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SILVER NANOPARTICLES: ANTIMICROBIAL SUBSTANCE WITH THE POSSIBILITY TO OVERCOME ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* CLINICAL ISOLATES

The wide spread of multidrug-resistant bacteria species stimulates a search for alternative antimicrobial substances. Silver nanoparticles (AgNP) are among the leading substances possessing a great potential for such a purpose. At the same time, the current data about the possibility of some metal nanoparticles, including silver ones, to stimulate the horizontal transfer of antibiotic resistance genes stipulate the necessity to know the nanoparticles' influence on the antibiotic resistance profile of microorganisms when studying the biological activity of synthesized nanomaterials. ***The aim*** *of the study was to evaluate the antimicrobial activity of synthesized AgNP against the test strains and Escherichia coli clinical isolates — causative agents of infectious diseases of farm animals — and to estimate the nanoparticles' effectiveness in overcoming the antibiotic resistance and colicinogenic activity of E. coli isolates.* ***Methods.*** *AgNPs were synthesized by the chemical reduction method. Antimicrobial activity of the synthesized AgNP was tested via the method of serial dilutions in agar using E. coli ATCC 2592, Staphylococcus aureus MRSA ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633 test strains, and 12 E. coli clinical isolates. The alkaline lysis by the method of Birnboim and Doly followed by agarose gel electrophoresis was used for plasmid DNA screening in E. coli clinical isolates. Disk diffusion assay was used for bacteria antibiotic susceptibility testing. The ability of E. coli clinical isolates to produce colicins was studied by the method of deferred antagonism by Fredericq.* ***Results.*** *AgNP with an average particle size of 30 nm and spherical shape were synthesized. AgNP were characterized as noncytotoxic and nongenotoxic for eukaryotic cells. Antimicrobial activity of AgNP was revealed on the test strains as well as E. coli strains isolated from the pathological material of farm animals (swine, cattle). The changes in the profile of antibiotic resistance and colicinogenic activity were revealed after the treatment of bacteria cells by AgNP at concentrations of 25 and 50 μg/mL.* ***Conclusions.*** *The*

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revealed properties make synthesized AgNP a great alternative antimicrobial substance with the possibility to overcome antibiotic resistance and colicinogenic activity of E. coli clinical isolates.

Keywords: *silver nanoparticles, antimicrobial substance, clinical isolates, antibiotic resistance, R-plasmids, colicinogenic activity, plasmids elimination.*

Infectious diseases of farm animals provoked by different microorganisms can lead to a significant deterioration in animal health and be a cause of direct and indirect high economic losses. Together with the threat for animal health, the problem is complicated by a zoonotic potential of many pathogens and their resistance to antibiotics (Bonardi, 2017; Heredia & García, 2018). For instance, contaminated dairy and meat products are the main source of foodborne pathogens such as *E. coli* O157:H7, causing million cases of diseases and even death in humans around the world (Heredia & García, 2018).

Intensive use of antibiotics as the leading choice in treatment of infected farm animals, which is often not accompanied by identification and characterization of the causative agents, together with the intensification and globalization of their production, provokes a rapid worldwide spread of the strains with multiple resistance to traditional antibiotics in both humans and farm animals (Heredia & García, 2018; Yuan et al., 2017).

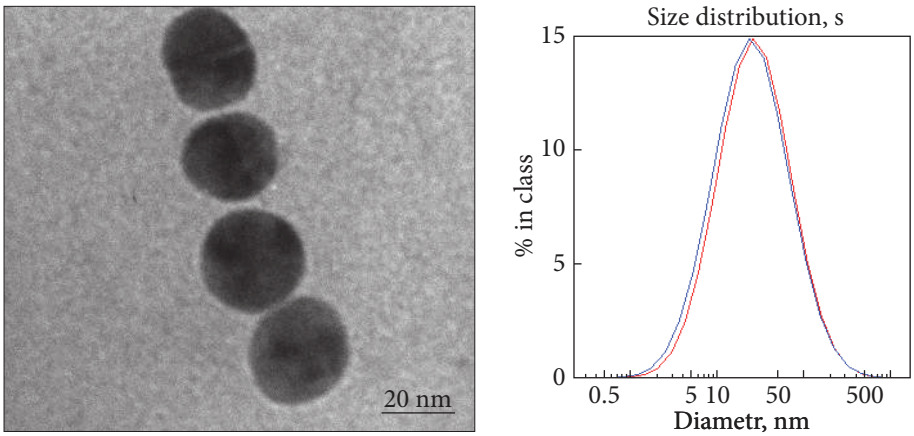
Despite the wide variations in the occurrence of bacteria with resistance to antibiotics across countries, *Escherichia coli* strains resistant to one or several antimicrobial groups are among the most commonly reported bacterial species distributed in both farm animals and humans (Silva et al., 2023; Nyirabahizi et al., 2020; EARS-Net, 2022). A wide spread of resistant *E. coli* strains, which are typically considered as benign commensals commonly found in the lower intestine of warm-blooded organisms, are of great importance. Due to resistance plasmids (R-plasmids) and other mobile genetic elements of *E. coli* genome, it is provided the distribution of antibiotic resistance genes among different microorganisms via the horizontal gene transfer. Such peculiarities make *E. coli* a sentinel microorganism for antimicrobial resistance surveillance (Nyirabahizi et al., 2020; Ramos et al., 2020).

It is also known that *E. coli* bacteria in natural populations have not only R-plasmids but also plasmids of colicinogenicity (Col-plasmids) possessed by similar characteristics (Clark & Pazdernik, 2013). The colicinogenicity of pathogenic bacteria strengthens their position in the fight against the beneficial microflora and colicins themselves and can be potentially important virulence factors of pathogenic *E. coli* (*Šmajs* et al., 2010). Therefore, such parameter as the ability of *E. coli* clinical isolates to produce colicins is a necessary characteristic when analyzing the pool of detected plasmids as well as when estimating potential effectiveness of new antimicrobial substances.

Silver nanoparticles today are well known nanomaterials demonstrating high antimicrobial activity against wide spectra of pathogen microorganisms such as *Escherichia coli*, *Staphylococcus aureus, Salmonella*, *Campylobacter,* and other strains, including antibiotic resistant ones (Yuan et al., 2017; Radzikowski et al., 2020; Kim et al., 2016; Farouk et al., 2020). Such properties stipulate special attention to AgNP for their extensive use in veterinary medicine and food processing industry in order to ensure prevention and treatment of infectious and foodborne diseases (Rodrigues et al., 2023; Danchuk et al., 2023; Carbone et al., 2016; Mikołajczuk-Szczyrba et al., 2019).

The **aim** of this study was to evaluate antimicrobial activity of the synthesized AgNP against the test strains and *Escherichia coli* clinical isolates, causative agents of infectious diseases of farm animals, and to estimate the nanoparticles' effectiveness in overcoming the antibiotic resistance and colicinogenic activity of *E. coli* isolates.

Materials and Methods. ***Silver nanoparticles' synthesis and characterization.*** Spherical mono-



a *b*

Fig. 1. Electron micrograph (A) (JEM-1400, «Jeol», Japan) and DLS data (B) (Zetasizer-3, «Malvern Instruments Ltd», UK) of the synthesized AgNP with an average particle size of 30 nm

disperse silver nanoparticles (AgNP): zeta potential –25.2±1.8 mV, an average particle size of 30 nm (Fig. 1) were synthesized via the chemical reduction of silver nitrate ($AgNO_3$) by tannic acid in the presence of potassium carbonate (K_2CO_3). It was obtained water ispersion with a concentration of silver nanoparticles of 8.0 mg/mL by metal**.**

The synthesized AgNP were characterized as biosafe for eukaryotic cells under *in vitro* tests using such criteria as cytotoxicity (MTT assay) and genotoxicity (Comet assay) in accordance with the Guidelines «Safety assessment of medical nanopreparations», 2013; ISO 10993 - 5:2009, Didenko, 2002.

Eukaryotic cell line L929 (fibroblast from mouse) from the collection of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine was used in an MTT assay. Eukaryotic cell lines L929, ST (Swine Testis), MDVK (calf kidney cell culture), and CHO-K1 (Chinese hamster ovary cells) from the collection of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine were used in the Comet assay. The synthesized AgNP did not show any cytotoxic effect in the concentration range 1.3 — 200 μg/mL. When studying genotoxicity of the nanoparticles, the absence of primary DNA damages in L929, ST, MDVK, and CHO-K1 eukaryotic cell cultures was observed under the influence of AgNP in the whole investigated concentration range (1.3—200 μg/mL by metal).

Electron microscopy images of the synthesized AgNP as well as their contact interaction with *E. coli* clinical isolates were recorded using transmission electron microscopes JEM-1230 and JEM-1400 («Jeol», Japan) in the Centers of collective usage, NAS of Ukraine. Dynamic light scattering (DLS) analysis of the nanoparticles was done using Zetasizer-3 («Malvern Instruments Ltd», UK).

Evaluation of AgNP antimicrobial activity. The method of serial dilutions in agar was used for evaluation of the antimicrobial activity of AgNP at the concentration range 40.0—160.0 **μ**g/mL by metal (Tenover, 2019). Muller-Hinton agar was used as a microbiological growth medium for AgNP antimicrobial activity testing. Test strains *Escherichia coli* ATCC 2592, *Staphylococcus aureus* MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, and 12 *E. coli* strains isolated from the pathological material of young and adult farm animals (swine, cattle) in the Laboratory of Anaerobic Infections of the Institute of Veterinary Medicine of the

NAAS of Ukraine were used for AgNP antimicrobial activity estimation. *E. coli* clinical isolates «Zhitomirska 4», «Niva», «Kalynivka», «Berdiansk», «Sergienko-Sumy», «Staro-Konstantinovka», «Zaporizka-12» No. 150, «Chernihiv-44» No. 154; «Rassvet-165» No. 153, «Malinovka-131» No. 152, «Mirgorod-1» No. 148, and «Donetsk-910» No. 149 were used in the work.

Screening of plasmid DNA; tests of antibiotic susceptibility and colicins production. For screening of plasmid DNA in *E. coli* clinical isolates and assessing the AgNP influence on plasmids' elimination, plasmid DNA was extracted from the investigated clinical isolates (overnight bacteria cultures) using the alkaline lysis by the method of Birnboim and Doly (Birnboim & Doly, 1979) followed by agarose (1%) gel electrophoresis.

Antibiotic susceptibility testing was performed with the disk diffusion assay according to the «Standard for antimicrobial disk susceptibility tests» (Clinical and Laboratory Standards Institute, 2009). The disks with antibiotics (Himedia Laboratories Pvt. Limited): ofloxacin (5 μg), ciprofloxacin (5 μg), nitrofurantoin (100 μg), imipenem (10 μg), ceftazidime (10 μg), ceftriaxone (10 μg), gentamicine (10 μg), amikacin (30 μg), neomycine (30 μg), tobramycine (10 μg), erythromycin (15 μg), and doxycycline (30 μg) were used to estimate antibiotic resistance of the clinical isolates.

The ability of *E. coli* clinical isolates to produce colicins was studied by the method of deferred antagonism by Fredericq. *E. coli* isolates were grown for 24 h at 37° C to individual macrocolonies on plates with 2% nutrient agar. The plates with the macrocolonies of the studied cultures were treated first with chloroform vapor for 50 min and then with UV light for 2.5 hours. 0.1 mL of 6-hour culture of *E. coli* K-12 indicator strain (plasmid-free strain, derivative of *E. coli* XL1-Blue) was mixed with 5 mL of 0.7% nutrient agar (melted and cooled to 48° C). The mixture was used to overlay the plates with the macrocolonies of the studied *E. coli* isolates (previously treated by chloroform and UV light). Then the cultures were incubated for 24 h at 37° C followed by summarizing the obtained results. When the inhibition zone of the indicator strain was observed around the microcolony, the studied isolate was considered a colicin producer. The levels of colicinogenic activity were expressed in relative units, from one to four pluses («+» — «++++»), where the lowest colicinogenic activity («+») was observed with a diameter of inhibition zone for the indicator strain of 7—16 mm; the medium one («++») — with the diameter 17—26 mm; high colicinogenic activity («+++») — with the diameter 27—36 mm; the extremely high colicinogenic activity («++++») — with the inhibition zone 37—45 mm. The colicinogenic activity was equal to zero («0») with the diameter of inhibition zone for the indicator strain less than 7 mm.

Elimination of plasmids. When studying the AgNP influence on elimination of plasmids (R-plasmids and Col-plasmids), the 18-hour cultures of bacteria were diluted in the nutrient broth in the ratio 1:50 and grown for 2 h to the titer $1—2\times10^9$ cells. Then 1×10^4 — 1×10^5 cells were added to the tubes with 2 mL of the nutrient broth containing AgNP at a concentration of 25 or 50 μg/mL with the subsequent incubation at 37° C for 24 h followed by plating 0.1 mL treated bacteria on the plates. Then the antibiotic susceptibility and colicinogenic activity of the microorganisms treated by AgNP were tested, and the inhibition zones were measured and compared to the initial values set for antibiotic resistance and colicinogenic activity. At the same time, the occurrence of the plasmids in the bacteria treated by AgNP was tested via the screening of plasmid DNA according to the method described above.

All experiments were performed in triplicate, and the obtained results were expressed as the average mean of three independent experiments. The experimental data were compared using Student's t-test. $P<0.05$ were considered statistically significant.

Results. When studying antimicrobial activity of the synthesized AgNP against test strains of microorganisms, it was shown a total inhibi-

tion of the cells' growth for *E. coli* ATCC 2592, *S. aureus* MRSA ATCC 43300, and *P. aeruginosa* ATCC 27853 strains in all studied concentration range of nanoparticles (40.0—160.0 μg/mL by metal), whereas in the case of the *B. subtilis* ATCC 6633 strain, the absence of bacteria growth was observed in the presence of at least 100.0 μg/mL of AgNP in the test medium (Table 1).

For *E. coli* clinical isolates, a high antimicrobial action of AgNP was revealed at all studied concentrations (40.0—160.0 μg/mL) against *E. coli* «Zhitomirska 4» strain (Table 2). For the strains *E. coli* «Kalynivka», «Malinovka-131», «Niva», «Berdyansk», «Sergienko-Sumy», «Staro-Konstantinovka», «Zaporizka-12», «Chernigiv-44»; «Rassvet-165, and «Donetsk-910» under the influence of AgNP at a concentration of 40.0 μg/mL, the growth of single colonies was observed, whereas the single colonies of *E. coli* «Mirgorod-1» were grown in the presence of all studied concentrations of the nanoparticles (Table 2).

An active contact interaction of the synthesized AgNP with *E. coli* cells obtained from the pathological material of young and adult farm

Table 1. **Antimicrobial activity of the synthesized AgNP against test strains of microorganisms**

Test strain	Concentration of AgNP in the growth medium, *μg*/mL by metal				Control of test strain growth
	160.0	100.0	80.0	40.0	
E. coli ATCC 2592	ø	ø	ø	ø	+++++
S. aureus MRSA ATCC 43300	ø	ø	ø	ø	+++++
P. aeruginosa ATCC 27853	ø	ø	ø	ø	+++++
B. subtilis ATCC 6633	ø	ø	+	+++++	+++++

«ø» — total inhibition of bacteria growth; «+» — growth of single colonies; «+++++» — intensive growth of microorganisms.

Table 2. **Antimicrobial activity of AgNP against *E. coli* clinical isolates from the pathological material of young and adult farm animals**

Clinical isolates	Concentration of AgNP in the growth medium, *μg*/mL by metal				Control of test strain growth
	160.0	100.0	80.0	40.0	
E. coli «Zhitomirska 4»	Ø	ø	ø	ø	+++++
E. coli «Kalynivka»	Ø	ø	ø	+	+++++
E. coli «Mirgorod-1»	+	+	+	+	+++++
E. coli «Niva»	Ø	ø	ø	+	+++++
E. coli «Berdyansk»	Ø	ø	ø	+	+++++
E. coli «Sergienko-Sumy»	Ø	ø	ø	+	+++++
E. coli «Staro-Konstantinovka»	Ø	ø	ø	+	+++++
E. coli «Zaporizka-12»	Ø	ø	ø	+	+++++
E. coli «Chernigiv-44»	Ø	ø	ø	+	+++++
E. coli «Rassvet-165»	Ø	ø	ø	+	+++++
E. coli «Donetsk-910»	Ø	ø	ø	+	+++++
E. coli «Malinovka-131»	Ø	ø	ø	+	+++++

«ø» — total inhibition of bacteria growth; «+» — growth of the single colonies; «+++++» — intensive growth of microorganisms.

animals was confirmed by the TEM data. Fig. 2 shows cells of the *E. coli* «Mirgorod-1» strain with the accumulated AgNP after 10 min of their contact in the growth medium.

Along with the analysis of AgNP bactericidal action the focus of the work was on evaluation of resistance to antibiotics and colicinogenic activity of *E. coli* clinical isolates as well as estimation the nanoparticles' influence on such properties.

Thus, the evaluation of the profile of antibiotic susceptibility of *E. coli* clinical isolates showed their multiple resistance to antibiotics. Seven *E. coli* isolates («Kalynivka», «Malinovka-131», «Mirgorod-1», «Rassvet-165», «Zaporizka-12», «Chernigiv-44», and «Donetsk-910») were resistant to nitrofurantoin, imipenem, ceftazidime, neomycin, tobramycin, erythromycin, and doxycycline and sensitive to ofloxacin, ciprofloxacin, ceftriaxone, gentamicin, and amikacin among the studied twelve antibiotics. The strain *E. coli* «Sergienko-Sumy» had a slight difference in the profile of antibiotic susceptibility. It was resistant to ciprofloxacin, nitrofurantoin, imipenem, ceftazidime, neomycin, and erythromycin and sensitive to ofloxacin, ceftriaxone, gentamicin, amikacin, tobramycin, and doxycycline. The revealed results of multiple resistance of *E. coli* clinical isolates to the different antibiotic groups correspond to the common tendency, in particular, observed across the European Union countries according to the EARS-Net report (EARS-Net, 2022).

Under the screening of plasmid DNA for eight *E. coli* clinical isolates from the studied twelve ones, there were identified small-sized plasmids that fit the signs of R-plasmids or Col-plasmids. The isolated plasmids were about 2.0 bp in size. Fig. 3 demonstrates analysis of plasmid profiles for eight *E. coli* clinical isolates where seven strains (tracks 4—10) contain the small-sized plasmids versus one strain (track 3).

5 of 8 studied plasmid-containing *E. coli* isolates had the ability to produce colicins (Table 3).

When studying AgNP antimicrobial activity against *E. coli* clinical isolates, it was observed a growth of single colonies of microorganisms under the influence of nanoparticles at a concentration of 40.0 **μ**g/mL by metal. So, the influence of AgNP on the *E. coli* profile of antibiotic resistance and ability to produce colicins was studied using two concentrations — 25 μg/mL and 50 μg/mL.

The treatment of *E. coli* clinical isolates by AgNP at a concentration of 25 μg/mL or 50 μg/mL led to significant changes both in the antibiotic resistance profile and in colicinogenic activity of the strains.

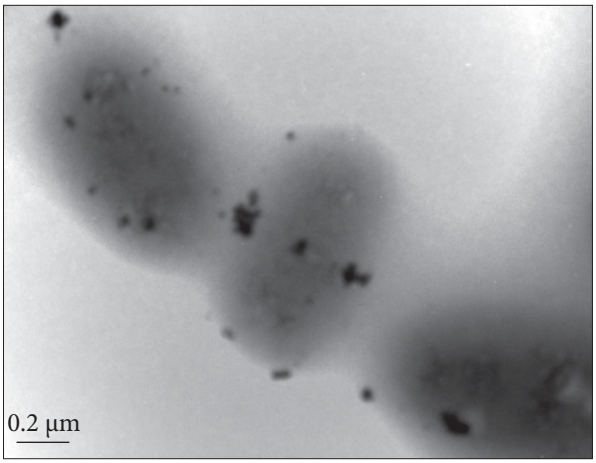


Fig. 2. TEM image of the cells of *Escherichia coli* «Mirgorod-1» clinical isolate after 10 min contact interaction with the synthesized AgNP

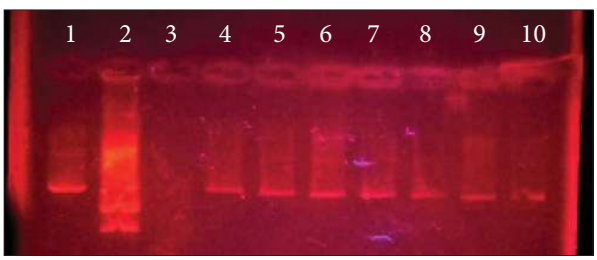


Fig. 3. Electrophoregram of DNA markers and isolated DNA plasmids of *E. coli* isolates: 1— plasmid marker pUC19, digested with enzyme Mva I (2686, 2073bp); 2 — plasmid marker pUC19, digested with enzyme Bgl I (1568, 1118 bp); 3— *E. coli* «Niva»; 4 — *E. coli* «Mirgorod-1»; 5 — *E. coli* «Rassvet-165»; 6 — *E. coli* «Zaporizka-12»; 7 — *E. coli* «Donetsk-910»; 8 — *E. coli* «Malinovka-131»; 9 — *E. coli* «Chernigiv-44»; 10 — *E. coli* «Sergienko-Sumy»

Thus, after treatment by AgNP, the colicinogenic activity of the studied *E. coli* isolates was equal to «0» with the diameter of inhibition zone 1—5 mm.

The selection of treated with AgNP plasmid-containing bacteria based on sensitivity to antibiotics showed a loss of antibiotic resistance in 50% of the isolates. The strains *E. coli* «Kalynivka», «Malinovka-131» and «Mirgorod-1» treated by AgNP became sensitive to nitrofurantoin, ceftazidime, and doxycycline (Table 4). *E. coli* «Mirgorod-1» and *E. coli* «Malinovka-131» also lost the resistance to erythromycin after AgNP treatment. *E. coli* «Rassvet-165» lost its resistance to nitrofurantoin, and doxycycline under the influence of AgNP (Table 4). The other studied plasmid-containing *E. coli* isolates did not change their antibiotic resistance profile.

Isolation of plasmid DNA followed by 1% agarose gel electrophoresis showed the absence of plasmids in all clinical isolates subjected to plasmid elimination using silver nanoparticles of average size of 30 nm.

Discussion. Effectiveness of AgNP antimicrobial activity, as well as their biosafety for eukary-

Table 3. **Colicinogenic activity of *E. coli* clinical isolates**

E. coli clinical isolate	Levels of *E. coli* colicinogenic activity
«Zaporizka-12»	0
«Chernigiv-44»	«+++» (31±2 mm)
«Rassvet-165»	«+++» (34±3 mm)
«Malinovka-131»	«++» (17±2 mm)
«Mirgorod-1»	0
«Donetsk-910»	«+++» (25±2 mm)
«Sergienko-Sumy»	«+++» (29±1 mm)
«Kalynivka»	0

Table 4. **Profile of *E. coli* clinical isolates' antibiotic resistance after their treatment with AgNP at a concentration of 25 μg/mL (1) and 50 μg/mL (2) in comparison with the initial profile of antibiotic resistance (control)**

Antibiotic	*E. coli* clinical isolate											
	«Kalynivka»			«Mirgorod-1»			«Malinovka-131»			«Rassvet-165»		
	Changes in antibiotic resistance after treatment of bacteria by AgNP at concentrations 25 μg/mL (1) and 50 μg/mL (2) in comparison with the control (C) by analysis of growth inhibition zones, (mm)											
	C	1	2	C	1	2	C	1	2	C	1	2
Nitrofurantoin	0	21±1	21±1	0	23±1	23±2	0	21±2	21±2	0	22±2	22±2
Imipenem	0	0	0	0	0	0	0	0	0	0	0	0
Ceftazidime	0	27±1	27±1	0	25±2	25±2	0	26±2	26±1	0	0	0
Neomycin	0	0	0	0	0	0	0	0	0	0	0	0
Tobramycin	0	0	0	0	0	0	0	0	0	0	0	0
Erythromycin	0	0	0	0	25±1	26±1	0	29±1	29±1	0	27±1	27±1
Doxycycline	0	23±1	23±1	0	22±2	22±1	0	23±1	23±1	0	21±2	21±1

otic cells, is determined by numerous factors, including the , shape, type of used reducing agents, method of synthesis, etc. (Cheon et al., 2019; Jeong et al., 2014; Qing et al., 2018). According to the obtained results, the synthesized spherical AgNP with an average particle size of 30 nm were possessed by high antimicrobial effectiveness both against test strains and *E. coli* clinical isolates — causative agents of infectious diseases of farm animals. The concentration values of antimicrobial action revealed for the synthesized AgNP are close to the data of Agnihotri et al. for 30 nm spherical silver nanoparticles synthesized using other reducers (Agnihotri et al., 2014).

High antimicrobial activity of AgNP is provided by the multiple mechanism of the nanoparticles' influence on bacteria cells: changes in the permeability and even disruption of the cell wall, inhibition of the main enzymes, activation of ROS, damage of DNA, etc. (Ahmad et al., 2020; Rai et al., 2012; Liao et al., 2019). Nevertheless, the release of Ag^+ ions by AgNP is often suggested among the basic reasons for such multiple ways of AgNP action when contacting with bacteria cells (Rai et al., 2012; Ahmad et al., 2020). This fact deserves a special attention against the background of the spread of antibiotic-resistant strains with the resistance to Ag^+ ions through the process of co-selection (McHugh et al., 1975; Silver, 2003) and the current data about the possibility of some metal nanoparticles to stimulate the horizontal transfer of antibiotic resistance genes (Zhang et al., 2019; Qiu et al., 2012). So, when studying the biological activity of synthesized nanoparticles, especially their antimicrobial properties, it is necessary to know the nanomaterials' influence on the antibiotic resistance profile of the tested microorganisms.

In our research, the presence of plasmids was shown in 8 out of 12 examined isolates of *E. coli*. Such plasmids were classified as R-plasmids and Col- plasmids, according to their sizes. The screening of plasmid DNA after treatment *E. coli* clinical isolates by the synthesized AgNP showed the absence of R- and Col-plasmids. These results can be explained by their elimination from the bacteria cells under the influence of AgNP. Thus, in the previous model, it was shown the changes in the structure of pUC19 and pBR322 model plasmids from globules to relaxed forms with the nanoparticles' localization on the unraveled DNA strands. The frequency of pUC19 and pBR322 plasmids elimination from *E. coli* XL1-Blue Kan bacteria under AgNP influence increased to 90—98% in comparison with the level of spontaneous elimination of 34—37% (Dybkova, 2014). The possibility of the synthesized AgNP to bind with R-plasmids can lead to inhibition of the horisontal transfer of antibiotic resistance genes. Similar data on the possibility of inhibition of the lateral transfer of antibiotic resistance genes as result of some types of metal oxide nanoparticles' binding with plasmids are discussed in (Hu et al., 2019).

The revealed elimination of R-plasmids from the clinical isolates under the influence of AgNP accompanied by the loss of antibiotic resistance can explain the synergistic or additive effect observed by some authors (Smekalova et al., 2016) when studying the effectiveness of combinations of AgNPs and antibiotics.

According to the experimental data on the evaluation of the effect of overcoming antibiotic resistance in clinical isolates of *E. coli* bacteria, presented in this paper, silver NPs were particularly effective in relation to doxycycline and ceftazidime.

Thus, our findings confirm the possibility of overcoming antibiotic resistance and colicinogenicity of *E. coli* bacteria, which are the causative agents of infectious diseases in animals, using silver nanoparticles. This effect can be considered a new effective way to solve the problem of the development of modern effective means and methods of antibiotic therapy in veterinary medicine.

Conclusions. The observed properties of the synthesized spherical AgNP with an average particle size of 30 nm, such as high antimicrobial activity and the ability to stimulate elimination

of R- and Col-plasmids, together with a simple method of synthesis and biosafety for eukaryotic cells according to the genotoxicity and cytotoxicity parameters, make such nanoparticles a great alternative antimicrobial means with the possibility to overcome antibiotic resistance. Also, it can be assumed a high potential of these nanoparticles in inhibition of the horizontal transfer of antibiotic resistance genes, as well as the synergistic or additive effect when combining AgNP and some types of antibiotics in clinical practice. Estimation of the metal nanoparticles influence on the pathogenic bacteria profile of antibiotic resistance and colicinogenic activity can be a prognostic and necessary test in order to avoid spread of resistant microorganisms.

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Conflicts of Interest. The authors declare no competing interest.

REFERENCES

Agnihotri, S., Mukherji, S., & Mukherji, S. (2014). Size-controlled silver nanoparticles synthesized over the range 5—100 nm using the same protocol and their antibacterial efficacy. *RSC Adv*, *4*, 3974—3983.

Ahmad, S. A., Das, S. S., Khatoon, A., Ansari, M. T., Afzal, M., Hasnain, S., & Nayak, A. K. (2020). Bactericidal activity of silver nanoparticles: A mechanistic review. *Materials Science for Energy Technologies*, *3*, 756—769.

Birnboim, H. C., & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*, *7*(6), 1513—1523.

Bonardi, S. (2017). Salmonella in the pork production chain and its impact on human health in the European Union. *Epidemiology and Infection*, 145(8), 1513—1526.

Carbone, M., Donia, D. T., Sabbatella, G., & Antiochia, R. (2016). Silver nanoparticles in polymeric matrices for fresh food packaging. *Journal of King Saud University — Science*, *28*(4), 273—279.

Cheon, J. Y., Kim, S. J., Rhee, Y. H., Kwon, O. H., & Park, W. H. (2019). Shape-dependent antimicrobial activities of silver nanoparticles. *Int J Nanomedicine*, *14*, 2773—2780.

Clark, D. P., & Pazdernik, N. J. (2013). Chapter 20 — Plasmids. In D.P. Clark, & N.J. Pazdernik (Eds.), *Molecular Biology (Second Edition)*, Academic Press, 616—648.

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard — Eleventh Edition. CLSI document M02-A11 Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 2012.

Danchuk, O., Levchenko, A., da Silva Mesquita, R., Danchuk, V., Cengiz, S., Cengiz, M., & Grafov, A. (2023). Meeting contemporary challenges: development of nanomaterials for veterinary medicine. *Pharmaceutics*, *15*(9), Article 2326.

Didenko, V. V. (2002). In Situ detection of DNA damage. Methods and Protocols. *Methods in Molecular Biology* 203(1st ed.).

Dybkova, S. M. (2014). [Gold and silver nanoparticles — effective agents for elimination the plasmids of antibiotic resistance]. *Bulletin of Problems Biology and Medicine*, *3*, 314—318. [In Ukrainian].

European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) — Annual Epidemiological Report 2020. Stockholm: ECDC; 2022.

Farouk, M. M., El-Molla, A., Salib, F. A., Soliman, Y. A., & Shaalan, M. (2020). The Role of silver nanoparticles in a treatment approach for multidrug-resistant *Salmonella* species isolates. *Int J Nanomedicine*, *15*, 6993—7011.

Guidelines «Safety assessment of medical nanopreparations». (2013). (approved by the Scientific Expert Council of the State Expert Centre of the Ministry of Health of Ukraine (protocol №8, 09.26.2013)), Kyiv, 108 p.

Heredia, N., & García, S. (2018). Animals as sources of food-borne pathogens: A review. *Anim Nutr*, *4*(3), 250—255.

Hu, X., Yang, B., Zhang, W., Qin, C., Sheng, X., Oleszczuk, P., & Gao, Y. (2019). Plasmid binding to metal oxide nanoparticles inhibited lateral transfer of antibiotic resistance genes. *Environ Sci: Nano*, *6*, 1310—1322.

ISO 10993 — 5:2009. Biological evaluation of medical devices — Part 5: Test for *in vitro* cytotoxicity. The standard was last reviewed and confirmed in 2022.

Jeong, Y., Lim, D. W., & Choi, J. (2014). Assessment of size-dependent antimicrobial and cytotoxic properties of silver nanoparticles. *Advances in Materials Science and Engineering*, Article 763807.

Kim, T. Y., Cha, S. H., Cho, S., & Park, Y. (2016). Tannic acid-mediated green synthesis of antibacterial silver nanoparticles. *Arch Pharm Res*, *39*(4), 465—473.

Liao, S., Zhang, Y., Pan, X., Zhu, F., Jiang, C., Liu, Q., Cheng, Z., Dai, G., Wu, G., Wang, L., & Chen, L. (2019). Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. *Int J Nanomedicine*, *14*, 1469—1487.

McHugh, G. L., Moellering, R. C., Hopkins, C. C., & Swartz, M. N. (1975). Salmonella typhimurium resistant to silver nitrate, chloramphenicol, and ampicillin. *Lancet*, *1*(7901), 235—240.

Mikołajczuk-Szczyrba, A., Kieliszek, M., Giurgiulescu, L., & Sokołowska B. (2019). Characteristics and application of silver nanoparticles in the food industry — Review. *Carpathian Journal of Food Science and Technology*, *11*(4), 153—160.

Nyirabahizi, E., Tyson, G. H., Dessai, U., Zhao, S., Kabera, C., Crarey, E., Womack, N., Crews, M. K., Strain, E., & Tate, H. (2020). Evaluation of *Escherichia coli* as an indicator for antimicrobial resistance in *Salmonella* recovered from the same food or animal ceca samples. *Food Control*, *115*, Article 107280.

Qing, Y., Cheng, L., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., Liu, H., & Qin, Y. (2018). Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *Int J Nanomedicine*, *13*, 3311—3327.

Qiu, Z., Yu, Y., Chen, Z., Jin, M., Yang, D., Zhao, Z., Wang, J., Shen, Z., Wang, X., Qian, D., Huang, A., Zhang, B., & Li, J-W. (2012). Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera. *Proc Natl Acad Sci U S A*, *109*(13), 4944—4949.

Radzikowski, D., Kalińska, A., Ostaszewska, U., & Gołębiewski, M. (2020). Alternative solutions to antibiotics in mastitis treatment for dairy cows — a review. *Anim Sci Pap Rep*, *38*(2), 117—133.

Rai, M. K., Deshmukh, S. D., Ingle, A. P., & Gade, A. K. (2012). Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *J Appl Microbiol*, *112*(5), 841—852.

Ramos, S., Silva, V., Dapkevicius, M. L. E., Caniça, M., Tejedor-Junco, M. T., Igrejas, G., & Poeta, P. (2020). *Escherichia coli* as commensal and pathogenic bacteria among food-producing animals: health implications of extended spectrum β-lactamase (ESBL) production. *Animals (Basel)*, *10*(12), Article 2239.

Rodrigues, M. P., Pinto, P. N., Dias, R. R. D. S., Biscoto, G. L., Salvato, L. A., Millán, R. D. S., Orlando, R. M., & Keller, K. M. (2023). The Antimicrobial applications of nanoparticles in veterinary medicine: a comprehensive review. *Antibiotics*, *12*(6), Article 958.

Silva, A., Silva, V., Pereira, J. E., Maltez, L., Igrejas, G., Valentão, P., Falco, V., & Poeta, P. (2023). Antimicrobial resistance and clonal lineages of *Escherichia coli* from food-producing animals. *Antibiotics*, *12*, Article 1061.

Silver, S. (2003). Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev*, *27*, 341—353.

Šmajs, D., Micenková, L., Šmarda, J., Vrba, M., Ševčíková, A., Vališová, Z., & Woznicová, V. (2010). Bacteriocin synthesis in uropathogenic and commensal *Escherichia coli*: colicin E1 is a potential virulence factor. *BMC Microbiol*, *10*, Article 288.

Smekalova, M., Aragon, V., Panacek, A., Prucek, R., Zboril, R., Kvitek, L. (2016). Enhanced antibacterial effect of antibiotics in combination with silver nanoparticles against animal pathogens. *Vet J*, *209*, 174—179.

Tenover, F. C. (2019). Antimicrobial susceptibility testing. In T.M. Schmidt (Ed.), *Encyclopedia of Microbiology (Fourth Edition)*, Academic Press, 166—175.

Yuan, Y. G., Peng, Q. L., & Gurunathan, S. (2017). Effects of silver nanoparticles on multiple drug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from mastitis-infected goats: an alternative approach for antimicrobial therapy. *Int J Mol Sci*, *18*(3), Article 569.

Zhang, S., Wang, Y., Song, H., Lu, J., Yuan, Z., & Guo, J. (2019). Copper nanoparticles and copper ions promote horizontal transfer of plasmid-mediated multi-antibiotic resistance genes across bacterial genera. *Environ Int*, *129*, 478—487.

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НАНОЧАСТИНКИ СРІБЛА: АНТИМІКРОБНА СУБСТАНЦІЯ З МОЖЛИВІСТЮ ПОДОЛАННЯ АНТИБІОТИКОРЕЗИСТЕНТНОСТІ КЛІНІЧНИХ ІЗОЛЯТІВ *ESCHERICHIA COLI*

Широке розповсюдження мультирезистентних мікроорганізмів стимулює пошук антимікробних субстанцій, альтернативних традиційним антибіотикам. Одними з найбільш перспективних субстанцій у цьому напрямку є наночастинки срібла (AgNP). Водночас, сучасні дані щодо здатності наночастинок деяких металів, зокрема срібла, стимулювати горизонтальне перенесення генів антибіотикорезистентності зумовлюють необхідність вивчення впливу наночастинок на профіль антибіотикорезистентності мікроорганізмів при дослідженні біологічної активності синтезованих наноматеріалів. **Метою** роботи була оцінка антимікробної активності синтезованих AgNP щодо тест-штамів та клінічних ізолятів *Escherichia coli* — збудників інфекційних захворювань сільськогосподарських тварин, а також оцінка ефективності наночастинок у подоланні антибіотикорезистентності та коліциногенної активності клінічних ізолятів *E. coli*. **Методи.** Наночастинки срібла синтезували хімічним відновленням нітрату срібла таніном у присутності карбонату калію. Форму і розмір синтезованих наночастинок визначали методами трансмісійної електронної мікроскопії та фотонної кореляційної спектроскопії. Біобезпечність AgNP для еукаріотичних клітин вивчали *in vitro* з використанням таких критеріїв, як цитотоксичність (МТТ-тест) та генотоксичність (метод ДНК-комет) згідно з методичними рекомендаціями «Оцінка безпеки лікарських нанопрепаратів». Антимікробну активність синтезованих AgNP тестували методом серійних розведень в агарі з використанням тестових штамів *E. coli* ATCC 2592, *Staphylococcus aureus* MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633 та дванадцяти клінічних ізолятів *E. coli*, виділених з патологічного матеріалу молодняку і дорослих сільськогосподарських тварин (свиней, великої рогатої худоби). Для скринінгу плазмідної ДНК у клінічних ізолятах *E. coli* та оцінки впливу AgNP на елімінацію плазмід використовували лужний лізис за методом Бірнбойма та Долі з наступним електрофорезом в агарозному гелі. Диско-дифузійний метод був використаний для тестування чутливості бактерій до антибіотиків офлоксацину, ципрофлоксацину, нітрофурантоїну, іміпенему, цефтазидиму, цефтріаксону, гентаміцину, амікацину, неоміцину, тобраміцину, еритроміцину і доксицикліну. Здатність клінічних ізолятів *E. coli* продукувати коліцини вивчали методом відстроченого антагонізму за Фредеріксом. **Результати.** AgNP сферичної форми середнього розміру 30 нм були синтезовані за допомогою простого, екологічного та економічно ефективного методу. В дослідженнях *in vitro* синтезовані AgNP були охарактеризовані як нецитотоксичні і негенотоксичні для еукаріотичних клітин. Антимікробна активність наночастинок срібла була виявлена відносно як тестових штамів, так і клінічних ізолятів *E. coli*, виділених з патологічного матеріалу сільськогосподарських тварин. Показано наявність змін профілю антибіотикорезистентності та коліциногенної активності після обробки клітин бактерій наночастинками срібла в концентраціях 25 та 50 мкг/мл. **Висновки**. Виявлені властивості свідчать, що синтезовані наночастинки срібла є перспективною альтернативною антимікробною субстанцією з можливістю подолання антибіотикорезистентності та коліциногенної активності клінічних ізолятів *E. coli*.

Ключові слова*:* наночастинки срібла, антимікробна субстанція, *клінічні ізолятиі,* антибіотикорезистентність, *R-плазміди,* коліциногенна активність, елімінація плазмід.