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Characterization of Biosynthesis of Silver Nanoparticles and their Antibiofilm Activity against *Enterococcus faecalis*

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ORIGINAL STUDY

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Characterization of Biosynthesis of Silver Nanoparticles and their Antibiofilm Activity against *Enterococcus faecalis*

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Abstract

Background: Due to the increasing presence of antibiotic resistance genes, treatment options for clinical infections are becoming increasingly limited.

Objectives: This study aimed to synthesize, optimize, characterize, and evaluate the bioactivity of biogenic silver nanoparticles (Ag bioNPs) against a clinical isolate of *E.faecalis*.

Materials and Methods: The formation of silver nanoparticles was confirmed under optimized conditions, with a UV-Vis absorption peak at 419 nm, and their crystal structures were verified by X-ray diffraction (XRD). Fourier transform infrared spectroscopy (FTIR) analysis demonstrated the interaction of protein molecules with the nanoparticles, and transmission electron microscopy (TEM) revealed monodisperse spherical silver nanoparticles with sizes ranging from 2 to 7.5 nm.

Results: The bacteria were capable of forming biofilms, with 37 samples exhibiting weak biofilm formation, 3 samples exhibiting moderate biofilm formation, and 10 samples exhibiting strong biofilm formation. However, adding 12.5 μg/ml of silver nanoparticles reduced bacterial growth and biofilm formation. Furthermore, increasing silver nanoparticles (AgNPs) dilution had an inhibitory effect on biofilm formation against *E.faecalis* samples.

Conclusion: This study concluded that naturally made silver nanoparticles could be a cheap, environmentally friendly, and strong alternative to traditional methods for fighting infections, especially those caused by hard-to-treat germs like *E.faecalis*.

Keywords: Enterococcus faecalis, Biosynthesis, Silver nanoparticles, FITR

1. Introduction

In recent years, nanotechnology has emerged as a transformative field in medicine, particularly in the development of novel antimicrobial agents. Among the various nanoparticles explored, silver nanoparticles (AgNPs) have garnered considerable attention due to their unique physicochemical, optical, and biological properties [1]. These properties not only enhance their antimicrobial efficacy but also enable their application across a wide range of biomedical and environmental fields. AgNPs possess broad-spectrum antibacterial, antifungal, antiviral, and even anticancer activities [2], making them valuable tools in addressing multidrug resistance (MDR) and persistent infections. The nanoscale dimensions of AgNPs result in an increased surface area-to-volume ratio, thereby improving their interaction with bacterial membranes, facilitating cellular penetration, and enhancing their reactivity with microbial components [3]. These interactions can disrupt membrane integrity, generate reactive oxygen species (ROS), inhibit respiratory enzymes, and lead to protein and DNA damage, ultimately causing bacterial cell death. These multifaceted mechanisms contribute to the effectiveness of AgNPs against both

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Scheme 1. Schematic illustration of biomass E.faecalis synthesis.

planktonic and biofilm-forming bacteria. Traditional methods for synthesizing AgNPs often involve the use of hazardous reducing agents and high energy inputs, which pose environmental and health risks [4]. In contrast, green synthesis approaches—employing plant extracts, fungi, bacteria, and other biological materials— offer eco-friendly, biocompatible, and cost-effective alternatives. These biological agents serve as both reducing and capping agents, thereby enhancing nanoparticle stability, reducing toxicity, and improving interaction with biological targets [5, 6]. A major concern in clinical microbiology today is the increasing prevalence of biofilm-associated infections. Biofilms are complex communities of bacteria embedded in a self-produced extracellular matrix composed of proteins, polysaccharides, and nucleic acids. This matrix not only facilitates adherence to surfaces but also significantly enhances bacterial resistance to antibiotics and host immune defenses [4]. Biofilm formation is particularly problematic in nosocomial settings, contributing to chronic infections and treatment failures. Among biofilm-forming pathogens, *E.faecalis* holds a significant place due to its role in healthcare-associated infections such as endocarditis, urinary tract infections, and failed root canal treatments [7]. The *E.faecalis* may also produce virulence factors that contribute to their pathogenicity and toxicity. It can accumulate multiple genetic elements encoding virulence factors and antibiotic resistance genes, as well as biofilm which play an important role in infection [8].Several studies have demonstrated that AgNPs possess potent antibiofilm activity against *E.faecalis* and other clinically relevant pathogens. Their antibiofilm effects are thought to arise from a combination of membrane damage, interference with quorum sensing, inhibition of biofilm gene expression, and degradation of the extracellular polymeric substance (EPS) matrix [9, 10]. Furthermore, the use of biologically synthesized AgNPs, especially those synthesized using bacterial biomass, has shown promise in improving nanoparticle penetration into biofilms and minimizing cytotoxic effects [11]. This research might provide new treatments that able to offensive biofilms in ways that regular antibiotics can't. This would be an important tool in the battle against bacterial infections that are resistant to drugs.

2. Materials and methods

2.1. Materials

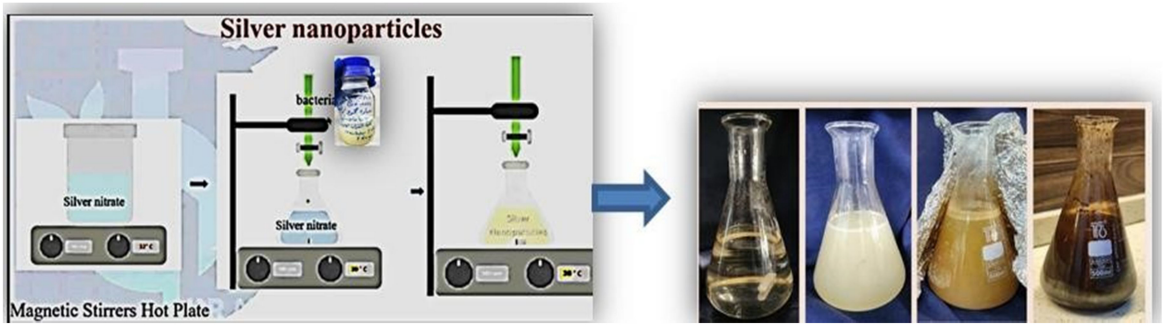
All chemicals and reagents used were of analytical grade: Silver nitrate ($AgNO_3$ ≥99.5%), Ethanol (70%), Brain Heart Infusion Broth (BHIb) and Agar (BHIA), Normal saline (0.9% NaCl), Glucose (1%), Phosphate-buffered saline (PBS), Crystal violet (1%), Glacial acetic acid (33%), Distilled deionized water (DDW).

2.1.1. Biomass preparation from E.faecalis bacteria

The *E.faecalis* was cultured on BHIA agar and incubated at 37°C for 24 hr. The harvested biomass was suspended in 100 mL of deionized distil water (DDT) and incubated in a shaker at 37°C for 3 days. Then the mixtures was sonicated for 3 minutes using an ultrasonic probe device (Fisher Scientific), the mixture then filtered through a 0.22 μm filter, Finally, the mixtures was stored for nanoparticle synthesis (step-1) as in Scheme 1.

2.1.2. Ag bioNPs synthesis procedure

The 0.72 g of silver nitrate was dissolved in 450 ml of de-ionized distilled water (DDW) inside the conical flask, the mixtures was fixed on Magnetic hotplate stirrer instrument without using heating until



Scheme 2. Schematic illustration steps of Ag bioNPs synthesis.

Scheme 3. Schematic illustration of stages of manufacturing biofilm synthesis.

the dissolving of all components within 30 minutes (step-2).The biomass production extracts (step-1) were dropped onto Ag bioNPs solution inside the conical flask (Step-2) for 1hr under heating until it reaching at 80°C in darkness conditions. The appearance of light yellowish-brown color which indicting the production of Ag bioNPs (step-3). After that, the precipitation washing 3 times with ethanol (70%) and 3 times with deionized water, and cooled using a cooling centrifuge at 20000 rpm for 5 min. The solution of Ag bioNPs was dried using oven at 80°C until it dries completely. Finally, the dried molecules were collected and preserved for more characterization procedure [10]. as arranged in Scheme 2.

2.2. *Antibiofilm activity assay of Ag bioNPs*

The antibiofilm activity was also performed using the 96-well plate. Each well loaded with 100 μL brain heart infusion broth with 1% glucose, and 100 μL Ag bioNPs with starting (25.6 μg /mL) dilution was loaded in the first well of Ag bioNPs and from this well the dilution process was started until 0.05 μg mL−1 dilution. After that, 10 μL of inoculum was added to each well. In the same way, in the next rows, samples containing the Ag bioNPs and were also prepared. Then, the microplate was incubated at 37°C for 24 hr. At the end of the incubation time, the wells were emptied, rinsed three times with PBS (phosphate buffer saline), and dried at 37°C for 24 hr. In the next step, wells were stained with crystal violet dye (0.1%) for 15 min. The wells were rinsed three times with PBS solution. After that, glacial acetic acid was added for 10 min for resuspension of biofilm. The intensity of the colored suspension was assessed by measuring the absorbance at 630 nm. The process is repeated for each *E.faecalis* strain 3 times [12]. as arranged in in Scheme 3. The reduction of biofilm is typically calculated by comparing the amount of biofilm formed in the treated group to a control group, using a formula like: % Inhibition = (ODcontrol - ODsample) / ODcontrol * 100, where OD represents optical density [12].

2.3. *Characterization of bacteriogenic Ag bioNPs*

Ultraviolet-visible (UV-vis) spectroscopy (CECL/English) was employed to confirm the biosynthesis of the AgNPs, which was done in the Ministry of Industry and Mineral / Ibn

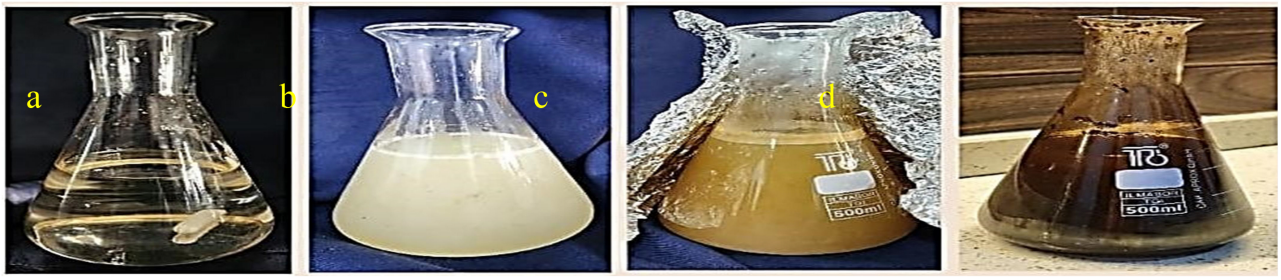


Fig. 1. Color change scenario of silver nanoparticle formation: [1] silver nitrate solution; [2] silver nitrate solution after putting in the stirrer devices 30 min colorless at the beginning; [3] light-orange (yellowish) color after 30 min after mixing with suspension of E.faecalis bacteria without heating; [4] brown color with some black sediment after 1 hr under heating at 80°C was a signal for the production of Ag bioNPs.

AL-Bittar research center. Fourier-Transform Infrared Spectroscopy (FTIR) (BRUKER/Germany), which was done in the Ministry of Industry and Mineral / Ibn AL-Bittar research center. FTIR showed the presence of functional groups indicating protein capping. Transmission Electron Microscope (TEM) (Zeiss-EM10C-100 KV/ Germany) revealed spherical particles sized 2-7.5 nm. done in the BPC Analysis Center.) X-ray Diffraction (XRD/Model PW1730 Philips, Netherland). The XRD confirmed crystallinity with peaks at 38.20°, 44.31°, 64.39°, and 97.90° for silver planes. done in BPC Analysis Center. Energy-Dispersive X-Ray spectroscopy (EDX, Bruker, Germany), and Field Emission Scanning Electron Microscopy (FESEM-MIR III/Czech) were at the BPC Scientific Research Center [13].

2.4. Statistical analysis

The Statistical Analysis System program (2021 version) was employed to deduce the effects of various components on the study's parameters. The chi-square test and FEP method were employed to assess the significant fractions. The level of significance was chosen as 0.05.

3. Results and discussion

3.1. Synthesis of biogenic silver nanoparticles by UV−Visible and FTIR spectroscopies

First, the study showed that silver nanoparticles can be made using *E.faecalis*, a type of bacteria. Color change from light yellow to dark brown (Fig. 1a-d). confirmed Ag+ reduction via biosynthesis [14–16]. The optical properties of UV−vis spectroscopy using Ag bioNPs solution analyzed nanoparticles. UV-Vis Spectroscopy confirmed SPR peak at 419 nm (Fig. 2B).

The FTIR analysis measurement of the Ag bioNPs synthesized by *E.faecalis* extracts showed stabilization and reduction of metal nanoparticles and a band at 3514.30, 3491.16 and 3452.58/ cm indicated bending N-H stretch. The band at 1747.51/ cm can assigned to be C=O stretching vibration of esters while bands 1627.92/ cm-1, indicated N-H bend while peak at 1512.19/cm showed C=C which indicated the formation of aromatic ring and alkene. While the peak at1384.89/cm revealed the formation of CH stretching. The analysis of FTIR provided evidence of protein coat on the stabilized and steady Ag bioNPs (Fig. 2D).This implicit that proteins of the *E.faecalis* extract has stronger affinity to bind with Ag+ions and thus could act as stabilizing and capping agents thereby decreasing the assemblage of NPs [17].

3.2. X-Ray diffraction (XRD) of Ag bioNPs

The green synthesis of Ag bioNPs was further supported by X-ray diffraction (XRD). In (Fig. 2-A) recorded four obvious diffraction peaks at values 38.20, 44.31, 64.39 and 97.90 for Ag bioNPs which were corresponded to 111, 200, 220 and 400 planes of silver. The XRD patterns indicated that the structure of bio-Ag bioNPs produced was spherical in shape. XRD pattern clearly showed that the Ag bioNPs formed by the reduction of Ag+ ions using *E.faecalis* extracts are crystalline in nature (Fig. 2A'). The production of silver nanoparticles of a larger size is evident from the observation that their diffraction profiles are broadened in comparison to those of bulk silver [17].

3.3. Transmission electron microscope TEM of Ag bioNPs

The TEM evaluated the size and morphology of the prepared Ag bioNPs. Transmission electron microscopy (TEM) revealed monodisperse spherical silver nanoparticles with sizes ranging from 2 to 7.5 nm (Fig. 2C). It was shown that the particle size influences the antibacterial activity of silver. As the size decreases, the toxicity markedly increases [18–20].

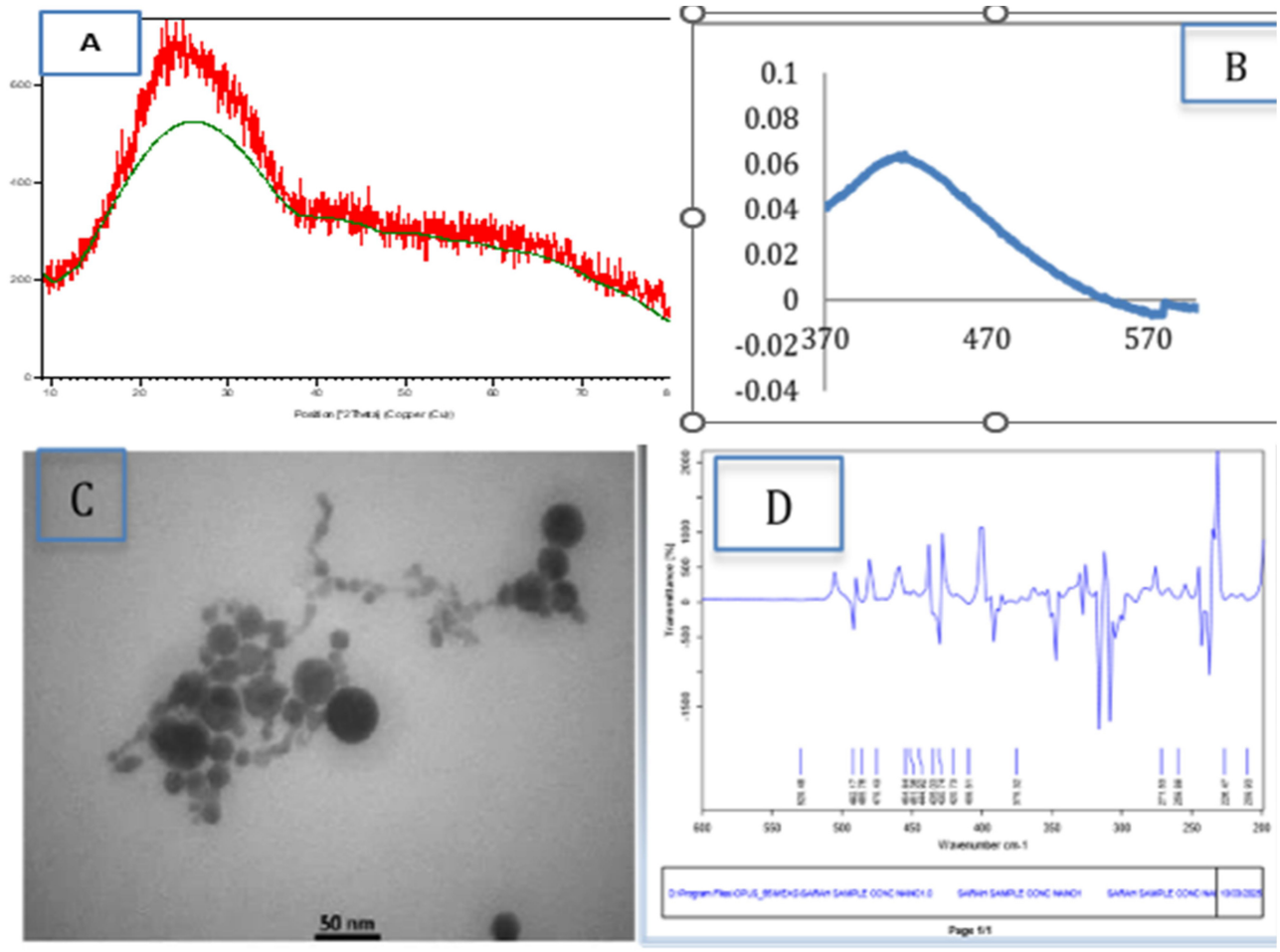


Fig. 2. From A, B, C and D about characterization for the production of Ag bioNPs. A: XRD pattern, B: UV-vis absorption peak at 419 nm,C: TEM and D: FTIR.

3.4. Data analysis using a field-emission scanning electron microscope and an energy-dispersive X-ray spectrometer

Silver nanoparticles of varying sizes and shapes were examined using FESEM to learn more about nano structured materials. Silver nano particles produced using the biogenic *E.faecalis* are illustrated in (Fig. 3a). From the FESEM images, the sample grain sizes were estimated to be 5 nm. The results demonstrated the wide range of sizes and shapes of the silver nanoparticles. In comparison to pure silver. It is also revealed that the overall crystal form is spherical in shape. The (Fig. 3b) shows the EDX spectra of the produced Ag nanoparticles. The sample was analyzed for its elemental composition using an EDX spectrometer attached to a field emission scanning electron microscope. The elements present in the produced Ag nanoparticles were quantified by EDX analysis (Table 1), which revealed 45.04% for Ag, 30.16% for oxygen, 14.99% for carbon, 1.31% for Cl and 4.39% for Zn [17, 21].

3.5. Antibiofilm activity assay

The results of the current study observed the anti-biofilm action of biogenic silver nanoparticles synthesized from clinically isolated *E. faecalis* bacteria and directed against the same strains. The results documented there was a reduction in the percentages of growth with decreasing dilution of biogenic silver nanoparticles. This was noted in the determination of the minimum inhibitory concentration (MIC), i.e., The lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic prevents bacterial growth from 25.6 (μg mL)% dilution to 0.8 (μg mL) % dilution among all the weak, moderate biofilm producing *E. faecalis* isolates with less action against strong biofilm producing isolates, statistically these differences were non-significant difference between each dilution with

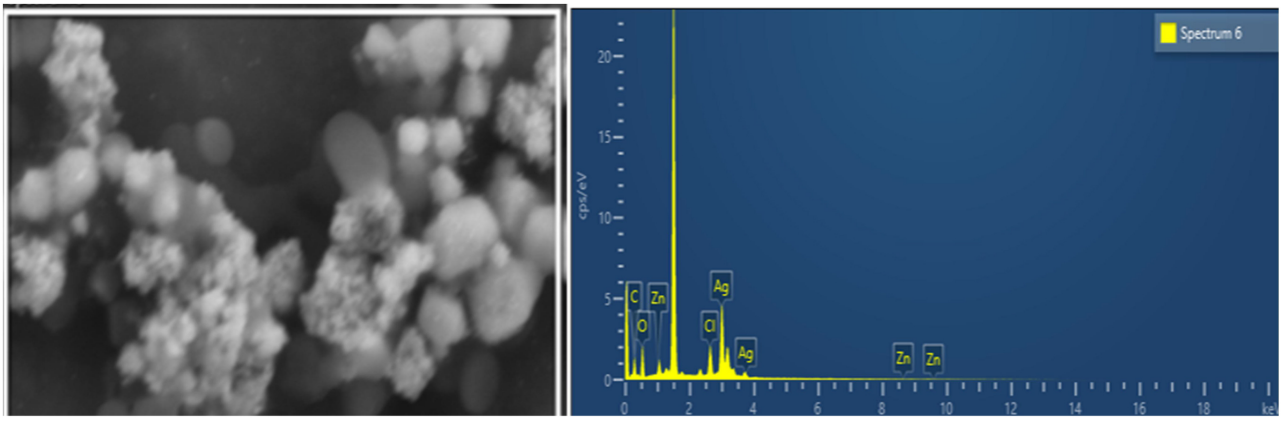


Fig. 3. (a) FESEM image of the biogenic Ag nanoparticles. (b) EDX spectrum pattern.

Table 1. Analysis of element of Ag nanoparticles using EDX.

Element	Line Type	Weight%	Atomic%
Ag	L-series	45.04	11.07
O	K-series	30.16	49.99
C	K-series	14.99	33.09
Cl	K-series	5.43	4.06
Zn	L-series	4.39	1.78

Table 2. Inhibition growth (%) after treatment with different concentration of silver nanoparticles against E.faecalis bacteria.

	(%) of inhibition growth-MIC (μg mL)					
Biofilm score	25.6	12.8	6.4	3.2	1.6	0.8
Weak (n = 37)	63.52 ± 9.54	58.17 ± 9.09	53.35 ± 10.17	47.37 ± 11.54	34.37 ± 13.19	18.83 ± 12.41
Moderate (n = 3)	66.86 ± 9.86	55.02 ± 8.31	50.12 ± 9.43	41.70 ± 6.67	34.141 ± 10.94	19.14 ± 11.82
Strong (N = 10)	60.03 ± 7.99	55.27 ± 8.12	50.07 ± 8.56	44.013 ± 10.07	31.831 ± 10.10	17 ± 3.22
P-value	0.43	0.57	0.59	0.41	0.9	0.93

scores of biofilms (P-value ≥0.05) as explained in Table 2.

Due to their difficulty in piercing a biofilm network, antimicrobial medicines are not as efficient in treating infections linked to biofilms. The application of nanoparticles for the suppression of biofilm is effective in resolving this issue. The mechanism of anti-biofilm action is believed to involve the generation of reactive oxygen species (ROS), which damage cellular components, and the penetration of nanoparticles into the extracellular polymeric substance (EPS), leading to degradation of the biofilm matrix. Furthermore, AgNPs may interfere with these findings suggest that biosynthesized silver nanoparticles could serve as a promising anti-biofilm agent against *E.faecalis* [4].

4. Conclusion

In summery; this study showed that silver nanoparticles can be made using *E.faecalis*, a type of bacteria. The bacteria helped to both create and keep the silver nanoparticles stable. Using tiny living organisms to make nanoparticles is a safe and environmentally friendly method [4]. It doesn't require harmful chemicals that are usually used in traditional ways of making nanoparticles. The made silver nanoparticles (Ag bioNPs) showed good physical and chemical properties, such as being small in size, having a crystal-like structure, and being covered with natural molecules. The antibacterial properties and especially the ability to stop biofilms of the created silver nanoparticles against *E.faecalis* was very important. *E.faecalis* is known to be a difficult germ to treat because it can resist many antibiotics and can easily create protective layers (biofilms) that help it survive in the body. The nanoparticles showed a clear decrease in the amount of biofilm as the dose increased, indicating that they could be useful for fighting biofilm. Also, future research could look into how combining silver nanoparticles with current antibiotics might work better to fight off drug resistance. Further in vivo studies and toxicity evaluations are recommended to confirm their clinical applicability and safety profile.

Ethical clearance

The Research Ethics Committee of Middle Technical University's Department of Pathological Analysis Techniques, College of Health and Medical Techniques -Baghdad, approved this study under the ethical guidelines of the Declaration of Ethical Committee of the College, Reference Number: (MEC:122) (2025P.C.:10074). Written consent was obtained from the Ministry of Health / Center of Training & Human Development before inclusion (2365 on 20-1-2025)-Baghdad Medical City.

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Conflict of interest

None.

References

1. Krishnan R, Muthuraj P, Manivasakan P, *et al.* Silver nanoparticles: Synthesis, characterization, and biomedical applications. Mater Sci Eng C. 2020;111:110713. https://doi.org/10.1515/biol-2020-0094.
2. Talapko J, Matijević T, Juzbašić M, Antolović-Požgain A, Škrlec I. Antibacterial activity of silver and its application in dentistry, cardiology and dermatology.Microorganisms. 2020;8(9). DOI: 10.3390/microorganisms8091400.
3. Khair-Allah DH, Al-Charrakh AH, Al-Dujaili NH. Antimicrobial activity of silver nanoparticles biosynthesized by *Streptomyces* spp. Annals of Tropical Medicine and Public Health. 2019;22(9):S301.
4. Karunakaran G, Sudha KG, Ali S, Cho EB. Biosynthesis of nanoparticles from various biological sources and its biomedical applications. Molecules. 2023;28(11):4527.DOI: 10.3390/molecules28114527.
5. Ahmed S, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. J Radiat Res Appl Sci. 2016;9(1):1–7. https://doi.org/10.1016/j.jrras.2015.06.006.
6. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnology advances. 2013 Mar 1;31(2):346–56. DOI: 10.1016/j.biotechadv.2013.01.003.
7. Khalil MA, Alorabi JA, Al-Otaibi LM, Ali SS, Elsilk SE. Antibiotic resistance and biofilm formation in Enterococcus spp. isolated from urinary tract infections. Pathogens. 2022 Dec 25;12(1):34. https://doi.org/10.3390/pathogens12010034.
8. Alzahrani OM, Al-Ghamdi MS, Al-Harbi AM, *et al.* Antimicrobial resistance, biofilm formation, and virulence genes in Enterococcus species from small backyard chicken flocks. Antibiotics. 2022;11(3):380.https://doi.org/10.3390/antibiotics11030380.
9. Halkai KR, Mudda JA, Shivanna V, Rathod V, Halkai R. Antibacterial efficacy of biosynthesized silver nanoparticles against Enterococcus faecalis biofilm: an: in vitro: study. Contemporary clinical dentistry. 2018 Apr 1;9(2):237–41. doi: 10.4103/ccd.ccd_828_17.
10. Fattah B, Arif H, Hamzah H. Antimicrobial and antibiofilm activity of biosynthesized silver nanoparticles against beta-lactamase-resistant Enterococcus faecalis. Appl Biochem Biotechnol. 2022;194(5):2036–46. DOI: 10.1007/s12010-022-03805-y.
11. Sánchez-López E, Gomes D, Nogueira A, *et al.* Metal-based nanoparticles as antimicrobial agents: An overview. Nanomaterials. 2020;10(2):292.DOI: 10.3390/nano10020292.
12. Abbas ZN. Comparison between phenotypic and genotypic methods in biofilm formation in MRSA isolates based on zinc oxide quantum dots. Cuestiones Fisioter. 2024;53(2):232–41. DOI: https://doi.org/10.48047/CU.
13. Chavali MS, Nikolova MP. Metal oxide nanoparticles and their applications in nanotechnology. SN Appl Sci. 2019;1:607. doi: 10.1007/s42452-019-0592-3. https://doi.org/10.1007/s42452-019-0592-3.
14. Hasan TI, Ahmed AA. Characterization, antibacterial and antibiofilm evaluation of biosynthesized silver nanoparticles from Pseudomonas aeruginosa against drug resistant Acinetobacter baumannii. Al Mustansiriyah J Pharm Sci. 2023;23(3):307–21. DOI: https://doi.org/10.32947/ajps.v23i3.1049.
15. Zare-Bidaki M, Zare-Bidaki M, Behzad M, *et al.* Photochemical synthesis of metallic silver nanoparticles using pistacia khinjuk leaves extract (pkl@agnps) and their applications as an alternative catalytic, antioxidant, antibacterial, and anticancer agents. Appl Organomet Chem. 2022;36:e6478. https://doi.org/10.1002/aoc.6478.
16. Paul B, Khan KA, Paul M. Study the impact of green synthesized silver nanoparticles on bio-voltaic cell. In: Lecture notes in electrical engineering. Cham: Springer. 2022:455–65. https://doi.org/10.1007/978-981-19-2308-1_45.
17. Ali MH, Abd-Algane M, Taki NM, *et al.* Analysis of crystallographic structures and properties of silver nanoparticles synthesized using PKL extract and nanoscale characterization techniques. ACS Omega. 2023;8(31):28133–40.https://doi.org/10.1021/acsomega.3c01261.
18. Menichetti A, Mavridi-Printezi A, Mordini D, Montalti M. Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles. J Funct Biomater. 2023;14:244. https://doi.org/10.3390/jfb14050244.
19. Angel BM, Batley GE, Jarolimek CV, Rogers NJ. The impact of size on the fate and toxicity of nanoparticulate silver in aquatic systems. Chemosphere. 2013;93:359–65. DOI: 10.1016/j.chemosphere.2013.04.096.
20. Xiu Z, Zhang Q, Puppala HL, Colvin VL, Alvarez PJJ. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett. 2012;12:4271–5. DOI: 10.1021/nl301934w.
21. Ramamoorthy RK, Lee J, Takeda H, *et al.* Ruthenium icosahedra and ultrathin platelets: The role of surface chemistry on the nanoparticle structure. Chem Mater. 2022;34:2931–44.https://doi.org/10.1021/acs.chemmater.1c03452.