Effect of a nanomodified antibiotic on field strains of E. coli and Enterobacter cloacae

L Lovtsova1, O Guliy1,2, O Larionova1, M Zabelina1, K Uskov1 and I Lovtsov1

1 Saratov State Vavilov Agrarian University, Saratov, Russia
2 Institute of Biochemistry and Physiology of Plants and Microorganisms of the Russian Academy of Sciences, Saratov, Russia

E-mail: larisalovtsova2018@mail.ru

Abstract. For the first time, a comparative analysis of the biological activity of selenium nanoparticles was carried out to create a new drug based on a non-toxic and effective means of intracellular delivery of selenium nanoparticles and the antibiotic polymyxin. Proven A method for the synthesis of selenium nanoparticles was developed using polyvinylpyrrolidone as an adsorbent, a stable form of their storage was achieved, microscopy of electron losses was carried out, the size of the synthesized preparation containing selenium nanoparticles was determined.

1. Introduction
At the moment, one of the most promising areas of biotechnology development is nanobiotechnology. The rapid development of nanobiotechnology is due to the demand for the proposed solutions in the applied aspect, as well as innovation and strategic focus [1].

Nanotechnology is of particular importance for medicine, when creating genetically engineered therapeutic and prophylactic drugs, immunobiological and biologically active substances, as well as new carriers and means of targeted delivery. The greatest relevance in this area of biotechnology is used in the diagnosis and treatment of cancer, allergic, cardiovascular and endocrine diseases [1].

An equally important task of biotechnology in medicine is the diagnosis and elimination of the microelement balance in an animal or human organism susceptible to disease. In view of the diversity of the function of mineral substances, they are included in the structure of various components, affect protective reactions, the work of the endocrine glands and the hematopoietic system. Therefore, the development of drugs based on nanoparticles of microelements is currently one of the priority areas for the development of biotechnology, which is confirmed by many government funding programs and grants.

The biochemical versatility of selenium is one of the reasons for the growing interest of the world community in this trace element. Immunostimulating, catalyzing, antioxidant and antitumor action, interaction with enzymes and vitamins, regulation of the absorption of vitamins and the rate of redox reactions, metabolism of proteins, carbohydrates and fats, and this is just a short list of its known functions.

There is a clear trend of selenium shortage all over the world. The content of this trace element in the soil is not enough to meet the needs of both humans and living animals. It is mainly found in the form of selenocyanates (SeCN -), Selenite oxyanions(SeO32 -), and selenate (SeO42 -). The element is part of selenium-containing amino acids, such as selenocysteine and Selenomethionine, and is an...
important factor in the diet of humans and animals due to its participation in the formation of glutathione peroxidase enzymes.

Selenium is a biologically active trace element, essential for human and animal life, and is a part of most hormones and enzymes. Selenium deficiency leads to the development of various processes of cell damage that underlie the occurrence of many pathological conditions. Given the achievements of science in recent decades, and especially the development of nanoscience, it is worth noting that the production of selenium in the nanoscale state will lead to the production of materials with a new level of physical and chemical characteristics. This is why there are many new methods for producing nanoscale selenium. Designed LPS have unique physical and chemical properties that make them extremely attractive for use in various areas of human life, including medicine, agriculture, food and perfume and cosmetics industries. When choosing the chemical form of selenium, you should pay attention to the effectiveness and safety, and also take into account the fact that the biochemical route of organic and inorganic forms of selenium is similar. The most promising is the use of selenium nanoparticles (size 20-70 nm), the main advantage of which, in comparison with other forms, is its lower toxicity, which allows it to be used in doses significantly exceeding the daily requirement. In addition, nanoselene has a so-called dimensional effect, which is manifested by the fact that the particles are biologically more active and accumulate better in the tissues.

The consequences of a lack of selenium in the body can be: heart disease, obesity, visual impairment, atherosclerosis, slowing down of metabolism, impaired liver function, multiple sclerosis, pancreatic diseases, infertility. In animals, deficient diseases in which selenium may play a role include muscular dystrophy in sheep and cows, exudative diathesis in chickens, and liver necrosis in pigs and rats. With selenium deficiency, the reproductive function decreases, and the growth of animals slows down. With a lack of selenium in the body, lipid, carbohydrate and fat metabolism are disrupted [2-4].

It is established that if the selenium content in the body is not enough, it can lead to the emergence and reproduction of atypical cells and cancer. If enough selenium is introduced into the body, the enzymes have time to calculate and destroy this atypical cell. That is, they not only prevent the formation of these cells, but also destroy the already formed ones. Selenium has such a positive effect in all tumor processes, including blood diseases and various benign neoplasms [5].

The use of elementary (zero-valent) selenium in the nanoscale state is promising to meet the physiological needs of humans [6]. During the transition to the nanoscale state, a number of fundamental characteristics of a substance change [4-6]. Selenium nanoparticles exhibit cytotoxic effects on tumor cells and are also considered as effective carriers for targeted in vivo delivery of drugs, genetic materials, proteins, etc. The highly "tunable" polyvalent surface structures of selenium nanoparticles provide a convenient platform for integrating multiple therapeutic drugs or biomacromolecules with covalent or non-covalent conjugation.

Nanoparticles have many positive properties: they can be easily transported, therapeutic and diagnostic substances, as well as immunoactive biomolecules can be attached to their surface. These unique properties of nanoparticles make them suitable for both diagnostics and therapeutic use in the treatment of various diseases [4].

Based on the fundamentally new properties of nanoparticles, such nanomaterials and nanocompositions are created that can radically change the diagnosis and treatment of diseases and thus open a new stage in the development of medical technologies, which is already commonly called nanomedicine. Therefore, the development of new methods for the synthesis of selenium nanoparticles is an urgent task and is of interest, both from an applied and fundamental point of view.

Another promising area of application is the diagnosis of selenium and a decrease in the activity of the human immunodeficiency virus. A decrease in selenium levels is an indicator of the progression of HIV infection or AIDS. Selenium deficiency is an independent indicator of mortality in adults and children infected with HIV-1. This essential trace element is associated with improved T-cell function and normalization of apoptosis in rats and mice. The enhancement of the immunomodulatory effect at a sufficient level of
selenium consists in stimulating the production of interleukins with a subsequent response of T-helpers.

The mechanism of action of molecular and nanoselene on the body is poorly understood, but the available data allow us to judge the effectiveness of this element in the fight against various tumor diseases.

Selenium increases the regulation of interleukins-2, stimulating their activation, spread, differentiation, and programmed death of T helper cells, while reducing the abnormally high levels of interleukins-8 and cancer alpha-factor observed in HIV [7].

Adding sodium selenide to water has a positive hepatoprotective effect on rats susceptible to diabetes. The biochemical parameters of diabetic rats differ from those of normal rats by 200-250%. In diabetic rats that received selenium-rich water, the deviation from the norm was 10-15%[4], which is a normal deviation when testing on laboratory nonlinear animals.

There are already several selenium-based drugs, such as Selenodine, DAPS-25K, E-Selenium, and several other substances that serve to make up for selenium deficiency in the body, but all of them are selenium-organic compounds and derivatives of inorganic selenium-containing compounds. The little-studied nature of selenium nanoparticles in comparison with organic selenium compounds is of interest, since published works show the high efficiency of nanoscale selenium in the fight against cancer and various tumors. The ability to conduct tests both on in-vitro cells and on animals allows for a variety of experiments.

Studies of the effect of nanoparticles on human Osteosarcoma cells MG36 showed a lower efficiency of selenium nanoparticles compared to gold at equal concentrations. This is due to the mechanism of action of drugs on cells and well shows the difference between them: gold causes damage to tissues or cells by producing free radicals, while selenium perfectly shows its biological relationship, stimulating protective mechanisms. In this regard, gold has a higher toxicity than selenium. This allows us to speak about the possibility of using the latter in high concentrations, without harm to health [7].

In tests of selenium nanoparticles stabilized with albumin against rats infected with liver cancer, a positive effect of the drug was found. When considering several key indicators of cancer activity, it was shown that 8-hydroxy-2-deoxyguanosine, against the background of an increase in other groups of animals, in the group that received the drug based on nanoselene, is at the same level as the control group. Expression of the Akr1b10, ING3, and Foxp1 genes is also normal, at the level of control animals.

The study of animal liver under an electron microscope revealed a violation of the cellular structure of hepatocytes in groups susceptible to cancer. The control and selenium-treated groups avoided this [8].

During tests aimed at determining the effect of selenium nanoparticles on MCF-7 cancer cells, a possible mechanism of particle penetration into the cell was established. Modification of nanoparticles with folic acid is expected to provide selectivity when delivering the particle to the cell. This is possible due to the fact that such a nanoparticle is attracted by the folic receptors of MCF-7 cells. Further, it penetrates into the cell membrane, where it is transported by vesicles through the cell, which leads to its staining. The remaining nanoparticles are released into the cytoplasm, but are not randomly distributed, but are redirected to the mitochondria, where they lead to the generation of free oxygen forms that release calcium ions. This causes damage to the mitochondria. All changes lead to increased activation of caspase 9 and later caspase 3 [8].

The use of microorganisms in the laboratory and on an industrial scale can speed up the production of many important organic compounds, such as enzymes, proteins and amino acids. The cultivation of microorganisms for the purpose of splitting a compound containing selenium to produce nanoparticles is one of the most studied methods. The disadvantage is the factor of sticking of particles inside the microorganism. When metabolized, selenium particles interact with each other within the microorganism, increasing in size. This makes it difficult to obtain a sufficient volume of particles, with a relatively large size spread. In addition, to extract the particle from the microorganism, it will
have to be destroyed. This makes it difficult to obtain a pure substance, without the remnants of cell walls and organelles. But, despite this, the distribution of selenium within the microorganism is quite easy to track, it is possible to diversify the original source of selenium. In addition, due to the characteristics of certain strains, it makes sense to use different classes of microorganisms.

One of the methods for obtaining selenium nanoparticles is biotransformation of a nutrient medium enriched with sodium Selenite by Enterobacter sp culture. Like any other microbiological synthesis of nanoselenium, this method is based on a redox reaction. The most optimal cultivation conditions: pH= 7.0 t = 37°C. Under these conditions, the maximum yield of nanoparticles is ensured. Changes in the temperature or pH of the medium up or down significantly impairs biosynthesis. The process takes 2-3 days. Nanoparticles remain stable during culture autoclaving. The size of the nanoparticles obtained in this way varies from 90 to 110 nm with an average of 108 nm. Under room conditions, the particles are stable for at least two months. At the same time, no mass clumping of particles was observed, the size for the most part remained the same, in the previously specified range [9].

It is also possible to biosynthesize selenium nanoparticles using Bacillus sp. MSh-1 cell culture with further centrifugation at 4000g, with 0.9% NaCl washing and stabilization. Such nanoparticles in mouse tests showed much lower toxicity (6-20 times) than those obtained by synthesis from SeO2. the LD50 of synthesized nanoparticles was 198.1 mg / kg-1 versus 7.35 mg/kg-1 for those obtained from selenium oxide. The biochemical parameters of the groups exposed to 2.5 mg / kg-1 are identical to those of the control group. Other mice that were administered 5, 10, and 20 mg / kg-1 had a relative control increase in all indicators by 5, 12.3, and 32.1% [9].

When cultivating the bacteria Azospirillum brasilense Sp7 and Sp 245 on a nutrient medium enriched with Na2SeO3, it was found that the SP7 strain had a biomass increase of 11.7-28.6% greater than that of the control sample, and Sp 245 by 23-34.4%. In addition, the strains grown on the enriched medium had a pronounced red color. X-ray fluorescence analysis showed a significant accumulation of selenium in bacterial cells. Electron microscopy showed the presence of selenium nanoparticles ranging in size from 50 to 400 nm in the cells of both strains [9].

According to the collected data, the duration of cultivation affects the size of nanoparticles. Observation of Shewanella sp HN-41, P. Agglomerans, and Clostridium Histolyticum Type 2 culture cells showed that during the first 18-20 hours of cultivation, selenium accumulates inside the cells, and after 24-28 hours it is removed from them as nanoparticles. During its stay in the cell, the particle changes in size from 5 to 300 nm [8].

The use of genetically modified microorganisms to achieve overproduction of the target compound has been actively used in recent decades. To increase the synthesis of selenium nanoparticles by Bacillus sp cell culture. genetic engineering methods were used in combination with changes in the concentration of selenium oxide in the nutrient medium. An improved strain of Bacillus cereus was used as a donor. The gene sequence responsible for producing the target substance was extracted from it and placed in a vector for subsequent encrustation.

As a result, the resulting strain of Bacillus sp. showed 41% more production of selenium nanoparticles, with the final particle size unchanged from 31nm to 335nm. Further studies were conducted in the direction of changing the conditions for the biodegradation of selenium oxide contained in the nutrient medium. The selenium oxide concentration, pH level, and cultivation temperature were used as variable parameters. As a result, it was found that the maximum level of selenium nanoparticle buildup is achieved at a selenium oxide concentration of 6.4 mM, a pH of 7-8, and a temperature of 33°C [10].

As part of the program for the development of nanotechnology in the Russian Federation, one of the areas of budget allocation is the creation of a promising antitumor conjugate for vaccines. There are already several selenium-based drugs, such as Selenodine, DAPS-25K, E-Selenium, and several other substances that serve to make up for selenium deficiency in the body, but all of them are selenium-organic compounds and derivatives of inorganic selenium-containing compounds. The little-studied nature of selenium nanoparticles in comparison with organic selenium compounds is of interest, since published works show the high efficiency of nanoscale selenium in the fight against
cancer and various tumors. The ability to conduct tests both on in-vitro cells and on animals allows for a variety of experiments.

In our work, DAPS – 25K was used as a source of selenium. This is a loose powder of white or light yellow color. The drug is not soluble in water, but it is well soluble in oils (for example, in vegetable oil), contains up to 93% 1.5-diphenyl-3-selenapentadion-1.5, and the selenium content is 25%. It is used together with food for all types of animals and birds. Based on the experiments of GNU "VNITIP", it is proved that the use of DAFS in diets for broilers in the amount of:

- 1.6 grams per ton of feed increases the productivity and safety of animals;
- 3.2 grams per ton of feed prophylactic dose for chronic mycotoxicosis of birds.

DAPS-25K is a moderately toxic substance, almost 30 times less toxic in comparison with sodium Selenite. This significantly increases the therapeutic capabilities of the DAFS feed additive (table 1).

| Indicator of acute toxicity for | Type of animal, method of administration | LD50 dose for DAFS-25K | LD50 dose of sodium Selenite |
|-------------------------------|-----------------------------------------|------------------------|----------------------------|
| LD50                          | White rats, per os                      | 385                    | 10                         |

Indications for the use of the Supplement are:

- Detection of selenium deficiency in the body (laboratory or clinical symptoms of white muscle disease);
- Diseases accompanied by protein, fat, and carbohydrate deficiencies (dystrophy);
- Identification of symptoms exposure to radiation, chemical, biological factors on the animal body;
- Symptoms of mycotoxicosis or feeding animals or birds with substandard food;
- Some States of immunodeficiency.

Preventive dosages of DAFS-25K significantly increase:

- Safety of young growth of animals and poultry;
- Egg production;
- Quality (vitamin and mineral saturation) of meat, dairy, and egg products;
- Reproductive properties of animals and birds.

Restrictions on the use of DAPS-25K together with antioxidants. The combination of these components reduces the level of free radicals

It is proved that with the correct supply of DAFS, the body's digestibility is 80-100%. In this case, DAPS is used in microdoses. For example, one kilogram is enough for 625 tons of feed (based on 1.6 g per ton of feed). This is extremely economical.

Polyvinylpyrrolidone was used as an adsorbent in the synthesis. To calculate the ratio of the adsorbent to the selenium source during synthesis, the adsorption capacity of each adsorbent was initially determined. For the experiment, a calibration schedule is required, for which a standard solution containing 1.5 g/ml of copper ions was made. When it was created, a sample of 0.16 g of CuSO4 was made on a Mettler Toledo al104 analytical balance (figure 2), placed in a 50 ml analytical flask and filled with 1% ammonia solution up to the risk of the lower meniscus. Further 2 consecutive dilutions were made, each subsequent of which contained 2 times less copper ions. Thus, 3 standard calibration solutions containing 1.5 g/ml, 0.75 g/ml and 0.325 g/ml of copper ions were obtained.
The measurement was performed on a Shimadzu UV 1280 spectrophotometer in the "photometry" mode at a wavelength of 600 nm in plastic disposable Sartorius cuvettes with an optical path length of 10 mm. During the analysis, a sample of adsorbents weighing 0.1 g was placed in a flat-bottomed cone-shaped measuring flask with a volume of 25 ml, then 20 ml of a solution containing 1.5 g/ml of copper ions prepared according to the previously specified method was poured into it. For measurement, 1.5 ml of the total volume was taken from each sample. Measurements were carried out every hour, for 5 hours with moderate stirring on the ES-20 Biosan orbital shaker incubator, with the starting point at the time of mixing the adsorbent with the solution and the final one, a day after the start of the experiment.

For the synthesis of selenium nanoparticles adsorbed on polyvinylpyrrolidone, isopropanol was selected as the solvent (JSC "Base No. 1 of chemical reagents"). In a flat-bottomed cone-shaped flask, polyvinylpyrrolidone powder (Sigma Aldrich) and isopropanol, preheated to 65°C, are mixed. They were added gradually, since the vapors of heated isopropanol affect the PVP, which leads to the substance sticking together. Next, a sample of DAFS-25K is added to the flask and mixed until completely dissolved. Then, with the help of ammonia, a reaction is initiated that proceeds until the yellow color of the contents of the flask is stabilized. Then the contents of the flask are cooled to stop the reaction. After that, it is placed in the freezer at a temperature of -53°C and freeze-dried.

The essence of the reaction is to create an alkaline environment. Being in such an environment, 1.5 diketones are decomposed, which is represented by DAPS-25K, with the release of selenium, which is adsorbed on a carrier dissolved in the medium. As a result, we get a powder, pale yellow in color, resembling sugar in structure. This substance is stable, water-soluble with a maximum concentration of 0.12 g/ml. When dissolved, it is a clear liquid with a pale yellow tint, without internal inclusions, which is convenient for both oral and parenteral administration. Determination of the size of nanoparticles on a transmission electron microscope showed particles in the range of 2-4 nm (figure 1).

![Figure 1](image.png)

Thus, the above data allow us to state that a stable conjugate for vaccines containing selenium nanoparticles with a size range from 2 nm to 4 nm has been obtained.

As you know, antibiotics are substances of microbial, animal or plant origin that can inhibit the growth of certain microorganisms or cause their death. But at present, a significant difference between new types of dosage forms and standard ones is the possibility of implementing technologies for targeted drug delivery to certain tissues, cells, and even intracellular organelles based on them. Since direct delivery of drugs and biomolecules to target organs is not very effective, which is associated with the enzyme degradation of active substances, their neutralization by the liver and excretion from the body by the kidneys, the search for effective and safe carriers to genes, cells and target organs is an important area of research.

This study is aimed at solving the problem of creating a new drug based on a non-toxic and effective means of intracellular delivery of selenium nanoparticles and the antibiotic polymyxin.
Antibiotics differ from other antimicrobial substances by selectivity of action, i.e. they suppress vital activity or have a destructive effect only on certain types of microbes. That is, unlike antiseptic substances (disinfectants), which are poisons that act on any living cells, antibiotics act selectively only on microorganisms. Just like chemotherapeutic agents, they act on enzymes, disrupt metabolic processes, growth and reproduction - only in certain microbial species.

Each antibiotic has its own spectrum of antimicrobial action. For example, erythromycin is effective only against gram-positive bacteria. The spectrum of antimicrobial action of streptomycin includes gram-negative bacteria and Mycobacterium tuberculosis. Some antibiotics have a broader spectrum of action. As levomycetin acts not only on gram-negative and gram-positive bacteria, but also on some viruses.

Of the vast number of antibiotics obtained and studied, only a few were found to be suitable for the treatment of infectious diseases. This is due to the fact that many of them were toxic or lost activity in the human body. The antagonist microbe produces an antibiotic only under certain conditions of cultivation: the nutrient medium, its composition and pH, temperature, aeration, etc. Different antibiotics differ from each other in the degree of their antimicrobial activity.

In the course of antibiotic therapy or under experimental conditions, when microbes are exposed to insufficient doses of the drug (sub-bacteriostatic concentrations), microbes may become resistant to these drugs. The degree of drug resistance can be very high. The drug resistance acquired by the microbe persists for a long time.

Polymyxins, being one of the first classes of natural AMPS, were obtained in the early 40s. They are characterized by a narrow spectrum of activity and high toxicity. Polymyxin B, intended for parenteral administration, has been considered for many years as a reserve drug used in the treatment of Pseudomonas aeruginosa. Polymyxin M was used orally for intestinal infections (figure 2). Currently, they are used only in limited quantities, more often in the form of "local" dosage forms.

![Polymyxins. Chemical structure.](image)

The mechanism of action of polymyxins begins with electrostatic interaction with LPS molecules of the outer membrane of a gram-negative bacterium, then there is a competitive substitution of Ca2+ and Mg2+ ions, which serve as stabilizers of the outer membrane. Violation of the permeability of the outer membrane and leakage of vital intracellular components from the cell leads to the death of the bacterium. The result of this action is blocking the biological effects of LPS (endotoxin).

Polymyxins are active against gram-negative bacteria such as E. coli, Salmonella, Shigella, Klebsiella, Enterobacter, Pseudomonas aeruginosa. Fusobacteria and bacteroids (except B. fragilis) are moderately sensitive. Proteus, Serratia, gram-negative cocci and all gram-positive flora are naturally...
resistant. Polymyxins are active against gram-negative bacteria such as E. coli, Salmonella, Shigella, Klebsiella, Enterobacter, Pseudomonas aeruginosa. Fusobacteria and bacteroids (except B. fragilis) are moderately sensitive. Proteus, Serratia, gram-negative cocci and all gram-positive flora are naturally resistant.

Polymyxins are not absorbed in the gastrointestinal tract, as well as when applied topically. However, with prolonged use in the form of ear or eye drops, partial absorption is possible. When administered parenterally, polymyxin B does not create high concentrations in the blood. Poorly penetrates the bile, pleural and synovial fluids, inflammatory exudates. It does not pass through the BBB, but is able to pass through the placenta and into breast milk in small amounts. Not metabolized, excreted by the kidneys unchanged. The half-life is 3-4 hours, with renal failure may increase to 2-3 days. Polymyxin M when taken orally is not absorbed and is completely eliminated by the gastrointestinal tract.

To obtain a means of intracellular delivery of a biologically active low-molecular compound, a solution of 0.1-5% concentration was prepared by adding a solvent-distilled water. Separately, prepare nanoparticles of selenium, a selenium-containing substance.

Bringing the pH of the product to 7.2-7.4 is due to the need to create a stable system, since the acidity values above or below the specified limits will lead to the destruction of the colloidal solution. In addition, this acidity is necessary for compliance with the physical parameters of injectable solutions.

The choice of boundary values for the solution of a biologically active low-molecular compound (0.1-5%) is due to the need to match the problem being solved. At values above or below the specified limits, the resulting product is stratified, it becomes inhomogeneous, and its stability is violated.

The selected Enterobacter cloacae is a genus of rod-shaped peritrichial asporogenic gram-negative chemoorganotrophic facultative anaerobic bacteria from the Enterobacter family. Escherichia coli is a gram-negative spore-forming rod-shaped bacterium of the genus Escherichia. Lives in the intestines of humans and animals, are saprophytes.

Determination of the sensitivity of microorganisms to antibacterial drugs (ABP) was carried out using the method of serial dilutions.

This method allows us to quantify the sensitivity of the isolated microbe to antibacterial agents and determine the MIC of the drug. Serial breeding methods are based on direct determination of the MIC value. To determine the value of MIC, the specified concentrations of antibiotics are introduced into the nutrient medium, which is then sown with the culture of the studied microorganism. After incubation, the presence or absence of visible growth is evaluated.

According to the method of serial dilutions, testing is carried out in the volume of 1 ml of each ABP dilution with a final concentration of the studied microorganism of approximately 5 x 10^5 CFU/ml.

When preparing a nutritious broth, 0.5 ml is poured into each test tube to determine the sensitivity. The number of test tubes is determined by the required range of ABP dilutions and increases by one to establish a "negative" control.

Figure 1 shows the scheme of the macro method of serial dilutions. The working solution of ABP is prepared from the main solution using a liquid nutrient medium. The concentration of the working solution is calculated based on the required maximum concentration in a series of serial dilutions, taking into account the dilution factor of the drug during subsequent inoculation. Then the working solution in the amount of 0.5 ml using a micropipette with a sterile tip is introduced into the first test tube containing 0.5 ml of broth. Mix thoroughly and transfer 0.5 ml of the ABP solution in the broth with a new sterile tip to a second test tube containing 0.5 ml of the broth initially. This procedure is repeated until all the necessary series of dilutions are prepared. 0.5 ml of broth is removed from the last test tube.

Thus, a number of test tubes with solutions of Ayup are obtained, the concentrations of which differ in neighboring test tubes by 2 times. At the same time, additional series of serial ABP dilutions
are being prepared for testing control strains. The series of dilutions must include borderline concentrations and acceptable ranges of MPC for control strains.

When preparing inoculum and inoculation, a standard microbial suspension is used, equivalent to 0.5 according to the Mcfarland standard, diluted 100 times in a nutrient broth, after which the concentration of the microorganism in it will be approximately $10^6$ CFU/ml.

0.5 ml of inoculum is added to each tube containing 0.5 ml of the corresponding ABP dilution, and to one tube with 0.5 ml of nutrient broth without an antibiotic ("negative" control). The final concentration of the microorganism in each test tube will reach the required one-approximately $5 \times 10^5$ CFU / ml. Inoculum should be introduced into test tubes with ABP dilutions no later than 15-30 minutes from the moment of preparation (figure 3-4).

**Figure 3.** Algorithm for determining the sensitivity of one culture under study to one ABP by dilution in a liquid nutrient medium.

**Figure 4.** Effect of nanomodified antibiotic on the concentration suppressing the growth of E. coli and Enterobacter cloacae.
Further, during incubation, the test tubes are closed with sterile cotton-gauze stoppers or metal caps, and all test tubes with the tested strains, except for the "negative" test tube, are incubated in a normal atmosphere at a temperature of 35°C for 16-20 or 20-24 hours (depending on the type of microorganism being tested). The "negative" test tube is placed in the refrigerator at +4°C, where it is stored until the results are recorded.

To account for the results and determine the presence of microbial growth, test tubes with crops are viewed in transmitted light. The growth of the culture in the presence of ABP is compared with a reference tube ("negative" control) containing the original inoculum and stored in the refrigerator. MPC is determined by the lowest concentration of ABP, which suppresses the visible growth of the microorganism.

A variation of the serial dilution method is also a method based on the use of only two concentrations of antibiotics, or even one concentration corresponding to the threshold (that is, concentrations that separate sensitive microorganisms from intermediate and intermediate from resistant). The method provides high-quality results that allow you to assign the studied microorganism to a certain category of sensitivity, and is often used in commercial test systems.

To determine the presence of microbial growth, test tubes with crops were viewed in transmitted light. The growth of the culture in the presence of ABP was compared with a reference tube ("negative" control) containing the original inoculum and stored in a refrigerator. MPC was determined by the lowest concentration of ABP, which suppresses the visible growth of the microorganism., and is often used in commercial test systems.

The results shown in figure 4 show that the lowest concentration of the antibiotic suppressing the visible growth of E. Coli was 0.8 mcg/ml for Polymyxin and 0.8 mcg/ml for Polymyxin + nSe, respectively.

As can be seen from the data presented in the figure, the concentration suppressing the visible growth of Enterobacter cloacae was 1.5 mcg/ml for Polymyxin and 0.8 mcg/ml for Polymyxin+nSe.

In our opinion, the data obtained clearly indicate that the antibiotic polymyxin + nSe is 1.9 times more effective than polymyxin against Enterobacter cloacae.

The interest of the world community in nanotechnology is due to the lack of knowledge of nanomaterials and high both applied and fundamental prospects of this field of science. In particular, selenium nanoparticles, as one of the most poorly studied materials in all aspects, showing enormous biological diversity. The use of selenium nanoparticles as an effective stimulant has been practiced by scientists around the world for the past 15 years. Despite the small number of published data, the results suggest a high probability of using these objects as enhanced antimicrobial drugs, adjuvants for vaccines, in the diagnosis and treatment of various diseases.

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