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ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES AS A SUBSTITUTE FOR SYNTHETIC ANTIBIOTICS IN POULTRY FARMING

Abstract

The present study investigated the antimicrobial properties of silver nanoparticles (AgNPs) against selected Gram-negative and Gram-positive bacteria using the agar diffusion method. AgNPs were applied at concentrations ranging from 10 to 50 ppm. Tetracycline (45 ppm) served as the positive control, while distilled water was used as the negative control. To determine the minimum inhibitory concentration (MIC), bacterial growth inhibition was evaluated spectrophotometrically by measuring optical density at 600 nm (OD_{600}). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, with significance established at $P < 0.05$.

The results demonstrated a pronounced antimicrobial effect of AgNPs against the Gram-negative strains Escherichia coli and Salmonella typhimurium. All tested concentrations (10–50 ppm) significantly suppressed bacterial growth ($P < 0.01$), and the lowest MIC value was identified as 6.25 ppm, at which OD_{600} values indicated complete inhibition of cell proliferation. In contrast, AgNPs had no significant inhibitory effect ($P > 0.05$) on the growth of the Gram-positive bacteria Lactobacillus acidophilus and Lactobacillus sp., highlighting the selective nature of their antimicrobial activity.

The observed selectivity suggests that silver nanoparticles can effectively target pathogenic Gram-negative bacteria while preserving beneficial lactic acid microflora. This is particularly relevant for poultry farming and veterinary medicine, where maintaining a balanced gut microbiota

is crucial for animal health and productivity. Furthermore, the strong inhibitory action of AgNPs supports their potential as a natural and efficient alternative to conventional synthetic antibiotics. The application of AgNPs at low, biologically safe concentrations may contribute to enhanced biosafety, reduction of antimicrobial resistance risks, and improved microbiological quality of animal-derived products.

Overall, the findings underscore the promise of silver nanoparticles as a multifunctional antimicrobial agent. Their incorporation into feed additives, veterinary preparations, or biosafety protocols could provide an innovative strategy for reducing antibiotic use while supporting sustainable and health-oriented livestock production.

Keywords: *silver nanoparticles, Escherichia coli, Salmonella typhimurium, Lactobacillus acidophilus, minimum inhibitory concentration, antimicrobial activity.*

Introduction. The global increase in antibiotic-resistant microorganisms has become one of the most pressing challenges for modern medicine, veterinary science, and animal production systems. According to the World Health Organization (WHO, 2022), antimicrobial resistance (AMR) is responsible for more than 1.2 million deaths annually, and this number continues to grow as resistance spreads across geographical regions and production sectors. The development of resistance is largely facilitated by the prolonged, excessive, or uncontrolled use of antibiotics in human and veterinary medicine, as well as in livestock and poultry production, where antimicrobial agents are frequently employed for therapeutic, prophylactic, or growth-promoting purposes [1–4].

Bacteria can rapidly modify their genetic material and exchange resistance determinants through mobile genetic elements such as plasmids, integrons, transposons, or via horizontal gene transfer mechanisms—including conjugation, transformation, and transduction. These adaptive processes enable bacteria to survive even under exposure to high antibiotic concentrations and promote the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-resistant strains. Among the most clinically and economically significant antibiotic-resistant pathogens are *Escherichia coli* [5], *Salmonella spp.* [6], *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, all of which are known to cause a broad spectrum of pathological conditions affecting the gastrointestinal, respiratory, integumentary, and urogenital systems of animals [7].

In livestock and poultry production, MDR *E. coli* and *Salmonella* spp. are particularly threatening, as they frequently cause enteritis, septicemia, and dysbiosis in young animals, leading to high morbidity and mortality rates and substantial declines in productivity [8]. These concerns emphasize the urgent need to develop alternative antimicrobial strategies that are effective, biologically safe, and capable of reducing antibiotic use while preserving the balance of beneficial intestinal microbiota.

One promising direction involves the application of nanotechnology, specifically the use of metal-based nanoparticles, which exhibit unique physicochemical properties at the nanoscale (1–100 nm). Nanoparticles demonstrate high surface reactivity, enhanced catalytic potential, electrical conductivity, and strong biological interactions that make them suitable for biomedical and veterinary applications [9]. Among metallic nanoparticles, silver nanoparticles (AgNPs) are the

most widely studied due to their potent broad-spectrum antimicrobial, antiviral, and antifungal activities, confirmed by numerous experimental and applied studies conducted over the past decade [10–12].

The antimicrobial effect of silver nanoparticles is multi-stage and involves several complementary mechanisms:

1. **Adsorption of Ag^+ ions onto bacterial cell surfaces.** Silver interacts with thiol (-SH) groups of membrane-bound proteins, resulting in enzyme inactivation and disruption of metabolic pathways.
2. **Damage to membrane integrity.** Electrostatic interactions between AgNPs and membrane phospholipids increase permeability, causing leakage of intracellular contents and osmotic imbalance.
3. **Induction of oxidative stress.** AgNPs stimulate the formation of reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, and hydrogen peroxide, which damage nucleic acids, membrane lipids, and proteins.
4. **Inhibition of respiratory chain enzymes.** Ag^+ ions bind to components of the cytochrome system, blocking ATP synthesis and inducing energy deprivation.
5. **Interference with DNA replication.** Silver ions interact with phosphate groups of DNA, disrupting replication and transcription processes [13-18].

These multi-target interactions not only inhibit microbial growth but also significantly reduce the probability of resistance formation, as bacteria are unable to simultaneously adapt to several lethal mechanisms. Importantly, AgNPs are generally biocompatible with mammalian host tissues and do not exert destructive effects on beneficial lactic acid bacteria (Lactobacillus spp., Bifidobacterium spp.) when used at optimal concentrations. This renders them attractive candidates for use in veterinary practice and poultry farming as natural antimicrobial agents and potential alternatives to conventional antibiotics.

Recent Ukrainian studies highlight the safety and functional potential of AgNPs in poultry production. Research by Shevchenko, Zheltonozhskaya, Dovbnia et al. (2023) demonstrated that hybrid silica/polyacrylamide carriers containing AgNPs (2.4 ± 1.0 nm) added to drinking water of laying hens resulted in mild accumulation of silver in eggshells but did not produce toxic effects on birds, nor did they compromise egg quality or mineral composition under controlled dosing [19]. Other studies (Kucheruk, Zaseikin, 2021–2023) have shown that colloidal silver nanoparticles may beneficially modulate the gut microbiota of broiler chickens, reducing pathogenic microflora and decreasing the need for antibiotic intervention [20-23].

Given these findings, investigating the physicochemical properties and antimicrobial efficacy of silver nanoparticles is of particular scientific and practical relevance for developing novel antimicrobial solutions in veterinary medicine. The current study aims to assess the antimicrobial activity of silver nanoparticles (AgNPs) against Gram-negative (*Escherichia coli*, *Salmonella typhimurium*) and Gram-positive (*Lactobacillus acidophilus*, *Lactobacillus sp.*) bacteria to evaluate their potential as alternatives to conventional antibiotics in veterinary applications.

The aim of the study was to evaluate the antimicrobial activity of silver nanoparticles (AgNPs) against Gram-negative (Escherichia coli, Salmonella

typhimurium) and Gram-positive (Lactobacillus acidophilus, Lactobacillus sp.) bacteria in order to determine their potential use as an alternative to antibiotics in veterinary medicine.

Materials and Methods.: Preparation of silver nanoparticles. Silver nanoparticles were synthesized using the conventional citrate method. In a round-bottom flask, 0.0425 g of $AgNO_3$ (1 mM) was dissolved in 250 mL of distilled water, and the solution was brought to a boil on a hot plate with vigorous stirring. After boiling, 5 mL of a 1% sodium citrate solution (w/v) was rapidly added to the hot solution, and boiling was maintained for 30 min until a stable yellow-brown color was achieved. The resulting suspension was cooled to room temperature and stored in a dark glass container at 4 °C. Particle size and morphology were determined using a Zetasizer Nano ZS particle size analyzer (Malvern Instruments, UK). The average diameter of the nanoparticles was 84.78 ± 1.54 nm, the polydispersity index was 0.23 ± 0.015, and the zeta potential was –22.03 mV, indicating high stability of the colloidal system [24].

Study of Antimicrobial Activity The antimicrobial activity of AgNPs was determined using the agar diffusion method (Kirby–Bauer) (CLSI, 2020).

The following test microorganisms were used: Salmonella typhimurium; Escherichia coli; Lactobacillus acidophilus; Lactobacillus sp.

Bacterial cultures were re-established from lyophilized forms on Nutrient Agar (NA) for Gram-negative bacteria and MRS Agar (de Man, Rogosa & Sharpe) for lactobacilli (manufacturer—HiMedia, India; distributor — Bio-Test-Ukraine LLC) [25].

Preparation of bacterial cultures Four isolated colonies from a pure culture were transferred to a tube containing 7 mL of liquid nutrient medium (NA or MRS) and incubated at 37 °C for 24 hours in a TS-80 thermostat (Ukraine). The inoculum was standardized to 0.5 McFarland, corresponding to 1.5×10^8 CFU/mL, using a DEN-1 photometer (Grant Instruments, UK) [26].

Test Procedure 0.1 mL of bacterial suspension was aseptically applied to the surface of pre-solidified agar medium and evenly spread with a sterile spatula. Paper discs (Ø 6 mm, Liofilchem, Italy) were saturated with the corresponding samples and placed on the agar surface:

Table 1

Composition of experimental groups in the study of the antimicrobial activity of silver nanoparticles

Option	Composition	Note
KN	distilled water	negative control
KP	tetracycline 45 ppm (manufactured by Darnitsa LLC, Ukraine)	positive control
P1–P5	AgNPs (10–50 ppm)	silver nanoparticles

After incubation at 37 °C for 24 hours, the diameter of the inhibition zones was measured using a Scan 500 automatic colony counter (Interscience, France).

Determination of the minimum inhibitory concentration (MIC) The minimum inhibitory concentration of AgNPs was determined by the microdilution method in 96-well microplates (CLSI, 2020).

Nutrient broth (NB) was used; 100 μL of the medium and corresponding dilutions of AgNPs (from 100 to 0.2 ppm) were added to each well.

50 μL of a standardized bacterial suspension was added to each well. Rows 1–10 contained sequential dilutions of AgNPs, row 11—a positive control (broth + bacteria), and row 12—a negative control (broth without bacteria). The plates were incubated at 37 °C for 24 hours in a Binder BD 56 CO_2 incubator (Germany). After incubation, the optical density (OD_{600}) was determined using a Multiskan Sky microplate spectrophotometer (Thermo Fisher Scientific, USA). The minimum inhibitory concentration was defined as the lowest concentration of AgNPs at which no visible bacterial growth was observed [27].

Statistical Analysis Statistical analysis of the results was performed using GraphPad Prism 9.0 (GraphPad Software, USA) and SPSS Statistics 21.0 (IBM, USA). Data were tested for normality of distribution using the Shapiro–Wilk test. Results are presented as mean ± standard deviation (M ± SD). To assess the significance of differences between means, one-way analysis of variance (ANOVA) was used, followed by Duncan's multiple range test. A p-value of <0.05 was considered statistically significant [28].

Research results. Statistical analysis of the results revealed statistically significant differences ($P < 0.01$) in the diameter of bacterial growth inhibition zones among the different treatment options (Table 2). The use of silver nanoparticles at concentrations of 10 ppm (P1), 20 ppm (P2), 30 ppm (P3), 40 ppm (P4), and 50 ppm (P5) resulted in a significant increase in the diameter of the inhibition zone ($P < 0.01$). At the same time, the efficacy of silver nanoparticles was lower ($P < 0.01$) than that of tetracycline (45 ppm, KP) when tested against Escherichia coli and Salmonella typhimurium. The antimicrobial effect of silver nanoparticles is due to their ability to interact with the bacterial cell membrane, disrupting its integrity and functional activity. Silver has a high affinity for sulfur-containing, carbonyl, and phosphate groups, which are components of proteins and lipopolysaccharides in the bacterial cell wall. Due to electrostatic interactions between positively charged Ag^+ ions and negatively charged components of the cell surface, nanoparticles adsorb onto the membrane, leading to a change in its permeability, loss of ionic balance, and leakage of intracellular contents. A distinctive feature of Gram-negative bacteria, such as E. coli and S. typhimurium, is the presence of porins—protein channels in the outer membrane through which silver nanoparticles can penetrate into the cell. Once inside the cytoplasm, the nanoparticles bind to membrane-bound enzymes, inhibit their activity by forming complexes with the sulfhydryl groups of proteins, and disrupt the functions of the respiratory chain and DNA replication. These changes lead to the inhibition of cellular metabolism, damage to nucleic acids, and, ultimately, the death of the bacterial cell.

Table 2

Inhibition zone diameter of silver nanoparticles against Gram-negative (Escherichia coli, Salmonella typhimurium) and Gram-positive (Lactobacillus acidophilus, Lactobacillus sp.) bacteria.

Medications	Gram-negative		Gram-positive	
	Escherichia coli	Salmonella typhimurium	Lactobacillus acidphopilus	Lactobacillus sp.
KN	0,00	0,00	0,00	0,00
KP	27,93	22,53	30,13	28,60
P1	18,33	7,33	0,00	0,00
P2	18,57	7,67	0,00	0,00
P3	18,93	8,59	0,00	0,00
P4	19,73	8,70	0,00	0,00
P5	20,60	9,40	0,00	0,00
P value	0,001	0,001	0,001	0,001

Note: Different superscripts in the same column indicate statistically significant differences (P<0.05). KN = negative control; KP = positive control (tetracycline 45 ppm); P6 = 10 ppm silver nanoparticles; P7 = 20 ppm silver nanoparticles; P8 = 30 ppm silver nanoparticles; P9 = 40 ppm silver nanoparticles; P10 = 50 ppm silver nanoparticles.

The results of the studies (Table 2) showed that treatment with silver nanoparticles at concentrations of 10 ppm (P1), 20 ppm (P2), 30 ppm (P3), 40 ppm (P4), and 50 ppm (P15) did not cause significant changes in the diameter of the inhibition zone ($P > 0.05$) compared to tetracycline (45 ppm, KP) against the Gram-positive bacteria Lactobacillus acidophilus and Lactobacillus sp.

The reduced sensitivity of Lactobacillus bacteria to silver nanoparticles may be attributed to a number of factors. One of these is the presence of a robust cell wall 20–80 nm thick, which acts as a barrier and limits contact between the nanoparticles and the cytoplasmic membrane. This structural feature reduces the affinity of silver nanoparticles for the bacterial surface, which, in turn, diminishes their antimicrobial effect. In addition, the metabolic activity of lactic acid bacteria, particularly fermentation processes and lactic acid production, creates a microenvironment less conducive to the action of silver nanoparticles. This may account for a reduction in their inhibitory effect on these microorganisms. At the same time, tetracycline at a concentration of 45 ppm demonstrated high efficacy in inhibiting the growth of Gram-positive bacteria. Its mechanism of action involves blocking protein synthesis at the ribosomal level, which provides both bacteriostatic and bactericidal effects. The results obtained indicate pronounced antimicrobial activity of silver nanoparticles against Gram-negative pathogens, confirming the potential for their use in veterinary medicine as an alternative antimicrobial agent.

Determination of the minimum inhibitory concentration (MIC) showed that the values for silver nanoparticles against Escherichia coli and Salmonella typhimurium ranged from 6.25 to 50 ppm. Bacterial growth was observed at concentrations below

3.125 ppm, as evidenced by an increase in turbidity and optical density (OD_{600}) at a wavelength of 600 nm within the range of 1.24–0.15. At a concentration of 6.25 ppm, the OD_{600} value was approximately 0.09, indicating nearly complete inhibition of bacterial cell growth.

Thus, it was determined that the MIC value for silver nanoparticles against both Gram-negative bacteria is 6.25 ppm, which corresponds to the concentration at which cell proliferation ceases and the optical density (OD_{600}) does not exceed 0.1. It is known that a decrease in OD_{600} below this threshold indicates effective inhibition of bacterial growth. As the concentration of silver nanoparticles increased to 50 ppm, a further reduction in the growth of E. coli and S. typhimurium was observed, confirming the high antimicrobial activity of silver against Gram-negative microorganisms. The results obtained are consistent with previously published data indicating the effectiveness of silver nanoparticles in the range of 3–7 ppm against these bacteria. It has been established that the antimicrobial action of silver nanoparticles is due to their ability to disrupt the integrity of the cell wall, penetrate the membrane, and affect DNA. This leads to the inhibition of pathogenic bacterial growth at relatively low concentrations of the preparation. The results obtained are of practical significance for veterinary medicine, particularly in poultry and livestock farming, where silver nanoparticles can be used as an alternative to antibiotics or as a feed additive with antimicrobial, anti-inflammatory, and immunostimulatory effects. The use of low concentrations (up to 4 ppm) in feed or during the treatment of hatching eggs potentially contributes to improving the immune status, digestion, and overall productivity of animals without disrupting the balance of the natural intestinal microflora.

Conclusions and future research perspectives. The results of the present study clearly demonstrate the high antimicrobial potential of silver nanoparticles (AgNPs) against pathogenic Gram-negative bacteria. Silver nanoparticles exhibited strong inhibitory activity toward *Escherichia coli* and *Salmonella typhimurium*, two clinically and economically significant pathogens in veterinary medicine and animal production systems. The minimum inhibitory concentration (MIC) for both bacterial species was identified as 6.25 ppm, at which the optical density (OD_{600}) remained below 0.1, indicating almost complete suppression of bacterial growth. The dose-dependent nature of the antimicrobial effect was further confirmed by progressively reduced bacterial proliferation at concentrations up to 50 ppm. In contrast, AgNPs showed minimal inhibitory activity against Gram-positive *Lactobacillus acidophilus* and *Lactobacillus sp.*, which are essential representatives of normal gastrointestinal microbiota in poultry and livestock. This selective antibacterial response highlights an advantageous property of silver nanoparticles—the ability to suppress harmful pathogens without significantly affecting beneficial commensal bacteria.

The mechanisms underlying the antimicrobial activity of AgNPs include several synergistic cellular disruptions: compromising the integrity of the bacterial cell wall and membrane; interacting with membrane proteins, phospholipids, and lipopolysaccharides; binding to sulfhydryl groups of key bacterial enzymes; inhibiting metabolic and respiratory pathways; promoting the generation of reactive oxygen

species (ROS); and interfering with DNA replication and transcription. This multifunctional mode of action distinguishes AgNPs from traditional antibiotics, which typically act on a single cellular target, and therefore reduces the likelihood of resistance development. These findings support the feasibility of using silver nanoparticles as an alternative antimicrobial agent in veterinary practice, either independently or as a component of feed additives. Their potential roles include suppressing pathogenic microflora, enhancing immune responses, reducing inflammation, and ultimately improving the overall productive and physiological status of animals.

Despite these promising results, several aspects require further investigation to ensure the safe and effective implementation of AgNPs in veterinary medicine and livestock production. Future research should prioritize evaluating potential long-term effects associated with the repeated or chronic administration of silver nanoparticles, particularly focusing on bioaccumulation in animal tissues, possible alterations in metabolic processes, and cytotoxicity toward host cells. Assessing oxidative stress markers, immune responses, and the integrity of vital organs following prolonged exposure will be crucial for establishing a comprehensive safety profile.

Another important area for further study is the refinement of dosage strategies, especially low-dose formulations (≤4 ppm), which may provide beneficial immunostimulatory effects while maintaining microbiome stability. Determining the threshold concentrations that enhance the growth of beneficial lactic acid bacteria without promoting dysbiosis will be essential for optimizing feed additive formulations. Moreover, advanced molecular and genomic studies are needed to elucidate the precise pathways through which AgNPs influence microbial cells and interact with probiotic species. Such insights may help identify potential synergistic effects between silver nanoparticles and conventional antimicrobial agents, thereby improving the efficacy of therapeutic interventions and reducing the required dose of antibiotics.

Furthermore, practical applications of AgNPs in commercial poultry and livestock operations warrant extensive field trials to validate laboratory findings under real production conditions. This includes evaluating their impact on growth performance, feed conversion efficiency, immune status, carcass quality, and overall animal welfare. Studies should also address environmental considerations, such as the fate of nanoparticles in manure, soil, and water systems, as well as their potential ecological impacts.

In summary, the outcomes of the current study reinforce the scientific and practical relevance of silver nanoparticles as a promising alternative to conventional antibiotics in veterinary medicine. With targeted future research aimed at understanding their safety, optimizing their application, and assessing their interactions with microbial ecosystems, AgNPs hold substantial potential to contribute to sustainable livestock production, reduce reliance on antibiotics, and improve the microbiological safety of animal-derived products.

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АНТИБАКТЕРІАЛЬНІ ВЛАСТИВОСТІ НАНОЧАСТИНОК СРІБЛА ЯК ЗАМІНИ СИНТЕТИЧНИХ АНТИБІОТИКІВ У ПТАХІВНИЦТВІ

Анотація

У цьому дослідженні досліджували антимікробні властивості наночастинок срібла (AgNPs) проти окремих грамнегативних та грампозитивних бактерій за допомогою методу дифузії в агарі Метод. AgNPs наносили в концентраціях від 10 до 50 ppm. Тетрациклін (45 ppm) служив позитивним контролем, а дистильована вода – негативним контролем. Для визначення мінімальної інгібуючої концентрації (МІК) пригнічення росту бактерій оцінювали спектрофотометрично, вимірюючи оптичну густину при 600 нм (OD_{600}). Статистичний

аналіз проводили за допомогою однофакторного дисперсійного аналізу (ANOVA) з подальшим використанням багатодіапазонного тесту Дункана, зі значущістю, встановленою при P < 0,05.

Результати продемонстрували виражений антимікробний ефект AgNPs проти грамнегативних штамів Escherichia coli та Salmonella typhimurium. Усі протестовані концентрації (10–50 ppm) значно пригнічували ріст бактерій (P < 0,01), а найнижче значення МІК було визначено як 6,25 ppm, при якому значення OD_{600} вказували на повне пригнічення проліферації клітин. На відміну від цього, AgNP не мали значного інгібуючого ефекту (P > 0,05) на ріст грампозитивних бактерій Lactobacillus acidophilus та Lactobacillus sp., що підкреслює селективний характер їхньої антимікробної активності.

Спостережувана селективність свідчить про те, що наночастинки срібла можуть ефективно впливати на патогенні грамнегативні бактерії, зберігаючи при цьому корисну молочнокислу мікрофлору. Це особливо актуально для птахівництва та ветеринарії, де підтримка збалансованої кишкової мікробіоти має вирішальне значення для здоров'я та продуктивності тварин. Крім того, сильна інгібуюча дія AgNP підтверджує їхній потенціал як природної та ефективної альтернативи звичайним синтетичним антибіотикам. Застосування AgNP у низьких, біологічно безпечних концентраціях може сприяти підвищенню біобезпеки, зниженню ризиків резистентності до антимікробних препаратів та покращенню мікробіологічної якості продуктів тваринного походження.

Загалом, отримані результати підкреслюють перспективність використання наночастинок срібла як багатофункціонального антимікробного засобу. Їх включення до кормових добавок, ветеринарних препаратів або протоколів біобезпеки може забезпечити інноваційну стратегію для зменшення використання антибіотиків, одночасно підтримуючи стале та орієнтоване на здоров'я тваринництво.

Ключові слова: *наночастинки срібла, Escherichia coli, Salmonella typhimurium, Lactobacillus acidophilus, мінімальна інгібуюча концентрація, антимікробна активність.*

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