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Research Article

Synthesis of Silver Nanoparticles by *Raoultella Planticola* and Their Potential Antibacterial Activity Against Multidrug-Resistant Isolates

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Background: Nanoparticles can be chemically, physically, or biologically synthesized. Biosynthesis of silver nanoparticles (AgNPs) utilizing microbes is a promising process due to the low toxicity and high stability of AgNPs. Here, AgNPs were fabricated by Gram-negative *Raoultella planticola*.

Objectives: This study aimed to assess the ability of *Raoultella planticola* to produce nanoparticles (NPs) and evaluate their antibacterial potential against multidrug-resistant pathogens (MDR). Additionally, the study aimed to compare the antibacterial activity of biosynthesized nanoparticles to well-known conventional antibiotics Azithromycin and Tetracycline.

Materials and Methods: AgNPs were characterized using visual observation, UV–visible spectroscopy (UV-vis), X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The TEM and SEM were used to determine the size and shape of the nanoparticles. The XRD data were recorded in the 2θ ranging from 20-80° to analyze the crystalline structure of nanoparticles. The antibacterial activity was detected using a 96-well microtiter plate.

Results: The UV–vis absorption recorded from the 300 – 900 nm spectrum was well defined at 420 nm, and the XRD pattern was compatible with Braggs's reflection of the silver nanocrystals. FTIR showed absorbance bands corresponding to different functional groups. TEM and SEM images showed non-uniform spherical and AgNPs of 10-80 nm. XRD data confirmed that the resultant particles are AgNPs. The AgNPs showed effective activity against multi-drug resistant (MDR) *Pseudomonas aeruginosa, Salmonella* sp., *Shigella* sp., *E. coli, Enterobacter* sp., *Staphylococcus aureus,* and *Bacillus cereus*. The AgNPs demonstrated effectiveness in lower concentrations compared to broad-spectrum antibiotics.

Conclusion: These data reveal that AgNP generated by *R. planticola* was more efficient against MDR microorganisms than commercial antibiotics. However, the cytotoxicity of these nanoparticles must be further studied.

Keywords: Antibacterial, Azithromycin, *Raoultella planticola*, Silver nanoparticles, Tetracycline

1. Background

Materials with at least one dimension within the nanometer scale (1-100 nm) are called nanoparticles (1). Due to their distinctive properties, nanoparticles (NPs) have been used in various fields. NPs can be synthesized chemically, physically, or biologically.

It has been reported that many eukaryotic and prokaryotic organisms have been used in synthesizing metallic NPs (2). Biosynthesized NPs show more significant advantages due to their cost-effectiveness, environmental friendliness, biocompatibility, and stability. Biosynthesized NPs are potential alternatives to conventional antibiotics due to the emergence of MDR bacteria. The antibacterial action of NPs comes, from direct contact with the bacterial cell wall, through which it overcomes the mechanisms of bacterial resistance (3). Furthermore, the antibacterial activity of NPs is due to their nano-size, which improves their biochemical, physical, and magnetic properties (4).

Several studies have reported that AgNPs exhibit antibacterial properties (5), with a greater focus on bio-AgNPs (6). Therefore, numerous scholars have focused on biosynthesizing AgNPs using different species of bacteria, including *Bacillus brevis* (7) and *Vibrio alginolyticus* (8). *Raoultella planticola*, a Gram-negative bacterium, has been used to fabricate silver particles. The genus *Raoultella* is facultative-anaerobic, catalase-positive, oxidase-negative bacteria and belongs to the family of *Enterobacteriaceae* that are closely related to *Klebsiella* species (9). For the first time, *R. planticola* was known as *Klebsiella planticola* in 1981 (10). *K. planticola* was renamed *R. planticola* based on sequence analysis of 16S rRNA and *rpo*B genes (11). This bacteria can degrade different organic compounds (12)ame>Mi, reducing inorganic compounds, including phosphorus and Nitrogen (13), and dissimilate and reduce iron and uranium in the presence of citrate as an electron donor (14). The biosynthesis of nanoparticles depends on microbial biomass and the growth phase. The presence of active biomass during the stationary phase of microbial growth helps to produce the highest number of nanoparticles (15). The biosynthesis of extracellular silver nanoparticles by microbes also depends on enzymes, including nitrate reductase, which helps to produce silver nanoparticles from silver nitrate (16). Nitrate reductase could be responsible for reducing Ag^{+} to Ag^{0}, where the NADH-dependent reductase is predicted to act as a carrier. At the same time, metals' bioreduction occurs by employing NADH's electrons (17).

2. Objectives

The hypothesis to be tested here was that heavy metals were tolerating *R. planticola* convert silver nitrate to silver nanoparticles extracellularly. Therefore, the current study's objective was to synthesize AgNPs via biomass of *R. planticola*. Additionally, AgNPs were characterized by UV-Vis, XRD, FTIR, TEM, and SEM. Further, AgNPs were inspected for their antigrowth action against common local MDR isolates, comparing their activity to the commonly prescribed commercial antibiotics.

3. Materials and Methods

3.1. Synthesis of AgNPs

R. planticola was isolated from the heavy metal polluted Tanjaro region, Sulaimani province of Kurdistan Region, Iraq. The isolated bacteria were diagnosed as R. planticola through manual microbiological and biochemical tests as well as VITEK 2 automated instrument (BioMerieux, USA) using a Gram-negative VITEK2 ID card. The bacterial biomass was collected by inoculating *R. planticola* into Luria-Bertani (LB) medium (10 $g.L^{-1}$ Tryptone, 10 $g.L^{-1}$ NaCl, 5 $g.L^{-1}$ Yeast Extract) (Sigma-Aldrich, Germany). The culture was incubated in a shaker incubator (Stuart, United Kingdom) at 33 °C/120 rpm for 24 hours. Then, the bacterial biomass was collected by centrifugation (Hettich, Germany) at 2000 g for 30 minutes. The supernatant was removed, and approximately 2 mg of the bacterial biomass was added to a sterilized Erlenmeyer flask that contained 100 mL of 1 mM of $AgNO_3$ solution. The mixture was dark incubated in a shaker incubator at 33 °C/120 rpm for 24 hours. Afterward, the mixture was centrifuged at 2000 g for 5 minutes to remove the bacterial biomass and any remnant debris, followed by high-speed centrifugation at 5000 g to collect the formed AgNPs. The AgNPs pellets were washed with deionized distilled water (ddH_2O) three times. Ultimately, the AgNPs were collected and air-dried [modified protocol from (17)]. AgNPs were then prepared for characterization and for conducting further experiments.

3.2. Characterization of AgNPs

AgNPs were first monitored and scanned by UV–Vis spectrophotometer (JENWAY, Hong Kong). The UV spectra were recorded from 300 to 900 nm, and ddH_2O was used as a blank. Crystalline metallic AgNPs were carried out by X-ray diffraction analysis using X'Pert PRO diffractometer (Malvern PANalytical, UK) with a Ni-FILTERED Cu Kα radiation (*l*=1.54o A in the range 10–80◦). FTIR measurements were examined by

NICOLET (iS10) spectrophotometer (ThermoFisher Scientific, USA). Morphology and size of AgNPs were investigated by utilizing high-resolution transmission electron microscopy (HR-TEM) images using (JEOL-JEM-2100, Japan) and SEM images using LEO instrument model 1455VP (ZEISS, Germany).

3.3. Antibacterial Activity of AgNPs

The antibacterial activity was performed against MDR local isolates. *Pseudomonas aeruginosa, Salmonella* sp*., Shigella* sp*., E. coli, Enterobacter* sp*., Klebsiella pneumoniae, Staphylococcus aureus,* and *Bacillus cereus* were obtained from hospitals in the Sulaimani province of the Kurdistan region of Iraq. Using VITEK-2 compact (Biomerieux, USA), the identification and antibacterial susceptibility were performed for isolates using the identification (ID) and antibacterial Susceptibility Testing (AST) cards, respectively. Identified strains showed resistance to most antibiotics. The antibacterial activity of the AgNPs was detected using 96-well (U-shape, 200 µL) microtiter plate method [modified procedure from (18)]. Each well contained 150 µL of Mueller Hinton broth, different concentrations of freshly prepared AgNPs (0, 2.5, 5, 7.5, 10, 20, 30, and 40 ppm), and 50 µL of culture broth of MDR isolated with 1×10^9 colony-forming unit per mL ($CFU.mL^{-1}$). Finally, the growth rate of MDR isolation was determined using a microplate reader (ELx808™, BioTek, USA) by measuring the absorbance at 600 nm. Broad-spectrum tetracycline and azithromycin antibiotics were used as controls (Sigma-Aldrich, Germany).

3.4. Statistical Analysis

Microsoft Excel (Microsoft 2016) was used for all the statistical computations. Four independent measurements of each antibacterial experiment of biosynthesized silver nanoparticles and antibiotics (Tetracycline and Azithromycin) were pooled and subjected to statistical analysis.

4. Results

4.1. Synthesis and Characterization of AgNPs

In the present study, *Raoultella planticola* was used to synthesize AgNPs. *R. planticola* was a gram-negative, rod shape, oxidase-negative, catalase-positive, and colorless nonmotile bacterium (**Supplementary Table 1**). Additionally, the VITEK 2 report confirmed that the species belongs to *R. planticola* (Supplementary report 1). The synthesis of AgNPs by *R. planticola* was confirmed through visual observation of the color change of the culture medium. It changed from light yellow to a brown color after incubating bacterial biomass supplemented with one mM $AgNO_3$ at 33 °C for 24 hours (**Fig. 1A**). In this study, AgNPs were characterized using UV–visible spectra recorded from 300 – 900 nm. The absorption spectrum was well defined at 420 nm (**Fig. 1A**).

Figure 1B illustrates the XRD pattern of AgNPs that showed four peaks at 2θ values of 38.23, 46.38, 64.48, and 78.03, which were allocated to diffraction planes of (111), (200), (220), and (311), respectively. FTIR analysis was used to characterize the functional groups within the biomolecules associated with AgNPs that accounted for the reduction of silver ions (**Fig. 1C**). Absorbance bands were seen at 3547 and 3413 cm^{-1}, corresponding to O-H stretching vibrations. The band at 1636 cm^{-1} corresponded to amide C=O stretching, and the peak at 1617 cm^{-1} was due to the aromatic C-C stretching of the phenyl group. SEM and TEM examined the morphology and size distribution of AgNPs. The TEM and SEM images demonstrated that the shapes of nanoparticles were spherical, roughly spherical, and cubic, with non-uniform sizes ranging between 10-80 nm (**Fig. 2A and 2B**).

4.2. Antibacterial Activity of AgNPs

In this study, AgNPs were synthesized using a biological system, and their antibacterial efficacy was investigated against different local Gram-negative and positive multidrug-resistant bacterial pathogens. Results demonstrated that bio-AgNPs have antibacterial activity against all MDR pathogens and showed resistance to other antibiotics classes (**Supplementary Table 2**). **Figure 3** revealed that Gram-negative bacteria were more susceptible to biosynthesized AgNPs than Gram-positive isolates. Moreover, the minimum inhibitory concentration (MIC) for most MDR isolates started from 7.5 ppm of AgNPs, which was significantly lower than those of commercial antibiotics for *Pseudomonas aeruginosa, Salmonella* sp*., E. coli, Enterobacter* sp*., Klebsiella pneumoniae* with p-values of 0.000, 0.000, 0.000, 0.000, and 0.001 respectively. The minimum bactericidal concentration (MBC) of AgNPs was 10 ppm. However, the MIC against *Shigella* started from

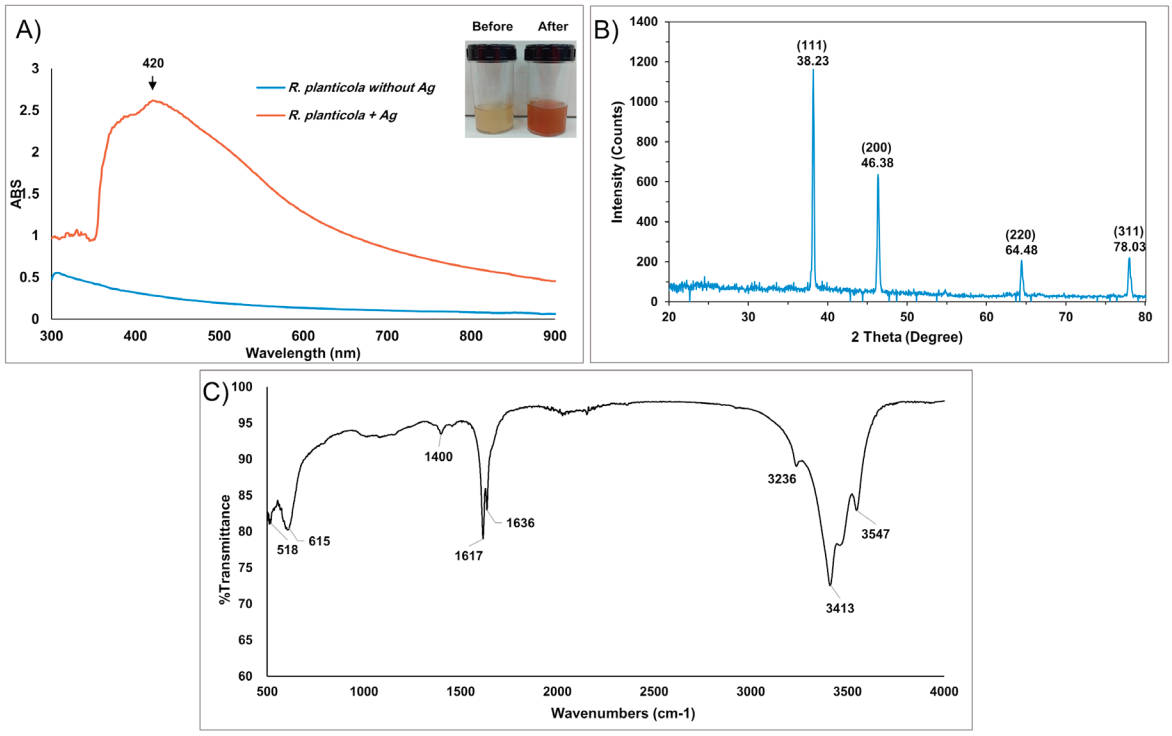

Figure 1. Characterization of biosynthesized AgNPs. **A)** UV-visible spectra showing a peak at 420 nm, **B)** X-ray diffraction (XRD) pattern showing four different peaks at 2θ values, **C)** Fourier-transform infrared spectroscopy (FTIR) of absorbing bands of functional groups

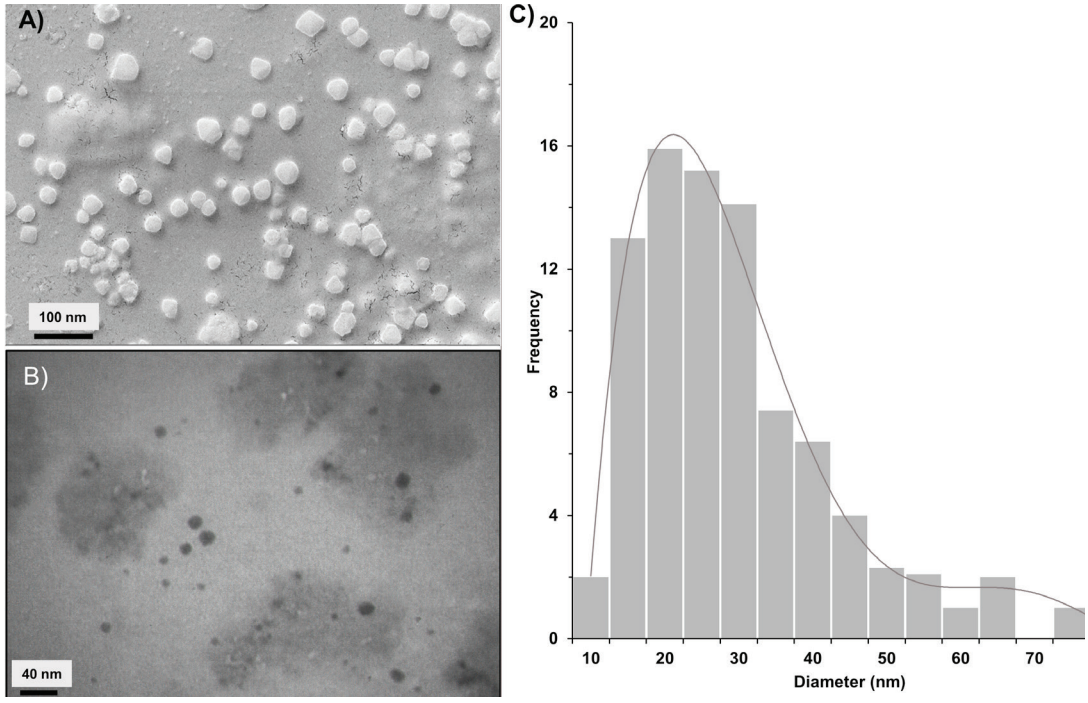

Figure 2. Morphology and size of biosynthesized AgNPs. **A)** Scanning electron microscope, **B)** Transmission electron microscope showed different nanoparticle shapes, **C)** Size of biosynthesized AgNPs between 10-80 nm

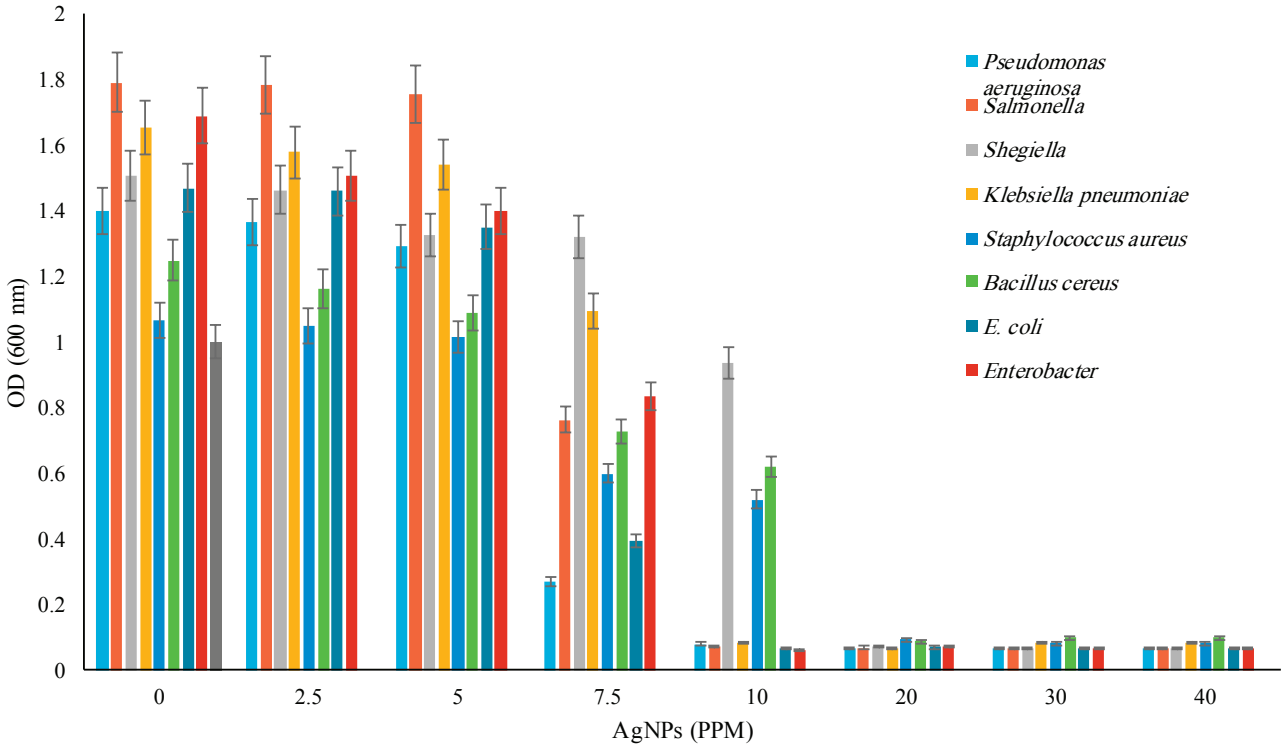

Figure 3. The antibacterial activity of AgNPs against multidrug-resistant bacterial isolates illustrated the MIC and MBC of nanoparticles in ppm against bacterial isolates. (OD: Optical Density)

10 ppm of AgNPs, while its MBC started from 20 ppm. Likewise, the MBC of AgNPs for Gram-positive MDR *Staphylococcus aureus* and *Bacillus cereus* started from 20 ppm.

The MIC for the MDR, as mentioned earlier with Tetracycline, was 10 ppm, excluding *Salmonella*, whose MIC started at 20 ppm. However, the MBC of Tetracycline for almost all MDR was 20 ppm, except for, *Salmonella,* which was more than 50 ppm (**Fig. 4**).

On the other hand, The MIC of Azithromycin against MDR isolates was 10 ppm, excluding *Salmonella* and *K.pneumonia* with MICs of 20 and 30 ppm, respectively. Nevertheless, the MBC of Azithromycin for MDR bacteria was different; the MBC of *Shigella, S. aureus, B. cereus,* and *E. coli* was 20 ppm, whereas it was 30 and 50 ppm for *P. aeruginosa* and *Enterobacter*, respectively. The MBC of Azithromycin for *Salmonella* and *K. pneumonia* was more than 50 ppm (**Fig. 5**).

5. Discussion

Scientists and researchers aim to develop and fabricate AgNPs with a broad spectrum of action against pathogenies. Several studies have reported that biosynthesized AgNPs are cheaper, safer, and could be an alternative to commercial antibiotics (19). The present paper is the first study, to our knowledge, investigating the use of *Raoultella planticola* as a biocatalyst for the synthesis of AgNPs. The results indicate that *R. planticola* can potentially optimally reduce $AgNO_3$ to AgNPs at 33 °C. This optimal *R. planticola* cell growth has recently been reported (20). Moreover, the brown color was developed in a few hours due to the synthesis of AgNPs. The developed color was due to the extracellular synthesis of AgNPs (21) and the excitation of the AgNPs' surface plasmon resonance (22).

AgNPs absorbed light of various wavelengths and were stimulated by charge density at the conductor-insulator interface, resulting in a peak at 420 nm, which was compatible with a previously conducted study (23). The XRD technique was used to confirm the exact nature of the biosynthesized AgNPs. As mentioned above, the XRD pattern exhibits various unique peaks at 2θ values. All the reflection planes matched and were

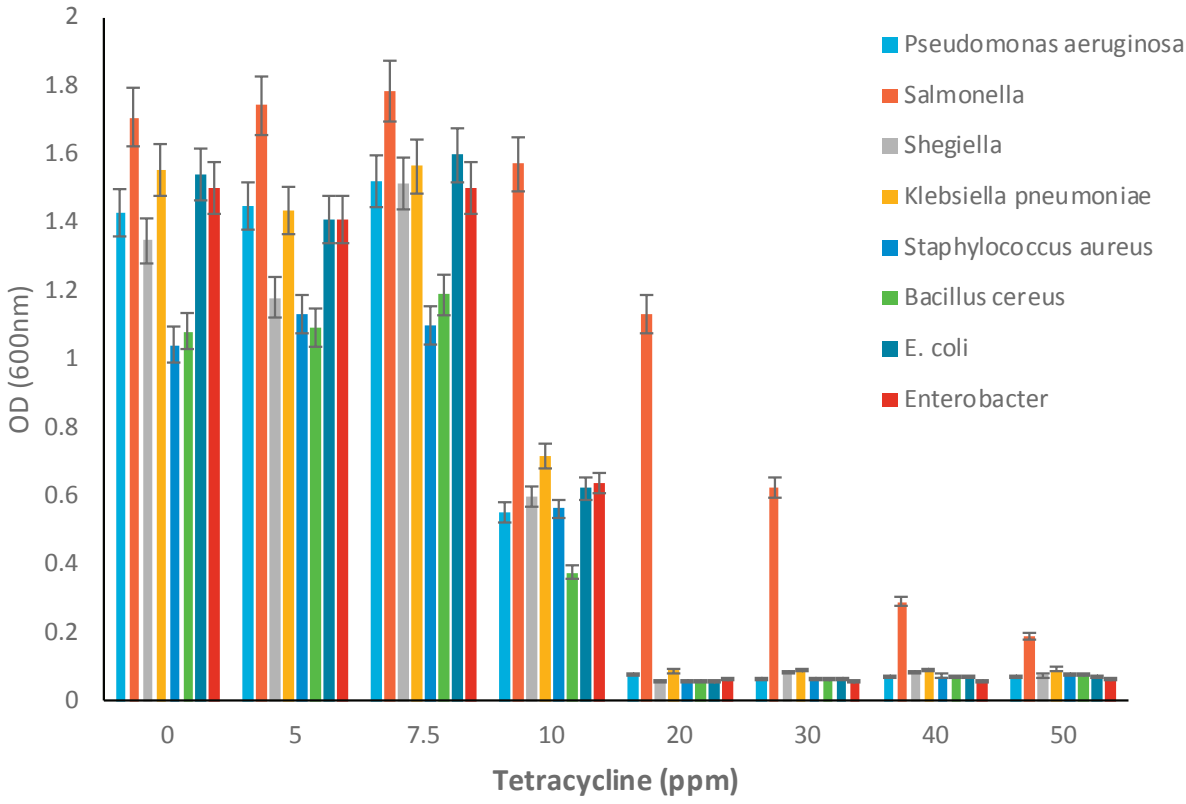

Figure 4. Antibacterial activity of Tetracycline against multidrug-resistant isolates demonstrated the MIC and MBC of the antibiotic in ppm against bacterial isolates. (OD: Optical Density)

consistent with the face-centered cubic phase of the pure crystalline silver structure in the Joint Committee on Powder Diffraction Standards (JCPDS) database. This pattern was compatible with Braggs's reflection of the silver nanocrystals. Our findings are similar to a previous study about AgNPs characterization by XRD (24). FTIR analysis was performed to discover the putative biomolecules responsible for stabilizing silver nanoparticles. Carbonyl, amide, and amino groups tend to bind to metal particles. According to a prior study, this binding aids in coating metal nanoparticles, ensuring their stability and agglomeration (25). Amides and other functional groups in the extract may affect AgNPs' interaction with peptides or carbohydrates. It has been reported that carboxyl and hydroxyl groups from a *Chlorella Vulgaris* extract were implicated in AgNP production (26). The size and shape of AgNPs were determined by TEM with an average size of 10-80 nm. Similar spherical, roughly spherical, and cubic-shaped silver nanoparticles have been obtained by a previous study with non-uniform sizes ranging between 10-100 nm (27).

The antibacterial potencies of bio-AgNPs are well known against various microbial pathogens. Our results showed that Gram-negative bacteria are more susceptible to AgNPs than Gram-positive isolates, possibly due to cell wall composition differences. Previous scholars showed a wide range of antibacterial activity of AgNPs against *P. aeruginosa,* starting from 1.5 to 400 ppm(28, 29). Foroohimanjili *et al*. (2020) reported that the biosynthesized AgNPs had MIC values between 6.25 and 100 µg.mL against *K. pneumoniae* (30). Previously, researchers reported the antibacterial activity of AgNPs against *Salmonella* sp. and *Shigella* sp. in the range of 8-16 ppm (31). However, the antibacterial activity of AgNPs against *E. coli* was between 6.25 to 100 ppm (32, 33). In addition, biosynthesized AgNPs against gram-negative *Enterobacter* sp. with MIC at 20 ppm and MBC at 40 ppm were reported by Gopinath *et al*. (2015) (34). Previous studies reported a range of different concentrations of antibacterial activity of AgNPs against gram-positive *Staphylococcus aureus*

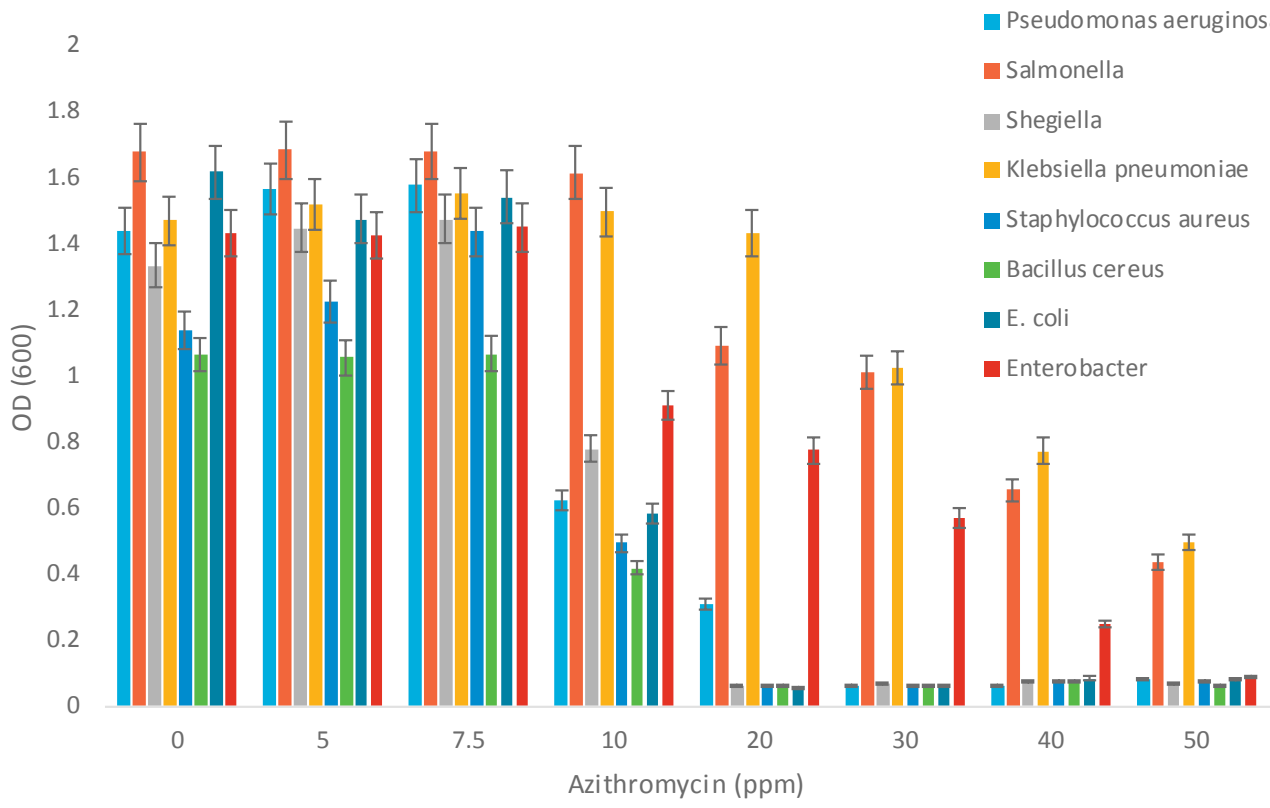

Figure 5. Antibacterial activity of Azithromycin against multidrug-resistant isolates demonstrated the MIC and MBC of the antibiotic in ppm against bacterial isolates. (OD: Optical Density)

(15-100 ppm) and *Bacillus cereus* (25-50 ppm), respectively (28, 35-37).

Recently, MDR pathogens have become a significant threat to public health as they evolved various mechanisms to withstand the toxicity of antibiotics. Biosynthesized silver nanoparticles can be used to overcome the limitations of industrial antibiotics. Since silver is toxic to microbes and barely toxic to animal cells, these features of Ag particles may be more advantageous for AgNPs in developing more effective medications against infections. The specific mechanism of the antibacterial effect of AgNPs against pathogenic microbes is unclear. Few studies have suggested that the bactericidal effects may be due to the electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles (38). The other proposed mechanisms for the effective antibacterial activity of AgNPs are inactivation of cellular proteins, vital metabolic enzyme inhibition, reactive oxygen species (ROS) generation, and breakage of DNA (39). AgNPs are assumed to have attached to the bacterial cell membrane and disrupted its functions, such as permeability and respiration. AgNPs could easily penetrate the bacteria and cause further damage, possibly by interacting with sulfur- and phosphorus-containing compounds, such as DNA, resulting in cell death (40). Another possible mechanism of the antibacterial activity of AgNPs might be due to releasing a cluster of highly reactive silver cations and radicals inside the pathogen body or the cell surface (37). These free radicals form pores in the bacterial cell walls and membranes, creating extremely permeable bacterial cell membranes responsible for final bacterial destruction and death.

6. Conclusion

The AgNPs synthesized by *R. planticola* were roughly spherical and cubic, with an average size ranging between 10 and 80 nm. Simultaneously, pathogenic bacteria develop resistance to a long list of antibiotics and threaten the world's health. Bio-AgNP by *R. planticola* showed effective antibacterial activity against

MDR microbes used in the present study compared to known antibiotics (Tetracycline and Azithromycin). It was also evident that synthesized AgNPs can inhibit bacterial growth at a lower concentration than conventional antibiotics. However, the toxicity of AgNPs on eukaryotic cells compared to bacteria should be investigated.

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References

1. Edmundson M, Thanh NTK, Song B, Nanoparticles based stem cell tracking in regenerative medicine. *Theranostics*. 2013;**3**:573-582. doi:10.7150/thno.5477
2. Thakkar KN, Mhatre SS, Parikh RY, Biological synthesis of metallic nanoparticles. *Nanomedicine*. 2010;**6**:257-262 doi:10.1016/j.nano.2009.07.002
3. Wang L, Hu C, Shao L, The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine*. 2017;**12**:1227-1249. doi:10.2147/ijn.s121956
4. Dobrovolskaia MA, McNeil SE, Immunological properties of engineered nanomaterials. *Nat Nanotechnol*. 2010:278-287. doi:10.1142/9789814287005_0029
5. Hong X, Wen J, Xiong X, Hu Y, Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. *Environ Sci Pollut Res*. 2016;**23**:4489-4497. doi:10.1007/s11356-015-5668-z
6. Hamzah HM, Salah RF, Maroof MN, Fusarium mangiferae as new cell factories for producing silver nanoparticles. *J Microbiol Biotechnol.* 2018;**28**:1654-1663. doi:10.4014/jmb.1806.06023
7. Punjabi K, Yedurkar S, Doshi S, Deshapnde S, Vaidya S, Biosynthesis of silver nanoparticles by Pseudomonas spp. Isolated from effluent of an electroplating industry. *IET Nanobiotechnol*. 2017;**11**:584-590. doi:10.1049/iet-nbt.2016.0172
8. Rajeshkumar S, Malarkodi C, Paulkumar K, Vanaja M, Gnanajobitha G, Annadurai GJNN, Intracellular and extracellular biosynthesis of silver nanoparticles by using marine bacteria Vibrio alginolyticus. *Nanoscience and Nanotechnology: An International J*. 2013;**3**(1):21-25. doi:10.1155/2013/317963
9. Castanheira M, Deshpande LM, DiPersio JR, Kang J, Weinstein MP, Jones RN, First descriptions of blaKPC in Raoultella spp. (R. planticola and R. ornithinolytica): report from the SENTRY Antimicrobial Surveillance Program. *J Clin Microbiol*. 2009;**47**(12):4129-4130. doi:10.1128/jcm.01502-09
10. Bagley ST, Seidler R, Brenner D, K. planticola sp. nov.: a new species of Enterobacteriaceae found primarily in nonclinical environments. *Curr Microbiol*. 1981;**6**(2):105-109. doi:10.1007/bf01569013
11. Drancourt M, Bollet C, Carta A, Rousselier P, Phylogenetic analyses of Klebsiella species delineate Klebsiella and Raoultella gen. nov., with description of R. ornithinolytica comb. nov., R. terrigena comb. nov. and R. planticola comb. nov. *Int J Syst Evol Microbiol.* 2001;**51**:925-932. doi:10.1099/00207713-51-3-925
12. Bao W, Zhou Y, Jiang J, Xu Z, Hou L, Leung FCC, Complete genome sequence of Raoultella ornithinolytica strain S12, a lignin-degrading bacterium isolated from forest soil. *Genome Announc*. 2015;**3**:1-2. doi:10.1128/genomea.00104-15
13. Xie J, Zhao X, Song X, Wei J, Cultivation in SBR system and screening of simultaneous nitrogen and phosphorus removal strain with identification. *Adv Mat Res.* 2012;**518-523**:5347-5350. doi:10.4028/www.scientific.net/amr.518-523.5347
14. Sklodowska A, Mielnicki S, Drewniak L, Raoultella sp. SM1, a novel iron-reducing and uranium-precipitating strain. *Chemosphere*. 2018;**195**:722-726. doi:10.1016/j.chemosphere.2017.12.123
15. Vaidyanathan R, Gopalram S, Kalishwaralal K, Deepak V, Pandian SRK, Gurunathan S, Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. *Colloids Surf B*. 2010;**75**:335-341. doi:10.1016/j.colsurfb.2009.09.006
16. Paulkumar K, Rajeshkumar S, Gnanajobitha G, Vanaja M, Malarkodi C, Annadurai GJIJoGC, *et al*., Eco-friendly synthesis of silver chloride nanoparticles using Klebsiella planticola (MTCC 2277). *Int j green chem bioprocess*. 2013;**3**(1):12-16. doi:10.1186/2193-8865-3-30
17. John MS, Nagoth JA, Ramasamy KP, Mancini A, Giuli G, Natalello A, *et al*. synthesis of bioactive silver nanoparticles by a pseudomonas strain associated with the antarctic psychrophilic protozoon euplotes focardii. *Mar Drugs*. 2020;**18**. doi:10.3390/md18010038
18. Qurbani K, Hamzah H, Intimate communication between Comamonas aquatica and Fusarium solani in remediation of heavy metal-polluted environments. *Arch Microbiol.* 2020;**202**:1397-1406. doi:10.1007/s00203-020-01853-8
19. Bala M, Arya V, Biological synthesis of silver nanoparticles from aqueous extract of endophytic fungus Aspergillus Fumigatus and its antibacterial action. *Int J Nanomaterials and Biostructures*. 2013;**3**:37-41.
20. Bustamante D, Segarra S, Montesinos A, Tortajada M, Ramón D, Rojas A, Improved Raoultella planticola Strains for the Production of 2,3-Butanediol from Glycerol. *Fermentation.* 2019;**5**. doi:10.3390/fermentation5010011
21. Quinteros MA, Aiassa Martínez IM, Dalmasso PR, Páez PL, Silver Nanoparticles: Biosynthesis Using an ATCC Reference Strain of Pseudomonas aeruginosa and Activity as Broad Spectrum Clinical Antibacterial Agents. *Int J Biomater*. 2016;**2016.** doi:10.1155/2016/5971047
22. Singh P, Kim YJ, Singh H, Wang C, Hwang KH, Farh ME-A, *et al*., biosynthesis, characterization, and antimicrobial applications of silver nanoparticles. *Int J Nanomedicine*. 2015;**10**:2567. doi:10.2147/ijn.s72313
23. Ramalingam V, Rajaram R, Premkumar C, Santhanam P, Dhinesh P, Vinothkumar S, *et al*., biosynthesis of silver nanoparticles from deep sea bacterium Pseudomonas aeruginosa JQ989348 for antimicrobial, antibiofilm, and cytotoxic activity. *J Basic Microbiol.* 2014;**54**:928-936. doi:10.1002/jobm.201300514
24. Singh H, Du J, Yi TH, Biosynthesis of silver nanoparticles using Aeromonas sp. THG-FG1.2 and its antibacterial activity against pathogenic microbes. *Artif Cells Nanomed Biotechnol.* 2017;**45**:584-590. doi:10.3109/21691401.2016.1163715

25. El-Rafie HM, El-Rafie MH, Zahran MK, Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae. *Carbohydr Polym*. 2013;**96**:403-410. doi:10.1016/j.carbpol.2013.03.071
26. Xie J, Lee JY, Wang DIC, Ting YP, Silver nanoplates: from biological to biomimetic synthesis. *ACS Nano*. 2007;**1**:429-439. doi:10.1021/nn7000883
27. Khodashenas B, Ghorbani HR, Synthesis of silver nanoparticles with different shapes. *Arab J Chem*. 2019;**12**:1823-1838. doi:10.1016/j.arabjc.2014.12.014
28. Arokiyaraj S, Vincent S, Saravanan M, Lee Y, Oh YK, Kim KH, Green synthesis of silver nanoparticles using Rheum palmatum root extract and their antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. *Artif Cells Nanomed Biotechnol.* 2017;**45**(2):372-379. doi: 10.3109/21691401.2016.1160403
29. Ulagesan S, Nam T-J, Choi Y-H, Biogenic preparation and characterization of Pyropia yezoensis silver nanoparticles (Py AgNPs) and their antibacterial activity against Pseudomonas aeruginosa. *Bioprocess Biosyst Eng.* 2021;**44**:443-452. doi:10.1007/s00449-020-02454-x
30. Foroohimanjili F, Mirzaie A, Hamdi SMM, Noorbazargan H, Hedayati Ch M, Dolatabadi A, *et al*., Antibacterial, antibiofilm, and antiquorum sensing activities of phytosynthesized silver nanoparticles fabricated from Mespilus germanica extract against multi-drug resistance of Klebsiella pneumoniae clinical strains. *J Basic Microbiol.* 2020;**60**:216-230. doi:10.1002/jobm.201900511
31. Omara ST, Zawrah MF, Samy AA, Minimum bactericidal concentration of chemically synthesized silver nanoparticles against pathogenic Salmonella and Shigella strains isolated from layer poultry farms. *J App Pharm Sci.* 2017;**7**:214-221. doi:10.7324/japs.2017.70829
32. Alshareef A, Laird K, Cross RBM, Shape-dependent antibacterial activity of silver nanoparticles on *Escherichia coli* and Enterococcus faecium bacterium. *Appl Surf Sci.* 2017;**424**:310-315. doi:10.1016/j.apsusc.2017.03.176
33. Senthil B, Devasena T, Prakash B, Rajasekar A, Non-cytotoxic effect of green synthesized silver nanoparticles and its antibacterial activity. *J Photochem Photobiol B Biol*. 2017;**177**:1-7. doi:10.1016/j.jphotobiol.2017.10.010
34. Gopinath PM, Narchonai G, Dhanasekaran D, Ranjani A, Thajuddin N, Mycosynthesis, characterization and antibacterial properties of AgNPs against multi-drug resistant (MDR) bacterial pathogens of female infertility cases. *Asian J Pharm Sci*. 2015;**10**:138-145. doi:10.1016/j.ajps.2014.08.007
35. Mythili R, Selvankumar T, Kamala-Kannan S, Sudhakar C, Ameen F, Al-Sabri A, *et al*., Utilization of market vegetable waste for silver nanoparticle synthesis and its antibacterial activity. *Mater Lett*. 2018;**225**:101-104. doi:10.1016/j.matlet.2018.04.111
36. Abdelmoteleb A, Valdez-Salas B, Ceceña-Duran C, Tzintzun-Camacho O, Gutiérrez-Miceli F, Grimaldo-Juarez O, *et al*., Silver nanoparticles from Prosopis glandulosa and their potential application as biocontrol of Acinetobacter calcoaceticus and Bacillus cereus. *Chem Speciat Bioavailab*. 2016;**29**(1):1-5. doi:10.1080/09542299.2016.1252693
37. Patra JK, Baek K-H, Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. *Front Microbiol.* 2017;**8**:167. doi:10.3389/fmicb.2017.00167
38. Sondi I, Salopek-Sondi B, Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* 2004;**275**:177-182. doi:10.1016/j.jcis.2004.02.012
39. Swarnavalli GCJ, Dinakaran S, Raman N, Jegadeesh R, Pereira C, Bio inspired synthesis of monodispersed silver nano particles using Sapindus emarginatus pericarp extract–Study of antibacterial efficacy. *J Saudi Chem Soc.* 2017;**21**:172-179. doi:10.1016/j.jscs.2015.03.004
40. Swamy MK, Sudipta KM, Jayanta K, Balasubramanya S, The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from Leptadenia reticulata leaf extract. *Appl Nanosci*. 2015;**5**:73-81. doi:10.1007/s13204-014-0293-6