ЗЕМЛЕДЕЛИЕ, АГРОХИМИЯ, КОРМОПРОИЗВОДСТВО, АГРОЭКОЛОГИЯ, ЛЕСНОЕ ХОЗЯЙСТВО

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COMPARATIVE STUDY OF ANTIBACTERIAL EFFECTS OF MODIFIED PREPARATIONS CONTAINING METAL NANOPARTICLES

Annatation

In this article was studied the antibacterial properties of modified preparations with metal nanoparticles against pathogenic and opportunistic microorganisms (*Staphylococcus aureus, Escherichia coli, Salmonella dublin*) by using disk diffusion and agar diffusion methods. Using electron microscopy method were determined morphological characteristics (shapes, dimensions) and established the bactericidal effects of metal nanoparticles.

Keywords: nanoparticles, disk diffusion method, agar diffusion, pathogenic microorganisms and opportunistic pathogens, bactericidal effect.

Introduction

Currently the use of modified preparations based on metal nanoparticles as natural antibiotics is one of the actual problems [1]. Despite the fact that antibiotics exhibit a wide range of antibacterial activity nanoparticle preparations can not be introduced intravenously and intramuscularly since injection form of nanocluster preparations is not created yet. However, experiments of clinical testing nanopreparations have shown that combined application with antibiotics significantly enhances the antibacterial and therapeutic activity of the latter. It is caused by the the nanoparticles that act purposefully when entering the body, then find and neutralize pernicious virus or bacteria. Because the nanoparticles associating with immunocomponent blood cells which are responsible for protecting the body from invading bacterial and viral infection, enhance the immune status of the whole organism [2-4].

Mostly a sharp issue is the application of preparations with nanoparticles in medicine, especially in the treatment of common nosocomial infections in the world. Pathogens of these diseases are bacteria, antibiotic resistance of which is growing every year. Most common among them are multiresistant strains of bacteria *Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli* [5].

In this regard, the aim of our study was to investigate the antimicrobial properties of preparations with nanoparticles of metals against the agents of nosocomial infections. Preparations were provided by UkrSRINanobiotechnology and resource saving.

In order to achieve this objective were stated the following tasks:

- determine the presence of metal nanoparticles in the composition of each preparation using transmission electron microscope;
- study the antimicrobial activity of nanoparticles against nosocomial infections.

Materials and methods

The work was performed at Food and Environmental Safety Laboratory of Kazakhstan-Japan Innovation Centre (KazNAU).

In order to determine the presence of nanoparticles in samples was used transmission electron microscope JEM-1011 brand «JEOL» with the digital camera Morada (OLYMPUS). Sample preparation for transmission microscope was carried out according to the nanoparticle observation method by negative staining.

Antibacterial and antifungal activities were determined by disk diffusion and agar diffusion methods. As test strains were used standard sample cultures of microorganisms: *Staphylococcus aureus*, *Escherichia coli* and *Salmonella dublin*.

Strains were cultivated for 18-20h in the slant MPA (meat peptone nutrient agar) supplemented with 0.1% glucose. Then were slurried in physiological solution, cell concentration was brought to 10^9 per ml in accordance with the optical turbidity standard SSS 42-28-29-85 and was used freshly prepared chain of 10-fold dilutions up to 10^3 cell/ml. The specific antimicrobial activity of nanoparticles containing preparations was studied by standard serial dilution method on different test strains with different microbial loads (from 10 to 105 cells per ml).

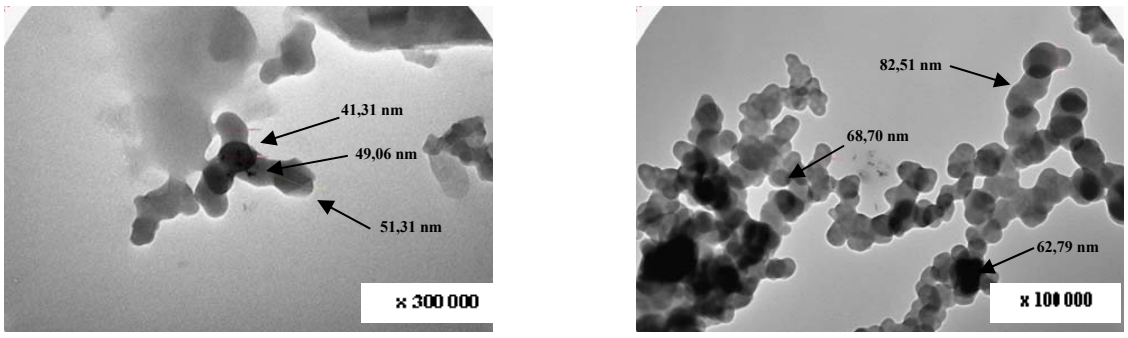
All drugs were used undiluted during the experiment since they were prepared as water solutions. Appropriate estimated amount of the medium with a double concentration of nutrients was used in order to compensate the reduction of the nutrient concentration in the culture medium.

Typical standard testing method: into test tubes with nutrient medium and the culture of the test strain were added studied preparations. Nutrient media was prepared according to the prescription using semi-finished products, in particular, production Titan Media (India). The most commonly used medium was meat peptone broth (MPB) supplemented with 0.1% glucose. Inoculation was carried out with a 18-hour test strain culture suspension rated from appropriate microbial load, usually 10^2 cells/ml. Sowing incubation was performed for 24-72 hours at 37^0C with the next confirming seeding on Petri dishes with nutrient agar.

Profitability analysis was held by visual assessment of the growth availability of the test strain in the experimental samples in comparison with the growth of the test strain in the positive control (nutrient medium with the test strain without preparation). Negative control was carried out in nutrient medium without test strain by control of the medium sterility and also performed preparations sterility control (nutrient medium without the test strain, but with the addition of preparation).

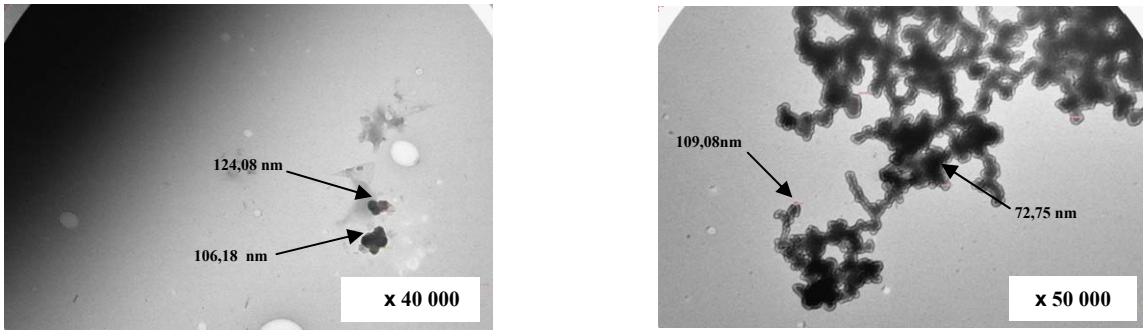
Results and discussion

The results of the study for the distribution of nanoparticles in preparations using transmission electron microscope shown in Figure 1-3.



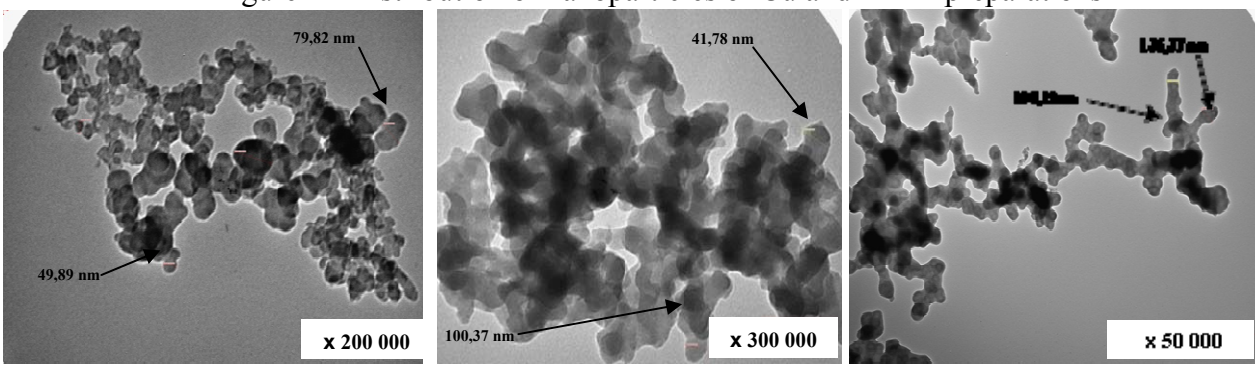
Au **Fe**

Figure 1 - Distribution of nanoparticles of Au and Fe in preparations



Cu **Mn**

Figure 2 - Distribution of nanoparticles of Cu and Mn in preparations



Mo Se Zn

Figure 3 - Structure microphotographs of preparations

The results electron microscopy studies indicate that the metal nanoparticles of preparations have the agglomerate form, mainly as chain structured. However, metals such as Cu and Au distributed in small clusters. These agglomerates consist metal nanoparticles ranging from 36 to 122 nm.

Table 1 and Figure 4-5 show the results of cell viability of microorganisms during combined cultivation with the metal nanoparticles for 24 and 48 hours. Experimental conditions: nutrient medium - meat-broth supplemented with 0.1% glucose; inoculation with 18-hour culture of test strain suspension rated of microbial load 10^2 cells/ml; incubation for 48 hours at 37^0C with the next confirming seeding on Petri dishes with nutrient agar.

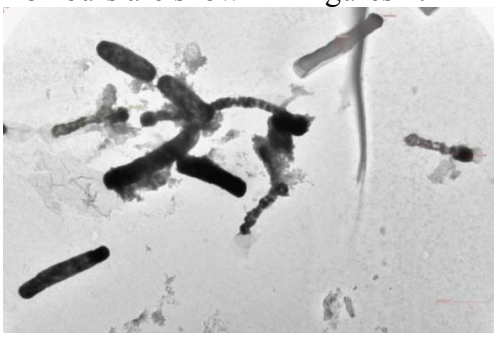
Table 1 - Cell viability of test cultures under the combined cultivation with metal nanoparticles (microbial load of 102 cells/ml).

Name	Growth availability of test strains					
	S.aureus		*S.dublin*		*E.coli*	
	24h	48h	24h	48h	24h	48h
Fe (n=3)	-	-	-	-	-	-
Co (n=3)	-	-	-	-	-	-
Mn (n=3)	-	-	-	-	-	-
Mo (n=3)	-	-	-	-	-	-
Cu (n=3)	-	-	-	-	-	-
Au (n=3)	-	-	-	-	-	-
Zn (n=3)	-	-	-	-	-	-
Mg (n=3)	+	+	+	+	+	+
Ge (n=3)	-	-	-	-	-	-
Se (n=3)	-	-	-	-	-	-

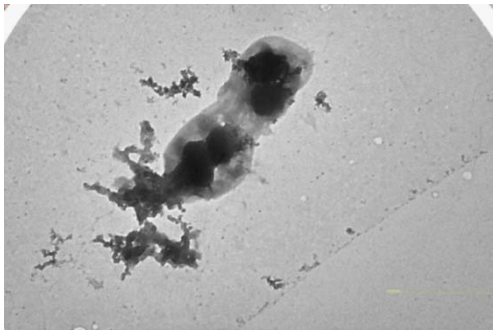
Note: (-) lack of CFU (colony forming unit), (+) presence of CFU

As shown in Table 1, only Mg has shown positive results, namely the growth of test cultures, all other microelements completely have inhibited the growth of cultures. Accordingly the same results were obtained from seeding on Petri dishes with nutrient agar.

Simultaneously was carried out the experimental observation in the transmission electron microscope, that is visually observed, the process of cell destruction, namely the bactericidal effect of metal nanoparticles. The effect results of cobalt nanoparticles during 24 and 48 hours are shown in Figures 4.

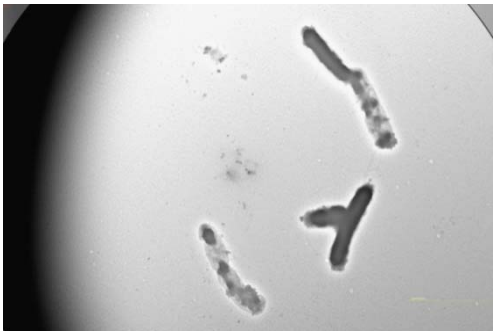


Salmonella dublin (x 20 000) in 24h



Salmonella dublin (x 40 000) in 48h

Escherichia coli (x 20 000) in 24h



Escherichia coli (x 15 000) in 48h

Figure 4 - Cell disruption of microorganisms *Salmonella dublin* and *Escherichia coli* in the interaction with Co nanoparticles

108,19 нм →

Study results of preparations by the antibacterial activity containing metal nanoparticles against the agents of nosocomial infections using disk diffusion and agar diffusion method are shown in Figures 5-8.

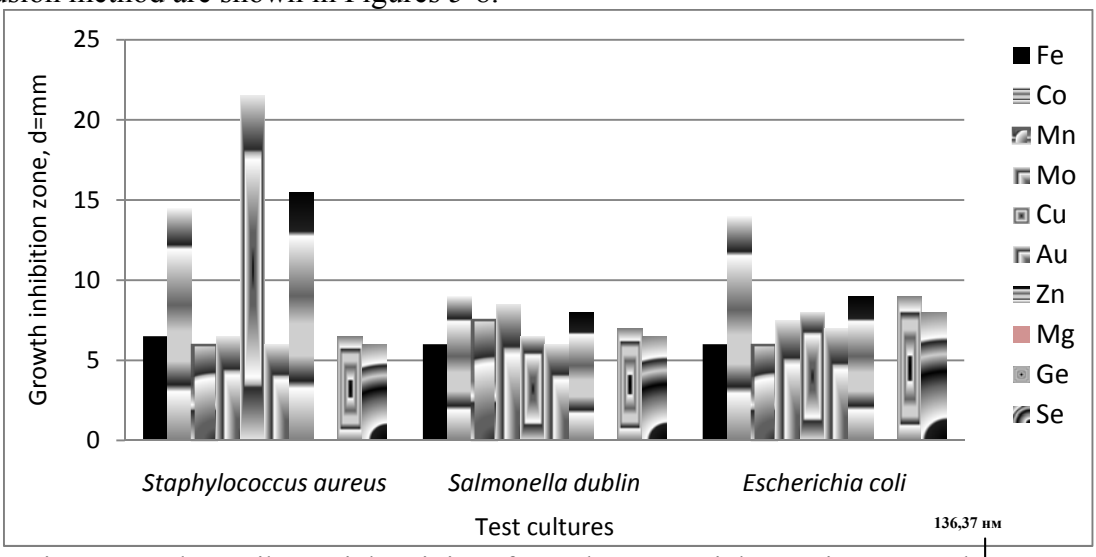
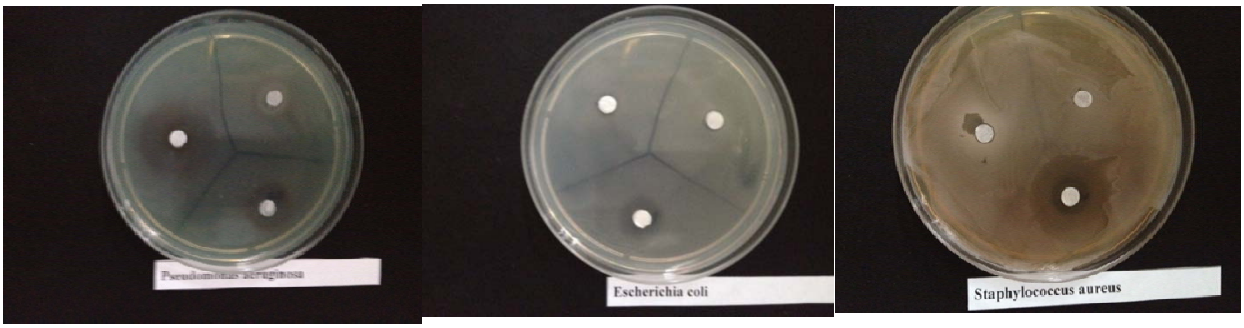


Figure 5 - The antibacterial activity of metal nanoparticles against test cultures *S.aureus* , *S.dublin* and *E.coli* (disk diffusion method)



P.aeruginosa *E.coli* *S.aureus*

Figure 6- The antibacterial activity of the nanoparticles against test cultures (disk diffusion method)

As seen from Figures 5 and 6 samples have shown slight antagonism to all bacterial test cultures. Only Mg has expressed no activity against bacterial test cultures and only Co had a pronounced antagonism against *S.aureus* (14,5 cm) and *E.coli* (14 cm), and Cu against *S.aureus* (21,5 cm).

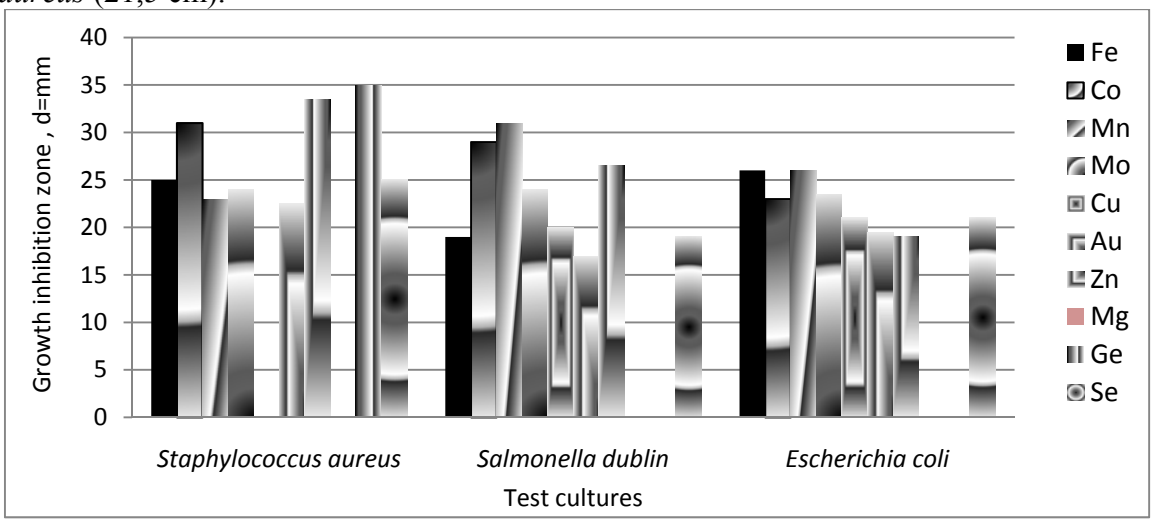
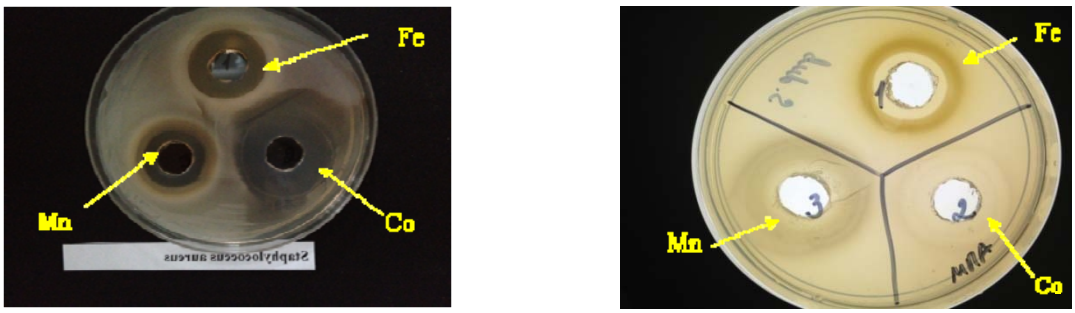


Figure 7 - The antibacterial activity of metal nanoparticles against test cultures *S.aureus* , *S.dublin* and *E.coli* (agar diffusion method)



Staphylococcus aureus *Salmonella dublin*

Figure 8 - Growth inhibition zones of test cultures

Figures 7-8 show that in comparison to disc-diffusion method 8 samples had shown clear expression of antagonism against all bacterial test cultures in agar diffusion method. As well Cu nanoparticles have shown no antagonism to *S. aureus*, Ge nanoparticles against *S.dublin* and *E.coli*, respectively. Only Mg has not expressed any antimicrobial activity against all test strains in the agar diffusion method.

Conclusions

The results of transmission electron microscope indicate the presence of metal nanoparticles in each preparation as chain structured agglomerates. Dimensions of nanoparticles ranged from 36 to 122nm.

It was found that all preparations containing metal nanoparticles have antimicrobial activity except Mg.

Specific effect of metal nanoparticles against bacterial cultures used as test strains were revealed. The highest antagonism against *Staphylococcus aureus* possess Ge, Zn, and Co nanoparticles, Mn, Co and Zn against *Salmonella dublin*, and Fe, Mn, Co, Zn and Se against *Escherichia coli*.

References

1. Ulberg Z.R. Biosafety metal nanoparticles in nanomedicine and nanobiotechnology // III ISC «Nanostructural materials -2010». – Ukraine, 2010. – 650 p.
2. Egorova E.M. Nanoparticles of metals in solutions: biochemical synthesis, properties and applications: 2011.: Abstract of diss. …Doc.Chem.Sc. Moscow, 2011. – 53 p.
3. Rakhmetova A.A. Biological activity of copper nanoparticles differing in dispersion and phase composition: 2011.: Abstract of diss. …Cand.Bio.sc. Moscow, 2011. – 24 p.
4. Shalyapina A. etc. Synthesis and characterization of nanoparticles based on Zn oxide / / J. Chemistry and Technology of Inorganic Materials. – 2011. - №6. – pp. 102-104
5. Reznichenko L.S. etc. Anti-microbial agents based on metal nanoparticles and their conjugates with antibiotics for the treatment of nosocomial infections // ISC «Nanostructural materials -2012». – St. Petersburg, 2012. – 408 p.

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СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ АНТИБАКТЕРИАЛЬНЫХ ЭФФЕКТОВ МОДИФИЦИРОВАННЫХ ПРЕПАРАТОВ, СОДЕРЖАЩИХ НАНОЧАСТИЦЫ МЕТАЛЛОВ

В данной статье изучено антибактериальные свойства модифицированных препаратов с наночастицами металлов по отношению к патогенным и условно-патогенным микроорганизмам (*Staphylococcus aureus*, *Escherichia coli, Salmonella dublin*). Электронно-микроскопическим методом были определены морфологические признаки (форма, размер) и установлены бактерицидные эффекты наночастиц металлов.

Ключевые слова: наночастицы, диско-диффузионный метод, метод диффузии в агар, патогенные и условно-патогенные микроорганизмы, бактерицидные действия.

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ҚҰРАМЫНДА МЕТАЛЛ НАНОБӨЛШЕКТЕРІ БАР МОДИФИЦИРЛЕНГЕН ПРЕПАРАТТАРДЫҢ АНТИБАКТЕРИАЛДЫ ӘСЕРІН САЛЫСТЫРМАЛЫ ЗЕРТТЕУ

Бұл мақалада құрамында металл нанобөлшектері бар модифицирленген препараттардың патогенді және шартты патогенді микрорганизмдерге (*Staphylococcus aureus*, *Escherichia coli, Salmonella dublin*) қатысты әсері зерттелді. Электронды микроскопия әдісінің көмегімен микроорганизмдердің морфологиялық белгілері (пішіні, көлемі) анықталып, металл нанобөлшектерінің бактерицидтік әсерлері белгіленді.

Кілт сөздер: нанобөлшектер, диск-диффузиялық әдіс, агардағы диффузия әдісі, патогенді және шартты патогенді микроорганизмдер, бактерицидтік әсер.