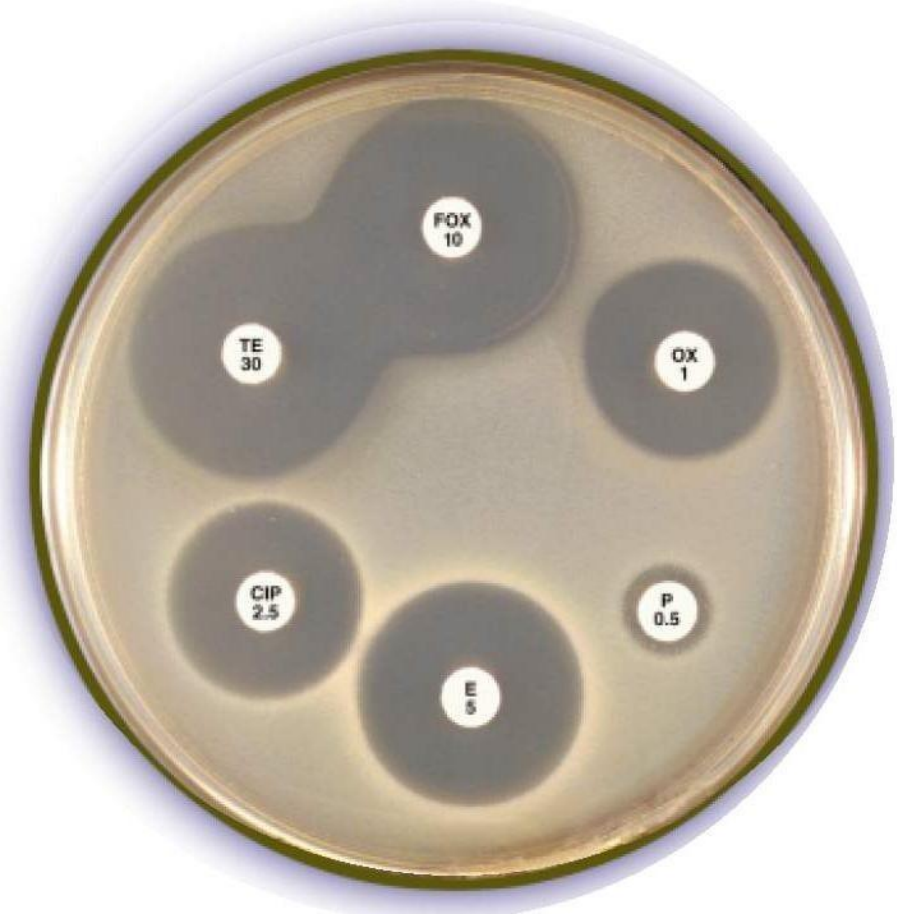
- One of the ways of antibiotic resistance prevention is evaluation of **antibiotic susceptibility** of microorganisms
- Qualitative and quantitative methods for antibiotic susceptibility testing exist.
- ***Qualitative method***. Disk-diffusion (Kirby-Bauer) is commonly used method.
- **Quantitative method.** Enables determination of minimal inhibitory and bactericide concentration of antibiotics.

Qualitative method

- **Disk-diffusion** (Kirby-Bauer) is commonly applied method. For this purpose paper disks with impregnated antibiotics(with determined concentration) are applied to solid media with inoculated culture of microorganism.
- Disks (no more than 6) are placed on 90-mm Petri dishes.
- Antibiotic susceptibility is evaluated on basis of growth of microorganisms around the disks after 1-day incubation.
- Antibiotic susceptible bacteria do not grow around disks and sterile zones of various diameter are observed.
- The diameter of sterile zone depends on susceptibility degree of microorganism

Paper disks with impregnated antibiotics

Disk diffusion method



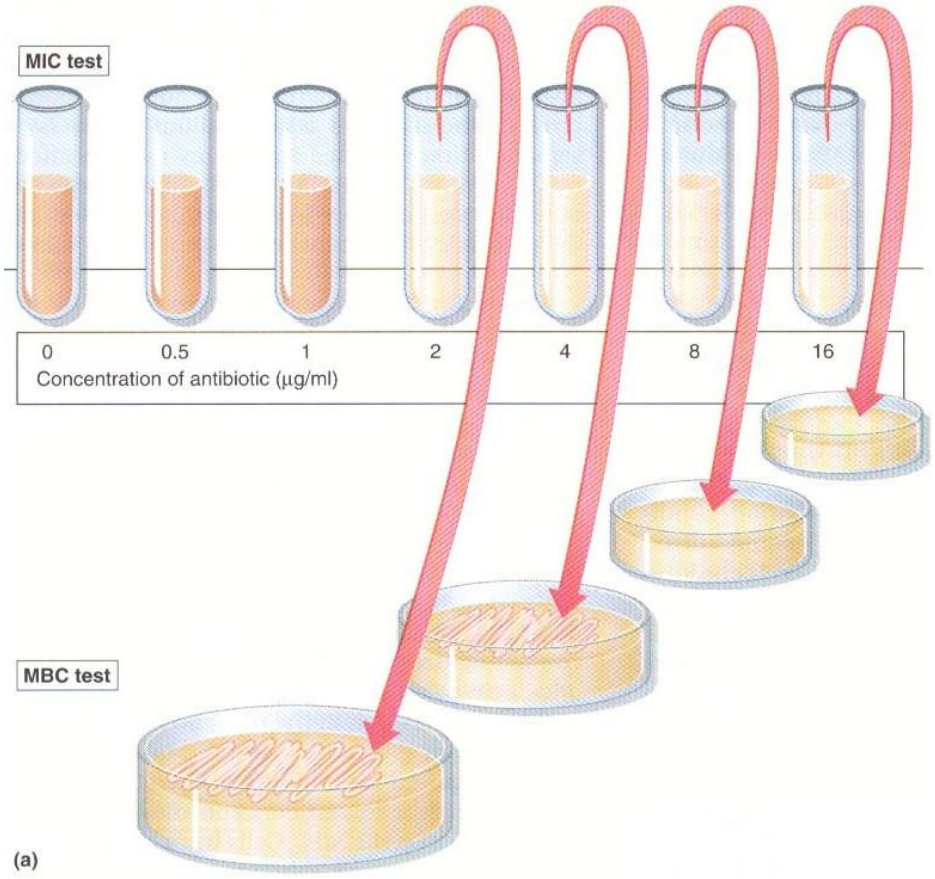
Quantitative method

Quantitative method *makes possible detection of minimal inhibitory concentration* ***(MIC)*** *of antibiotics****.***

- The principle of method is based on growth of microorganism in nutrition media with added antibiotics of different concentrations.
- The lowest concentration of antibiotic inhibiting growth of microorganism is considered as minimal inhibitory concentration (MIC), the lowest concentration killing microorganism – minimal bactericide(microbocide) concentration (MBC or MMC).
- These values are interpreted in mcg/ml. For some antibiotics international units are used. Unit of antibiotic is the lowest dose inhibiting growth of microorganism. Commonly 1 IU is equal to 1 mcg.

Detection of MIC by serial dilution method.

The principle of method based on growth inhibition of microorganism by different concentrations of antibiotics added to nutrient broth.



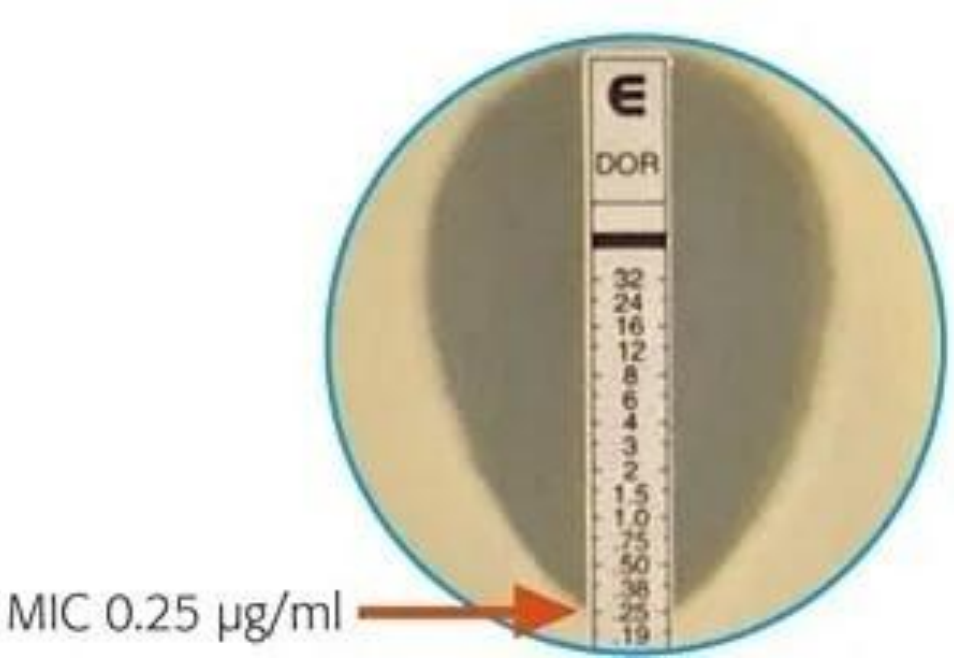
Serial dilutions method

- By serial dilutions method minimal concentration of antibiotic inhibiting growth of microorganism is detected.
- For example, in order to detect MIC of tetracycline for *Staphylococcus aureus* double lethal concentration of this antibiotic is added to test tubes with nutrient broth.
- Content of first tube is added to 2nd, content of 2nd - to 3rd and so on. As a result series of diluted concentrations are obtained.

Epsilometric method (E-test)

- E-test method is based on usage of paper strips with impregnated gradient of antibiotic concentration (128, 64, 32, 16, 8, 4, ..., mcq/ml).
- These strips are applied on nutrient media with inoculated culture of microorganism (as in disk diffusion).
- After incubation, bacterial growth becomes visible, symmetrical inhibition ellipse along the strip is seen. The MIC value is read from the scale in terms of µg/ml where the ellipse edge intersects the strip.

E-test

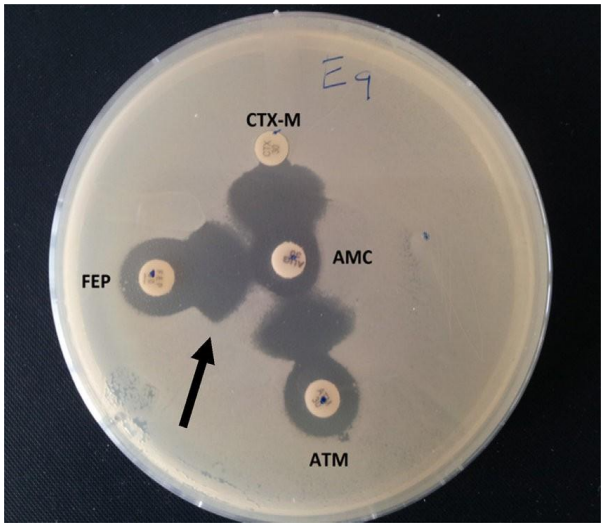


Investigation of antibiotic resistance of microorganisms.

- Production of enzymes breaking down antibiotics is one of the mechanisms responsible for antimicrobial resistance.
- **Beta-lactamases** that break down the beta-lactam ring in beta-lactam antibiotics and inactivate them are one of the examples of such enzymes.
- Production of these enzymes are encoded by plasmid genes.
- Recently, the number of microorganisms that synthesize extended-spectrum beta-lactamase (ESBL) is increasing. Unlike conventional beta-lactamases, ESBL breaks down antibiotics that are resistant to beta-lactamases causing resistance to them.

Detection of ESBL synthesis in microorganisms (*phenotypic test)*

- Two disks – beta-lactame (exp. Cefipime) disk and antibiotic+beta-lactamase inhibitor (exp. Amoxiclav) containing disk are placed in nutrient agar with inoculated culture.
- Result is interpreted after 1-day incubation.
- In case of ESBL synthesis enhancement of sterile zone(in amoxiclav direction) around cefipime is observed.



Possible complications after antibiotics usage and ways to prevent them

- **Hipersensitivity reactions- allergic reactions**

  - consideration of hypersensitivity reactions

- **Disbiosis and disbacteriosis**

  -combination of antibiotics with antifungals in case of prolonged treatment

  -use of normal flora representatives – eubiotics during prolonged antibiotic treatment

- **Toxic effect**

  - consideration of contraindications and side effects

Standard approachs on antimicrobial susceptibility testing

- There are standards for antibiotic susceptibility testing
- **EUCAST** (*European Committee on Antimicrobial Susceptibility Testing*).
- EUCAST recommends concentrations of antibiotics impregnated in paper disks, composition of nutrition media used, sterile zone breakpoints, list of antibiotics used for susceptibility (selective antibiogram).
- EUCAST principles are periodically updated

Chemotherapy principles of viral infections

- According to action type and clinic significance drugs used to treat viral infections can be divided into the following groups:
- **Etiotropic (antiviral) drugs;**
- **Pathogenetic drugs (affecting mechanisms responsible for disease development);**
- **Simptomatic (eliminating symptoms of disease).**

Etiotropic drugs

- Etitotropic drugs used for viral diseases treatment can be divided into the following groups:
- **Chemical drugs**
- **Interferons and their inducers**

Chemical antiviral drugs

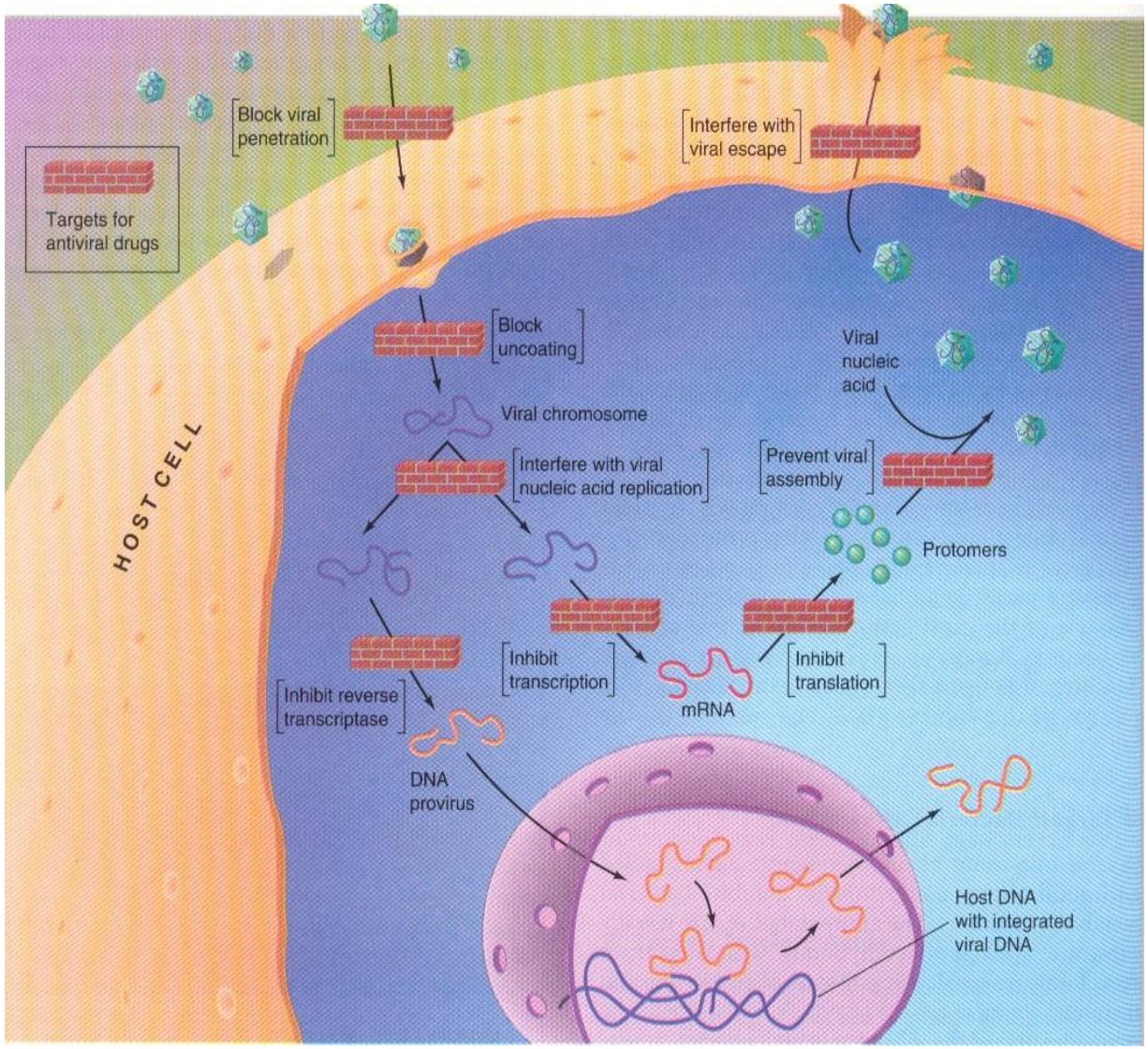
Antiviral drugs **selectively inhibit different stages of viral reproduction** without considerable effect on macroorganism. Based on this feature synthetic antiviral drugs can be divided into the following groups:

- **Inhibitors of viral adsorption to host cell**
- **Inhibitors of viral deprotenization in host cell** *(amantadin and remantadin)*
- **Inhibitors of viral DNA-polimeraze**

  - nucleoside (purine and pyrimidine bases) analogs(idoxuridin, vidarbin etc.)

  -selective drugs acting on virus infected cells (acyclovir, ganciclovir, famcyclovir, ribavirin, foscarnet etc.)
- **Reverse transcriptase inhibitors** - azidothymidine (zidovudine), zalcitabine, lamivudine etc.
- **Viral proteases inhibitorsinhibitorları** (saquinavir, ritonavir etc.)
- **Inhibitors of late viral proteins sybthesis** (metisazonum and marboran)

Antiviral drugs action targets



İnterferons

- A.Isaacs and J.Lindenmann in 1957 revealed that viral interference (the cell infected with one virus becomes insensitive to other viruses)is due to protein with antiviral activity – interferon.
- Interferon – is protein-glycolipid with molecular weight 15-70 κD and synthesized by immune system and connective tissue cells.
- Depending on producing cells interferons can be divided to 3 types:

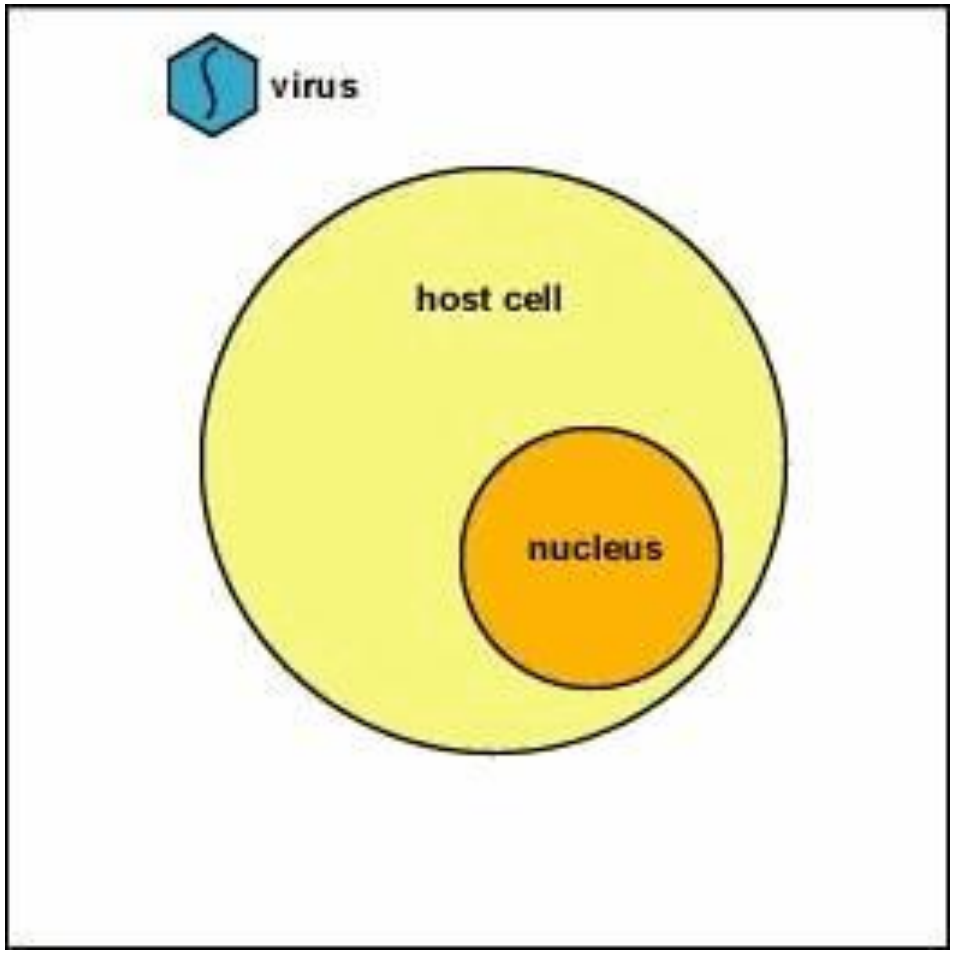
İnterferons

- **Alfa-interferon** produced by leucocytes(leucocyte interferon);
- **Beta-interferon** produced by connective tissue cells (fibroblasts interferon);
- **Gamma-interferon** immune interferon, produced by immune cells - activated T-lymphocytes, macrophages, natural killers.

Mechanism of action of interferon

- Interferon binds to specific cell receptors and inhibits viral reproduction at **protein synthesis stage.**
- Along With antiviral effect interferon inhibits proliferation of cancer cells, stimulates phagocytosis, natural killers, production of antibodies, activates major histocompatibility complex expression **(immune modulating effect)**

Interferon action mechanism



İnterferon inducers - interferonogens

- Viral infection of cell considerably stimulates interferon production.
- Interferon production is stimulated also by inducers – for example by DNA, RNA, polimers etc.
- Such inducers are called **interferonogens**. Currently, synthetic interferonogens (cycloferon, etc.) are widely used in medical practice.