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Extracellular Biosynthesis and Characterization of Silver Nanoparticles from Clinical *Serratia marcescens* Isolates Recovered from Urinary Tract Infections and Their Selective Antibacterial Activity Against Gram-Positive Bacteria

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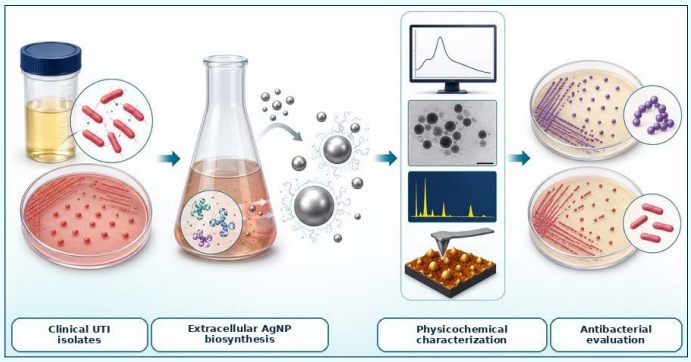
Abstract

Introduction: Green microbial synthesis of silver nanoparticles (AgNPs) is an attractive alternative to chemical methods because it is simple, cost-effective, and more environmentally friendly. However, clinically derived bacterial isolates remain insufficiently explored as biofactories for AgNP production and antibacterial testing. This study aimed to biosynthesize and characterize AgNPs using clinical *Serratia marcescens* isolates recovered from urinary tract infection patients and to evaluate their antibacterial activity against selected Gram-positive and Gram-negative bacteria.



Methods: One hundred urine samples were collected from intensive care unit patients. Serratia marcescens isolates were identified by conventional methods. Antibiotic susceptibility was assessed. AgNPs were synthesized extracellularly from bacterial supernatant and characterized by UV-Vis spectroscopy, EDX, FE-SEM, and AFM. Antibacterial activity was tested by agar well diffusion using AgNP concentrations of 125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/ml.

Results: Sixteen of 100 urine samples (16%) yielded S. marcescens. The isolates showed high resistance to co-amoxiclav (100%), cefotaxime (62.5%), and ceftriaxone (56%), while the highest sensitivity rates were observed for amikacin (100%), gentamicin (94%), and ciprofloxacin (81%). Biosynthesized AgNPs showed a surface plasmon resonance peak at 445 nm. EDX confirmed silver with an atomic weight percentage of 17.9%. FE-SEM showed predominantly spherical particles with an estimated size range of 50-100 nm, whereas AFM indicated nanoscale surface features ranging from 0.0 to 14.1 nm. In antibacterial testing, the strongest inhibition was recorded against Staphylococcus aureus at 125 µg/ml (28.33 ± 0.33 mm), followed by S. lugdunensis, S. haemolyticus, and S. epidermidis.

Conclusion: Clinical S. marcescens isolates were able to extracellularly synthesize AgNPs with confirmed nanoscale characteristics. The biosynthesized AgNPs showed selective antibacterial activity against Gram-positive bacteria. These findings support the biomedical relevance of clinically derived bacterial systems for green nanoparticle production while also highlighting the need for broader characterization and expanded biological validation.

Keywords: AgNps, *Serratia marcescens,* Urinary tract infections, UV-Vis, EDX, FE-SEM, AFM

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Introduction

The genus Serratia comprises ubiquitous Gram-negative rod-shaped bacteria belonging to the Enterobacterales order (1). Bacterial survival is increased by catheter colonization, particularly in biofilm communities. Due to its ability to block antibiotic penetration, biofilm also causes antibiotic resistance (2). Silver nanoparticles display exceptional antibacterial action, even at low concentrations, and are effective against a variety of illnesses caused by *Serratia marcescens*, including pneumonia, sepsis, wound infections, meningitis, endocarditis, and eye infections (3). They also possess economic viability and have demonstrated minimal immune response and cytotoxicity (4). Consequently, silver nanoparticles hold substantial potential for various biomedical applications (5). They find utility in areas such as molecular diagnostics, medical imaging, and drug delivery (6). Moreover, they play a role in the development of therapeutic products like surgical mesh, the fabrication of

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artificial joint replacements, wound dressings, and wound-healing-promoting medications (7).

Recent studies indicate that green nanomaterials are attracting more interest regarding their use as biocompatible antimicrobial, antiviral, anticancer, and drug-delivery carriers. A growing number of bio-nanomaterials and polymer-based bio-nanomaterials platforms are being created for antibacterial, photothermal therapy, biosensing, and controlled drug delivery. Among the biogenic nanomaterials, microbially produced AgNPs are valued in large part because they can be synthesized at relatively mild temperatures and remain surface-capping molecules that are biologically active. The antimicrobial efficacy of nanoparticles, however, is largely dependent on the choice of microorganism, synthesis conditions, and the particular microbial target, making it necessary to focus on clinically relevant bacteria as nanoparticle-producing entities (8).

While prior research has documented the biological synthesis of AgNPs by bacteria, relatively little has been studied regarding clinically-derived isolates, particularly the opportunistic pathogens from the Intensive Care Unit (ICU). More specifically, there is a paucity of knowledge surrounding whether clinically isolated, multidrug-resistant (MDR) Serratia marcescens, can act as an effective extracellular biofactory for the synthesis of AgNPs, and simultaneously offer nanoparticles with significant antibacterial activity against relevant clinical pathogens (5). The originality of this research stems from the ability to incorporate four unique elements, in a single workflow, to capture clinical S. marcescens isolates from urinary tract infection (UTI) samples, characterize antibiotic resistance patterns, extracellularly biosynthesize AgNPs and evaluate the antibacterial activity (if any) of the synthesized nanoparticles against clinically relevant strains of Gram-positive and Gram-negative bacteria. This multidisciplinary approach provides local clinical and nanobiotechnological relevance and enhances our understanding regarding the biosynthesized AgNPs selective antibacterial properties.

Another reason for the choice of Serratia marcescens as a model organism for the biosynthesis of nanoparticles is the organism's clinical significance and metabolic versatility. Clinical isolates, especially those obtained from sites of infections, are subjected to a high level of stress and may produce a range of different extracellular substances, some of which may be involved in the bioreduction of metal ions and the biological stabilization of nanoparticles. If clinically relevant bacteria incorporate metal ions as a bioreduction mechanism, then research of these isolates may determine the potential of such bacteria to be used as a biofactory for nanoparticle synthesis. Furthermore, the research of nanoparticles synthesized from pathogenic microorganisms provides an opportunity to study their applicability against clinically relevant bacteria, which is a necessity in the context of translational biomedicine.

Recent studies have focused less on the antimicrobial activity of silver nanoparticles and more on the relevant molecular and biophysical mechanisms of silver nanoparticles. The mechanisms through which silver nanoparticles disrupt bacterial membranes, such as increased permeability, and leakage of intracellular constituents cause the membranes to undergo more oxidative damage from the generation of reactive oxygen species (ROS). Proteins, lipids, and DNA are also affected by the oxidative stress caused by silver nanoparticles. Furthermore, released silver ions from nanoparticles can inhibit the activity of thiol-containing enzymes that are crucial in cell metabolism. The size, shape, and surface characteristics of nanoparticles are therefore important to the understanding of their mechanisms of antimicrobial activity (9).

The objective of this study was, therefore, to isolate and characterize clinical strains of Serratia marcescens from the urine of Intensive Care Unit patients, determine their antibiotic resistance patterns, biosynthesize extracellular silver nanoparticles, characterize the silver nanoparticles produced, and evaluate their antibacterial properties against selected Gram-negative and Gram-positive bacteria.

The study aimed to accomplish three objectives; to complete the cellular research biosynthesis of silver nanoparticles via clinical Serratia marcescens isolates, carry out the physicochemical characterization of the synthesized nanoparticles using various analytical techniques, and assess the antibacterial activity of synthesized nanoparticles against selected bacterial species. Such clearly defined objectives would result in a more precise understanding of the outcomes and the ability to correlate specific characteristics of the nanoparticles to their biological activity.

The unique feature of this research involves the synthesis of extracellular silver nanoparticles as measured against biosynthesis and selective antibacterial testing of Serratia marcescens isolates of clinical origin from a given regional location. This combines clinical patterns of antibiotic resistance and regional nanobiotechnology intersects with local epidemiological relevance and provides insights on the interactions between nanoparticles and bacteria.

Material and Methods

Study design, sample collection, and isolate identification

From September 1, 2022, to February 1, 2023, one hundred (100) urine samples were collected from patients who were admitted to the intensive care unit (ICU) for a cross-sectional laboratory study. All samples were processed under sterile conditions. For the initial identification of the bacterial isolates, the identification was based on the morphology of the colonies, Gram stain, and standard biochemical tests, and were confirmed using the Vitek 2 system. Only confirmed isolates for Serratia marcescens were considered for the nanoparticle biosynthesis stage of the study.

From the 16 confirmed isolates of Serratia marcescens, one representative isolate was chosen for biosynthesis of nanoparticles because of showing consistent growth and a positive biochemical profile.

Antibiotic susceptibility testing

The antibiotic susceptibility testing for the confirmed Serratia marcescens isolates was carried out by the method of Kirby-Bauer disk diffusion and was done on Mueller-Hinton agar as per Bauer et al. (10), and the interpretation of the results was done according to CLSI (11). The bacterial inoculum was adjusted to 0.5 McFarland standard turbidity before the inoculation. The antibiotics that were tested were amikacin, ciprofloxacin, cefotaxime, imipenem, cefepime, co-amoxiclav, ceftriaxone, gentamicin, and nitrofurantoin. Each assay was done in triplicates and the average of the inhibition was recorded.

Negative controls and a standard antibiotic were used to control for nonspecific inhibition. These controls made sure that the antibacterial effects were actually from the AgNPs synthesized and not from anything left over from the medium used to grow the bacteria or any of the bacterial metabolites.

Biosynthesis of Extracellular Silver Nanoparticles

The method for the extracellular biosynthesis of silver nanoparticles follows Song and Kim (12) with the exception of the bacterial culture supernatant modifications. Cell free culture supernatant from S. marcescens was combined with aqueous silver nitrate and incubated in the dark until the solution changed color indicating the formation of the nanoparticles. This reaction was performed at controlled laboratory conditions for temperature and time of incubation and neutral pH. The negative control, without bacterial supernatant, was used to demonstrate the absence of biological silver reduction. All experiments for nanoparticle synthesis were conducted in triplicate.

The method of extracellular synthesis was based on the reducing and stabilizing properties of the biomolecules that are released into the bacterial supernatant, and while the individual components have not been chemically identified in this study, it is assumed that the reduction of silver ions and stabilization of the formed nanoparticles were due to extracellular proteins, enzymes, and other organic metabolites secreted by Serratia marcescens. These biomolecules are likely to have acted as reducing and capping agents, thus aiding in the formation and stabilization of the nanoparticles. The presence of carbon and oxygen signals in the EDX spectra is consistent with the association of organic biomolecules with the surface of the nanoparticles.

Characterization of AgNPs

The biosynthesized AgNPs underwent characterization by means of UV-Vis spectroscopy, EDX, FE-SEM and AFM. UV-Vis spectroscopy detects the peak of surface plasmon resonance of the AgNPs while EDX determines the presence of elemental silver and surfaces bound biomolecules. The particle morphology and estimated particle size range were examined by FE-SEM while AFM evaluated the nanoscale surface topography and grain size distribution. Each of the methods explained above assess differing structural characteristics and, therefore, the obtained size values were interpreted in a comparative sense and not as strictly identical values.

Antibacterial activity assay of AgNPs

The antibacterial activity of biosynthesized AgNPs was tested using the agar well diffusion method against Gram-positive bacteria (Staphylococcus aureus, Staphylococcus lugdunensis, Staphylococcus epidermidis, and Staphylococcus haemolyticus) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa). Fresh bacterial suspensions were prepared to the 0.5 McFarland standard (approximately $1.5 \times 10\text{^}8$ CFU/ml). 0.1 ml of each of the prepared suspensions was used to evenly inoculate the Mueller-Hinton agar plates. A sterile cork borer was used to create wells with a diameter of 6 mm. AgNP suspensions were placed in the wells at the following concentrations: 125, 62.5, 31.25, 15.6, 7.8, and 3.9 µg/ml. A negative control was created using a well containing sterile distilled water or the nanoparticle-free reaction mixture. A standard antibiotic was used as a positive control to validate the assay. The diffusion was allowed to occur at room temperature for 1 hour and then the plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zone was measured (in mm). Each test was done in triplicate and results were recorded as mean ± standard error.

Statistical analysis

Statistical analysis was performed using SAS Software version 9.1. Data from the antibacterial assays were analyzed by means of two-way ANOVA to determine the effect of bacterial species, AgNP concentration, and their interaction effect. If the means were found to differ significantly, the least significant difference method (LSD) was used to compare the means. The results were considered significant for $P \leq 0.05$. The means, variability. The results from each of the three trials are reported as Mean ± Stand Error. For the two-way ANOVA, the main factors were the bacterial species and nanoparticulate concentration, and their interactions were analyzed.

Results

The examination of the *Serratia marcescens* isolate yielded affirmative outcomes for indole production, urease activity, citrate assimilation, motility, and catalase presence. Conversely, negative results were obtained for the methyl red and Voges Proskauer tests, oxidase function, and TSI reactions which displayed either alkaline/acidic or acidic/acidic characteristics, accompanied by the absence of gas or hydrogen sulfide (H2S) production.

According to antimicrobial susceptibility test results, cefotaxime (62.5%), ceftriaxone (56%), and co-amoxiclav (100%) had the highest levels of resistance.

Figure 1 showed results of antibiotics susceptibility test for *Serratia marcescens*. The full sensitivity 100% for amikacin and 0% for co-amoxiclav (full resistance). The percentages of sensitivity to other antibiotics varied as follows: gentamicin (94%), ciprofloxacin (81%), cefotaxime (38.5%) and ceftriaxone (44%).

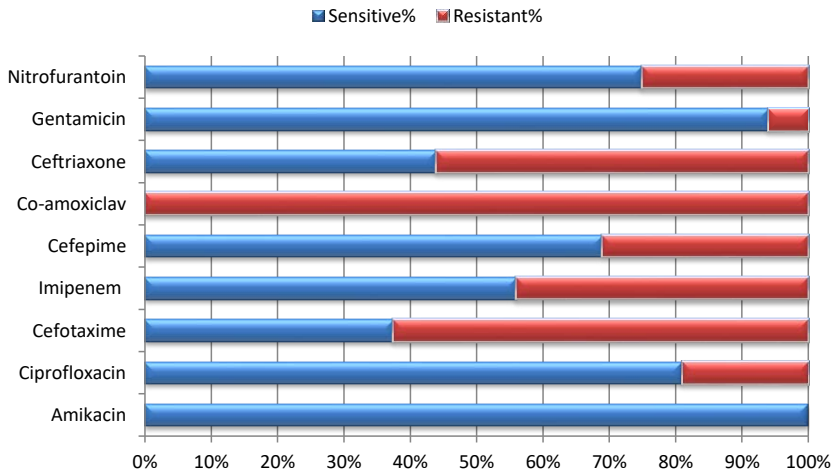

Figure 1. The proportion of Serratia marcescens antibiotic susceptibility profiles

Ultraviolet-visible (UV-Vis) spectroscopy

S. marcescens was observed through a UV spectrometer, manifesting its presence as the highest peak at a wavelength of 445 nanometers, as depicted in Figure 2.

This observation further substantiates the identification of biologically synthesized silver nanoparticles (AgNPs) resulting from bacterial activity.

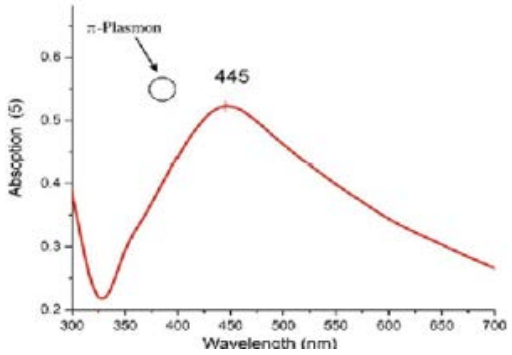

Figure 2. UV-Vis spectrophotometer analysis of silver nanoparticles

Energy-Dispersive X-Ray Analysis

The composition of the silver nanoparticles was additionally characterized through the utilization of EDX analysis. AgNPs produced by *S. marcescens* have a silver atomic weight percentage of 17.9%, according to EDX examination. Around 3 keV, a typical peak of metallic silver nanocrystallites, significant signals from the silver atoms are seen. The carbon and oxygen signals were also discovered in the EDX spectra of silver nanoparticles. Table 1 and Figure 3.

The morphology, structure, size, shape, and dispersion of the generated nanoparticles were scrutinized through the application of a field emission scanning electron microscope (FESEM). Figure 4 visually presents the outcomes of the investigation into silver nanoparticles conducted through field emission scanning electron microscopy (FE-SEM). The findings elucidate the morphology of the silver nanoparticles, with the average particle size ranging from (50-100) nm within the examined region.

Atomic Force Microscopy (AFM)

The prepared silver nanoparticles were tested by (AFM) technique for vertical analysis and the surface structure of these nanoparticles. The average grain size of produced nanoparticles was (0.0-14.1) nm, according to statistical analysis of AFM data for silver nanoparticle diameter (Figure 5).

Atomic force microscopy showed a generally homogenous nanoscale surface distribution of the synthesized AgNPs on the substrate. The smaller AFM grain-size values compared with FE-SEM size estimates are likely because AFM measures surface topography and nanoscale features rather than the complete lateral diameter of particle aggregates that are larger than the FE-SEM obscured top view of aggregates.

The FE-SEM images, while sufficient to understand the general morphology and size range of the nanoparticles, are somewhat limited as a high-resolution image would make size characterization and structural interpretation more accurate. This limitation is acknowledged, and such a limitation should be considered in the morphological analysis.

Distinct measurement principles from the two techniques explain the difference between the two values obtained from FE-SEM and AFM, where FE-SEM observes the structure in an aggregated form and AFM obtains information on surfaces at a small enough scale to be at the nanoscale. Because of this, the difference is complimentary rather than directly comparable.

Antibacterial activity test for colloidal AgNPs (in vitro)

Antibacterial efficacy of silver nanoparticles (AgNPs) was assessed against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus lugdunensis, Staphylococcus epidermidis,* and *Staphylococcus haemolyticus*) and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa)*. Colloidal AgNP*s* with different concentrations (125, 62.5, 31.25, 15.6, 7.8, 3.9) µg/ml, each concentration was introduced into a well. The inhibition zones were significantly different due to bacterial species and the concentration of AgNPs ($P<0.05$). Of the Gram-positive

Table 1. Elemental Composition and Quantitative Analysis

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	48.2	0.7	33.1	0.5
N	10.2	1.8	8.1	1.4
O	32.5	1.0	29.7	0.9
Na	1.3	0.1	1.7	0.2
Mg	1.3	0.1	1.8	0.1
Al	0.9	0.0	1.4	0.1
Ca	2.8	0.1	6.3	0.1
Ag	2.9	0.1	17.9	0.3

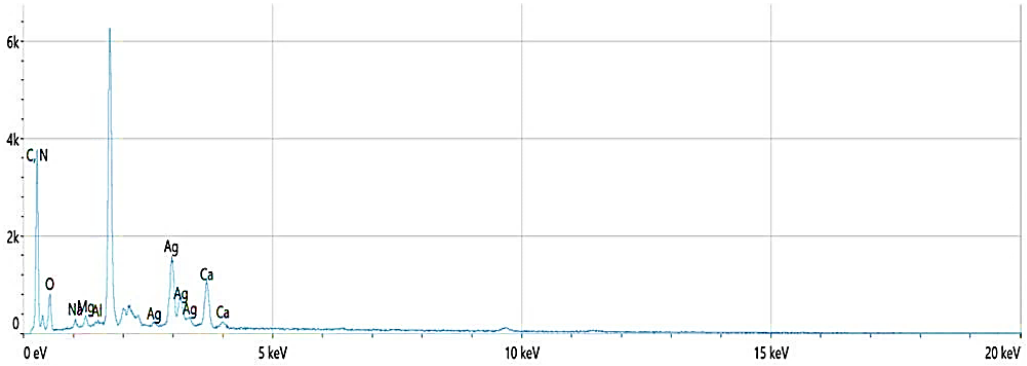

Figure 3. EDX analysis of biosynthesis AgNPs in the present study

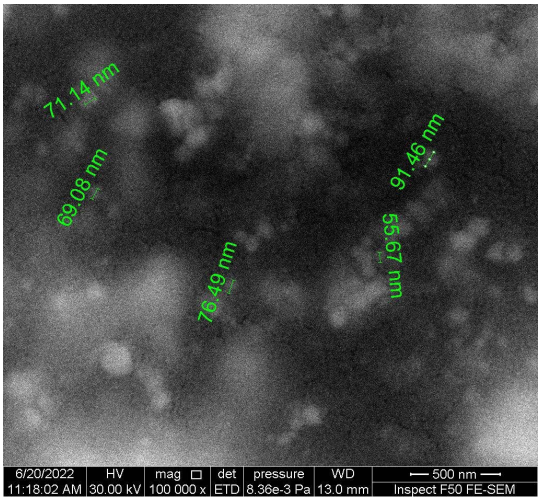

Figure 4. The characteristics of synthesized AgNPs by FE-SEM image in the present study

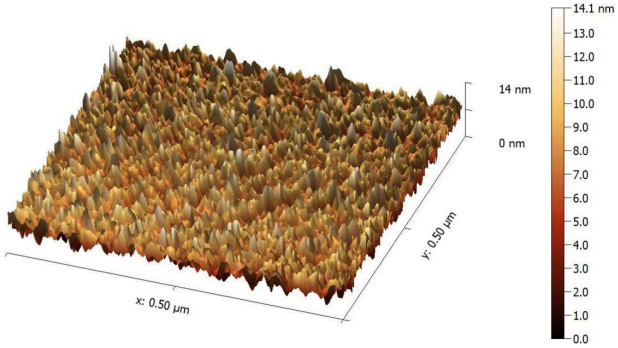

Figure 5. Characteristics of synthesized AgNPs by AFM

bacteria, the strongest activity was at Staphylococcus aureus at 125 µg/ml (28.33 ± 0.33 mm). There were no inhibition zones for Escherichia coli or Pseudomonas aeruginosa at any of the AgNPs concentrations tested (Table 2).

Means in the same column with different small letters are significantly different ($P < 0.05$).

Means in the same row with different capital letters are significantly different ($P < 0.05$).

The assessment of the antibacterial data illustrated a selective and concentration-based response of the tested Gram-positive bacteria. At the concentration of 125 µg/ml, the biggest inhibition zone was reported for Staphylococcus aureus (28.33 ± 0.33 mm) followed by Staphylococcus lugdunensis (24.00 ± 0.00 mm), Staphylococcus haemolyticus (18.00 ± 0.00 mm), and Staphylococcus epidermidis (10.00 ± 0.00 mm). At the 62.5 µg/ml concentration, inhibition was observed only for S. lugdunensis (15.00 ± 0.00 mm), S. haemolyticus (14.33 ± 0.33 mm), and S. epidermidis (8.00 ± 0.00 mm) while S. aureus had no inhibition detectable at this level. At the 31.25 µg/ml concentration, residual activity was remaining against S. lugdunensis (10.00 ± 0.00 mm) and S. hemolyticus (9.66 ± 0.33 mm) while no Gram-negative bacteria had inhibition against them at any concentration. This confirms that the biosynthesized AgNPs antibacterial effects were restricted primarily to the Gram-positive organisms targeted in the Table 2 experimental conditions.

Antibacterial activity for this study was measured against AgNPs using the standard agar well diffusion method. Although this paper features no photographs of the inhibition zones, it was noted during the experiment that inhibition zones were most prominent with Gram-positive bacteria at the highest concentration of AgNPs. Including

Table 2. Diameter inhibition zone of silver nanoparticle against various bacteria

Bacterial species	Diameter of inhibition zone (mm)				
	125 µg/ml	62.5 µg/ml	31.2 µg/ml	15 µg/ml	7.8 µg/ml
E. coli	A0.00 ± 0.00e	A0.00 ± 0.00d	A0.00 ± 0.00c	A0.00 ± 0.00a	A0.00 ± 0.00a
Pseudomonas aeruginosa	A0.00 ± 0.00e	A0.00 ± 0.00d	A0.00 ± 0.00c	A0.00 ± 0.00a	A0.00 ± 0.00a
Staph aureus	A28.33 ± 0.33a	B0.00 ± 0.00d	B0.00 ± 0.00c	B0.00 ± 0.00a	B0.00 ± 0.00a
Staph lugdunensis	A24 ± 0.00b	B15 ± 0.00a	C10 ± 0.00a	D0.00 ± 0.00a	D0.00 ± 0.00a
Staph epidermidis	A10 ± 0.00d	B8 ± 0.00c	C0.00 ± 0.00c	C0.00 ± 0.00a	C0.00 ± 0.00a
Staph haemolyticus	A18 ± 0.00c	B14.33 ± 0.33b	C9.66 ± 0.33b	D0.00 ± 0.00a	D0.00 ± 0.00a
LSD	0.29				

photographs of the inhibition zones would be helpful in future papers.

Discussion

The findings of identification of *Serratia marcescens* and its antibiotic susceptibility are in agreement with a study by Muslem et al. who indicated the sensitivity of *S. marcescens* isolates to ciprofloxacin, amikacin and gentamicin (13). Other studies indicated an increase in resistance to antibiotics in *S. marcescens* and more than one antibiotic should be taken to treat its infection in most situations. So, scientists recommended that antibiotic usage be maintained under medical supervision and guidance and administered in moderate dosages for the appropriate duration of time (2).

The multidrug resistance profile displayed by clinical Serratia marcescens isolates emphasizes the need for alternative antimicrobial strategies. In this study, biosynthesized AgNPs showed no inhibitory activity towards the tested Gram-negative multidrug resistant isolates, suggesting that, under the conditions tested, exposure to AgNPs was insufficient to overcome the protective resistance-associated barriers. This suggests that the antibacterial activity of AgNPs cannot be attributed solely to the presence of resistance mechanisms to antibiotics, but also to other structural and physiological characteristics such as outer membrane permeability, efflux pump activity, and biofilm-associated tolerance. Thus, the present study suggests that while AgNPs may have selective antibacterial activity, their use should not be taken to mean that they can be used to overcome multidrug resistance in all bacterial populations (14).

Silver nanoparticles have antibacterial properties through a number of ways. First, they attach to surfaces of bacteria cells, creating small holes in the bacterial cell wall which cause the bacterial cell interior to leak out. Also, AgNPs cause the production of reactive oxygen species (ROS) which cause oxidative stress and damage to proteins, cell membranes, and DNA. Released silver ions also precipitate the activity of thiols in enzymes which shuts down reactions and alters the entire metabolism of the bacteria.

The antibacterial activity seen in this research can be due to the difference in composition of the various groups of bacteria. Compared to Gram-negative (GN) bacteria Gram-positive (GP) bacteria have a single cell membrane and no outer membranes, thus no barrier to allow AgNPs to easily interact with and modify the cells. This in large part explains the lack of antibacterial activity and the insufficiency of the antibacterial activity seen in the Gram-negative (GN) bacteria compared to the Gram-positive (GP) bacteria.

The creation of AgNPs in this work was first validated by employing the surface plasmon resonance phenomenon, and the results of Ultraviolet-Visible (UV-Vis) spectroscopy are compatible with Zivkovic et al (15). Various investigations have proven that the resonance peak of AgNPs emerges around this area. For the first characterization of produced nanoparticles, UV-vis spectroscopy is a highly beneficial and trustworthy approach (9).

Due to the presence of carbon and oxygen, the EDX result would indicate the existence of Ag NPs capped with extract biomolecules. The results, according to the EDX, were consistent with earlier studies of the EDX spectra of silver nanoparticles (9). Additionally, the produced silver nanoparticles had excellent dispersion along the substrate surface and were mostly spherical (16).

Silver accounted for 17.9 wt% in the EDX analysis, while other elements such as carbon, oxygen, nitrogen, and trace minerals were also detected. These additional signals likely originate from biomolecular capping agents, residual medium components, or sample preparation artifacts. Therefore, the EDX result confirms the presence of silver nanoparticles but does not indicate complete chemical purity.

This interpretation also indicates that the bacterial supernatant had a double function during synthesis, assisting reduction of silver ions and the formation of an organic coating around the surface of the nanoparticles. Such capping biomolecules can enhance dispersion and minimize the rate of agglomeration of the nanoparticles, which is critical for their sustained physicochemical stability. Nonetheless, since no FTIR or protein-centric studies were conducted in the present work, the specific types of biomolecules involved will need to be addressed in future studies.

Atomic force microscopy was one of the most crucial tools for examining, altering, and imaging materials at the nano size. AgNPs' shape and size were described using it (17). Atomic force microscopy was one of the most crucial tools for examining, altering, and imaging materials at the nano size. AgNPs' shape and size were described by it (18).

The internal structure, size distribution, surface charge,

and colloidal stability of the synthesized AgNPs could be ascertained from the additional characterization techniques of dynamic light scattering (DLS), zeta potential analysis and transmission electron microscopy (TEM) which are not included in the present study due to lack of instrumentation. These techniques should be utilized in order to improve the characterization of nanoparticles in future studies. This would help to determine the physicochemical properties to a desirable level.

The study's observed antibacterial activity can be explained in part by the physicochemical properties of the synthesized nanoparticles. The way in which the size, shape, and surface characteristics of an antibacterial agent affect their interaction with bacteria is very intricate and constituent specific. Generally, smaller sized particles will have a larger surface area which can be advantageous for a stronger attachment to the bacteria's cell membrane especially when it comes to the release of an active substance. Throughout the document, heterogeneous structures aid in explaining the antibacterial activity that is selective for Gram-positive Bacteria.

Sondi and Salopek-Sondi initially reported their findings on the impact of AgNPs against Escherichia coli, highlighting the creation of "pits" in the bacterial cell wall. This led to the accumulation of AgNPs in the cellular membrane, resulting in increased permeability of the cell wall and eventual cell demise (19).

The antibacterial findings from this study indicate that the biosynthesized AgNPs had a selective effect rather than a broad-spectrum effect under the conditions studied. Clear inhibition zones were observed against the Gram-positive isolates, with Staphylococcus aureus showing the highest activity at the highest tested concentration. This concentration-dependent activity pattern is consistent with literature that shows the antibacterial effect of AgNPs is influenced by particle size and dose, surface properties of the AgNPs, and the susceptibility of the target organism. The observed activity against Staphylococcus supports the notion that AgNPs can dismantle the cell membrane, alter membrane permeability and disturb key cellular activities and processes (20-24).

On the contrary, no inhibition zones were observed against the Gram-negative isolates tested, which were Escherichia coli, and Pseudomonas aeruginosa. This result should be explicitly stated as a limitation of the antibacterial activity spectrum observed in this study. These results could be explained by the heightened intrinsic resistance often seen in these multi-drug resistant isolates, including the outer membrane protective barrier, and the potential biofilm-related tolerance which may reduce the activity of penetrating nanoparticles (14). Thus, the data indicate that the produced AgNPs were not broad-spectrum antibacterial agents against the tested bacteria, and that Gram-positive bacteria were more susceptible while Gram-negative, multi-drug resistant bacteria were less susceptible. Under the given conditions, the AgNPs were not detected to have any antibacterial activity toward the Gram-negative bacteria.

A point that is important, but was not investigated in this study, is whether the biosynthesized AgNPs can inhibit the growth of Serratia marcescens. Since the extracellular components used to create the AgNPs were derived from this specific bacterium, the producing strain might be able to withstand the effects of the nanoparticles. While this is plausible, it would need to be tested. Evaluating the antibacterial properties of AgNPs in combination with the producing organism would be beneficial with respect to understanding self-resistance and antibacterial activity of the nanoparticles, and this should be included in future studies.

While the biological pathway utilized in this research is seen as more sustainable than most chemical synthesis approaches, the AgNPs' safety must still be addressed. This research was limited to biosynthesis, characterization, and in vitro antibacterial studies, and did not assess mammalian cell cytotoxicity or the potential ecological impacts of released nanoparticles. For a biomedical application, future research is required to address toxicity, dose refinement, and assessment of nanoparticles in the bloodstream, and the potential oxidant or inflammatory effects in relevant cell cultures or animal models.

From an applied viewpoint, the particular method of extracellular biosynthesis described here may be advantageous, as it may be easier to scale compared to intracellular methods, while also not requiring the use of aggressive reducing agents. Utilization of bacterial supernatants could potentially simplify production and downstream processing. That being said, successful scale-up would still require synthetic yield, batch-to-batch consistency, storage stability of the nanoparticles, and production under cost-effective conditions to be optimized. Consequently, although the current data provides evidence of the biological method's plausibility at the lab scale, additional work is required to assess this method's gap to be feasible for use in clinical or industrial settings.

Study Limitations

The current study demonstrates some limitations that require recognition. To begin with, the recovery of only 16 Serratia marcescens isolates from 100 clinical samples limits the range of significance concerning the microbiological findings. Secondly, the methods used to characterize nanoparticles were UV-Vis, EDX, FE-SEM, and AFM. However, the addition of XRD, zeta potential, and stability assessments would yield more thorough analyses on the characterization of the AgNPs produced. Thirdly, while some differences in antibacterial activity were statistically significant, the addition of some graphical representations of error bars and P-values would perhaps, provide greater insight. Lastly, the findings of the antibacterial assay should be viewed as initial evidence of selective antibacterial activity, due to the fact that the assay was conducted in vitro and on a restricted panel of organisms, which limits the overall clinical applicability of the findings.

Conclusion

The current study illustrated that clinical Serratia

marcescens isolates obtained from samples of urinary tract infections were capable of biosynthesizing silver nanoparticles, with the process taking place in a laboratory environment. The isolates exhibited significant multidrug resistance, with the most significant resistance being exhibited in co-amoxiclav, cefotaxime, and ceftriaxone. However, amikacin, gentamicin, and ciprofloxacin exhibited the most activity. The characterization of UV-Vis, EDX, FE-SEM, and AFM confirmed that AgNPs were formed and suggested that biomolecules within the supernatant were actively reduced and capped during the formation of AgNPs.

In biological terms, produced AgNPs exhibited selective and concentration-dependent antibacterial activity, with the most potent inhibition noted against Gram-positive bacteria, particularly Staphylococcus aureus, while no inhibitory response was noted with the tested Gram-negative multidrug-resistant isolates. Thus, the current findings endorse the application of clinically derived bacterial isolates as a novel green approach for the synthesis of nanoparticles, albeit the findings do not support a claim of broad-spectrum antibacterial activity.

This work has scientific merit for the first time merging clinical microbiology with green nanobiotechnology using locally sourced isolates from Iraq. Future work is anticipated to address a wider range of isolates, incorporate more complete physicochemical characterization, including XRD and zeta potential, and assess the activity of the synthesized AgNPs against an expanded range of pathogens and strains known to form biofilms.

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Conflict of Interests

The authors declare that there are no conflicts of interest existed in this work.

Data Availability Statement

Data are available upon request from the corresponding author.

Ethical Approval

The ethics approval was granted by the College of Veterinary Medicine (No. 0815-22). Patient consent was acquired for sample collection. All clinical sample and bacterial isolate manipulations were performed in accordance to the standard microbiological safety procedures of the laboratory. Sample manipulations, bacterial culturing, and antibiotic susceptibility testing were performed using the required aseptic techniques and personal protective equipment to reduce the risk and extent of exposure and contamination. Controlled laboratory conditions were also used for the preparation and manipulation of the nanoparticles.

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