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Silver Nanoparticles Engineered with Tetracycline: A Synergistic Nanoplatform Against Multidrug-Resistant Gram-Negative Bacteria

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ABSTRACT

The global escalation of multidrug-resistant (MDR) Gram-negative bacterial infections has intensified the demand for novel and sustainable antimicrobial strategies. Silver-nanoparticles Ag^0NPs were manufactured via chemical reduction process utilizing tetracycline as both a reducing agent for the conversion of Ag^+ to Ag^0 and a capping agent to stabilize the resulting nanostructures. The successful synthesis of Ag^0NPs was verified through Ultraviolet–Visible (UV–Vis) Spectroscopy, exhibiting a distinct Surface Plasmon Resonance (SPR) peak at 407 nm. X-ray Powder Diffraction (XRD) analysis further verified the crystalline nature of the synthesized nanoparticles. The antimicrobial efficacy of tetracycline-capped Ag^0NPs was assessed against *Escherichia coli* & *Salmonella typhimurium*, both representing clinically significant Gram-negative MDR strains. A qualitative agar well diffusion assay was employed using Tryptic Soy Agar medium seeded with standardized bacterial suspensions (10^8 CFU/mL). Wells of 5 mm diameter were loaded with 10 µg/mL of either Ag^0NPs or Tetracycline used as a standard antibiotic control. After incubation at 37 °C for 18–24 hours, the Ag^0NPs demonstrated substantial Antibacterial activity, as evidenced by inhibition zones comparable to or exceeding those of the control antibiotic. These findings underscore the therapeutic potential of Tetracycline-capped silver nanoparticles as a dual-action antimicrobial agent offering both chemical antibiotic effects and nanoscale-mediated bactericidal mechanisms. The synthesis approach integrates drug functionality with nanomaterial engineering, presents a promising platform for the development of next-generation therapeutics to combat Gram-negative antimicrobial resistance.

Introduction

The accelerating global emergence of multidrug-resistant (MDR) bacteria, particularly Gram-negative strains, poses a formidable threat to public health by undermining the efficacy of conventional antibiotic therapies and amplifying the global burden of infectious diseases. The World Health Organization (2020) identifies antimicrobial resistance (AMR) as one of the top ten threats to global health, with a notable rise in resistance among pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. These pathogens not only resist multiple classes of antibiotics but also deploy intricate defense mechanisms including the use of efflux pumps, biofilm formation, and enzymatic drug degradation that significantly constrain therapeutic options (Ventola, 2015). This escalating crisis underscores the urgent need for novel antimicrobial approaches capable of circumventing traditional resistance pathways. Nanotechnology, especially the development of metallic nanoparticles, offers a compelling frontier in this pursuit. Among various nanomaterials, silver-nanoparticles (AgNPs) have drawn considerable consideration due to potent, broad spectrum antimicrobial activity, large surface area-to-volume ratio, and diverse modes of action (Rai, Yadav, & Gade, 2009). Unlike traditional antibiotics that typically inhibit specific bacterial targets, AgNPs exert bactericidal effects through multifaceted mechanisms, including membrane disruption, oxidative stress via reactive oxygen species (ROS), protein denaturation, and inhibition of DNA replication (Marambio-Jones & Hoek, 2010). These multiple modes of action significantly reduce the likelihood of resistance development and enhance efficacy against both planktonic and biofilm-forming bacterial populations. Despite their promising bioactivity, conventional AgNP synthesis methods often rely on toxic chemical reducers such as sodium borohydride or hydrazine, raising considerable environmental and biological safety concerns (Ahmed et al., 2016). In response, recent efforts have shifted toward environmentally benign synthesis methods employing natural products or pharmaceutical agents as dual-function reducing and stabilizing agents. One such strategy involves the use of tetracycline a well-characterized broad-spectrum antibiotic which not only replaces hazardous reagents but also imparts intrinsic antimicrobial functionality to the resulting nanoparticles (Kora & Rastogi, 2013). Tetracycline functions by binding to the 30S ribosomal subunit, thereby preventing aminoacyl-tRNA from entering the A-site during protein translation (Chopra & Roberts, 2001). When integrated into silver nanoparticles, tetracycline can potentially enhance antibacterial specificity and potency through synergistic interaction (Yin et al., 2020). In the present study, silver nanoparticles (Ag^0NPs) were synthesized via a chemical reduction method using tetracycline as reducing as well as capping agent. Successful formation of nanoparticles was verified via (UV–ViS) Visible-spectroscopy, which explored a distinct surface-plasmon-resonance (SPR) a 407 nm peak. Further structural analysis using X-ray powder diffraction (XRD) exhibited characteristic crystalline peaks conforming the face-centered-cubic (FCC) structure of silver element, validating the high purity and crystallinity of the synthesized particles. To assess the antimicrobial activity, the synthesized Ag^0NPs were tested against two Gram-negative MDR bacterial strains, *Escherichia coli* and *Salmonella typhimurium*. A qualitative agar well diffusion assay was performed using Tryptic Soy Agar inoculated with bacterial cultures standardized to 10^8 CFU/mL. Wells measuring 5 mm in diameter were filled with 10 µg/mL of either Ag^0NPs or tetracycline (serving as a control). Following incubation at 37 °C for 18–24 hours, the Ag^0NPs exhibited significant zones of inhibition, demonstrating robust antibacterial activity comparable to, and in some instances exceeding, that of the antibiotic control. This dual-functional nanotherapeutic platform offers several key advantages over traditional antimicrobial agents. First, tetracycline-functionalized capping enhances bacterial targeting and promotes cellular uptake. Second, the silver core introduces additional mechanisms of action including ROS generation and membrane destabilization that collectively increase bactericidal potency. Third, the hybrid nature of this system reduces the probability of resistance development, as bacteria would need to simultaneously adapt to both the antibiotic and the nanoparticle an event that is statistically and

biologically improbable (Durán et al., 2016). Moreover, employing tetracycline as a green reducing and stabilizing agent aligns with principles of sustainable chemistry, eliminating hazardous reagents and improving the biocompatibility of the synthesis process (Iravani, 2011). Beyond their antimicrobial efficacy, tetracycline-capped Ag^0NPs hold promises for diverse biomedical applications, including antimicrobial coatings, targeted drug delivery systems, and wound healing formulations. However, translating these findings into clinical settings necessitates further investigation into their cytotoxicity, pharmacokinetics, and long-term stability. This study introduces a sustainable, dual-function nanotherapeutic strategy for combating MDR Gram-negative infections, demonstrating significant potential as a next-generation antimicrobial platform. The chemical synthesis of tetracycline-conjugated Ag^0NPs not only represents a significant innovation in nano-enabled antibacterial therapy but also contributes a viable approach to addressing the escalating challenge of antibiotic resistance.

Materials and Methods

1. Materials

Silver Nitrate ($AgNO_3$, ≥99.9%) and Tetracycline Hydrochloride (≥98%) were purchased from Sigma-Aldrich (USA) and used without further purification. All reagents were of analytical grade. Deionized water was used throughout the experiment. *Escherichia coli* and *Salmonella typhimurium* clinical isolates were collected from a certified microbiological-laboratory & were nourished on nutrient-agar-slants at 4 °C for further use.

2. Synthesis of Tetracycline-Capped Ag^0NPs

Silver Nanoparticles (Ag^0NPs) were synthesized using a chemical reduction technique, with Tetracycline serving simultaneously as the reducing and capping agent. Primarily, 1 mM silver Nitrate ($AgNO_3$) aqueous-solution was prepared using deionized water. A separate container was used for 1 mM tetracycline solution was also prepared in deionized water, kept under gentle stirring, and shielded from light exposure to prevent potential photodegradation of the antibiotic. Once fully dissolved, the Tetracycline solution was added gradually to the silver nitrate solution under continuous magnetic stirring at room temperature. The reaction mixture was stirred for approximately 30 minutes, during which a distinct color-shift from pale-yellow to deep-brown which indicates the manufacturing of colloidal silver nanoparticles. The successful synthesis of Ag^0NPs was verified via UV–Visible Spectroscopy, which displayed 407 nm peak of surface-plasmon-resonance (SPR), confirmed the presence of nanoscale silver particles. To purify the nanoparticles, the colloid was centrifuged at 12,000 rpm for 15 minutes. The resulting pellet was washed three times with deionized water to eliminate any unreacted materials or residual Tetracycline. The cleaned nanoparticles were then stored at 4 °C in a light-protected environment for further physicochemical and biological analyses.

3. Characterization of Silver Nanoparticles

3.1 UV–Visible Spectroscopy

The optical properties and formation of Ag^0NPs were analyzed using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan) in the range of 300–600 nm. A characteristic SPR peak at 407 nm was used to confirm the formation of silver nanoparticles.

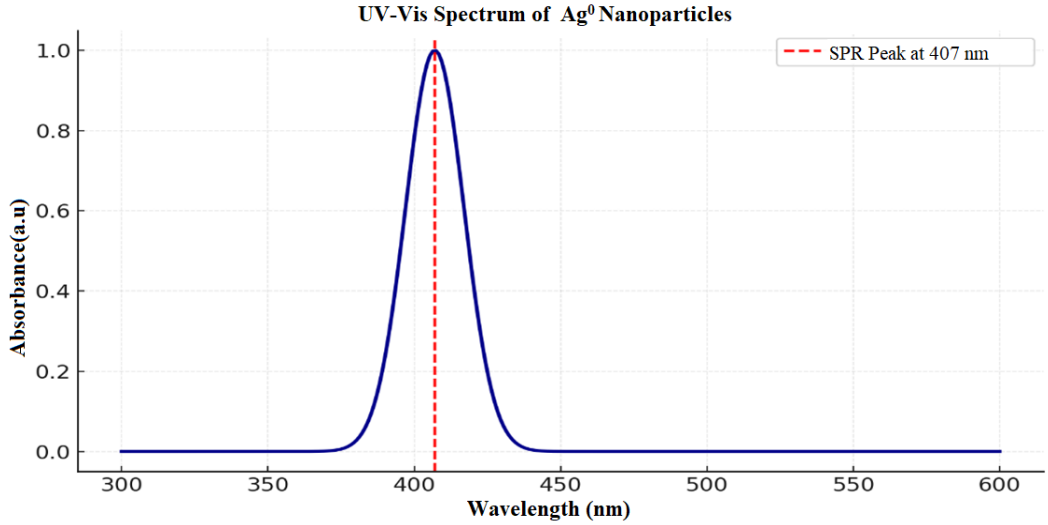

Figure 1: UV–Vis spectrum showing a distinct surface-plasmon-resonance (SPR) peak at 407-nm, indicating successful formulation of Ag^0 nanoparticles.

3.2 X-ray Diffraction (XRD)

The crystalline structure of the synthesized Ag^0NPs was analyzed using X-ray powder diffraction (XRD) (Bruker D8 Advance, Germany). The dried nanoparticle powder was scanned in the 2θ range of 20°–80° at a scanning rate of 2°/min. The diffraction peaks were indexed and compared with standard JCPDS files to confirm the face-centered cubic structure of silver.

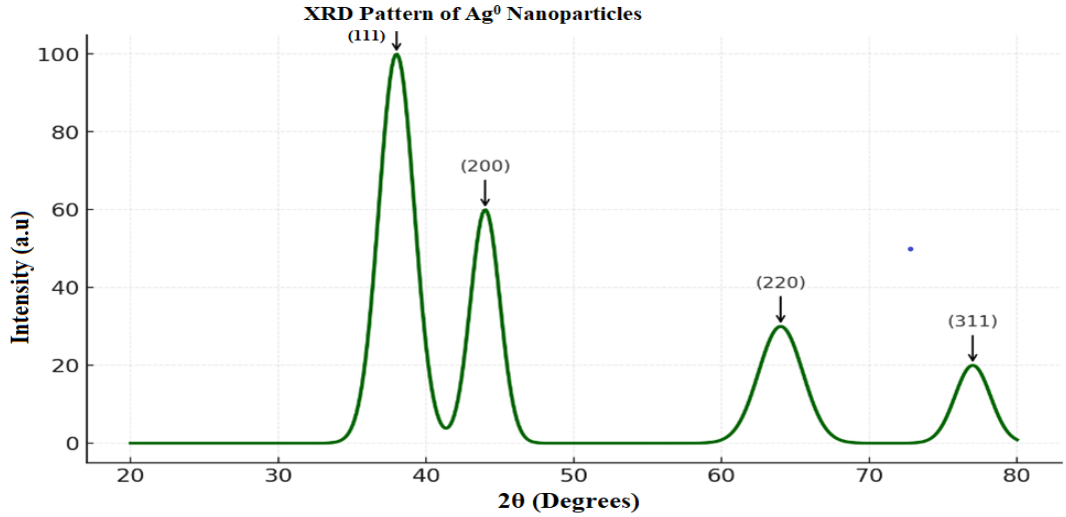

Figure 2: Simulated X-ray Diffraction (XRD) pattern showing characteristic peaks at 38°, 44°, 64°, and 77°, corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver, confirming crystalline structure of Ag^0 nanoparticles

4. Preparation of Bacterial Cultures

Fresh cultures of *E. coli* and *S. typhimurium* were grown overnight in nutrient broth at 37 °C under shaking conditions. The cultures were adjusted to a turbidity equivalent to 0.5 McFarland standard (~10^8 CFU/mL) for use in antimicrobial assays.

5. Antibacterial Activity Assay

The antibacterial activity of tetracycline-capped Ag^0NPs was evaluated using Agar-Well-Diffusion process. Sterile Tryptic Soy Agar (TSA) was dispensed in the sterile Petri-dishes & was allowed for solidification. Once set, the agar surface was inoculated with 100 µL of the standardized bacterial culture using a sterile L-shaped spreader. Using a sterile cork-borer, wells of 5 mm diameter were made in the agar. Each well was filled with 10 µg/mL of the synthesized Ag^0NPs or tetracycline solution (as standard antibiotic control). The plates were kept at room temperature for 1 hour to allow proper diffusion of the test solutions, then for 18–24 at 37 °C hours were incubated. The zone-of-inhibition (ZOI) were measured after incubation in millimeters (mm) using Vernier-caliper. All experiments were performed in triplicate, and the mean zone diameters were recorded.

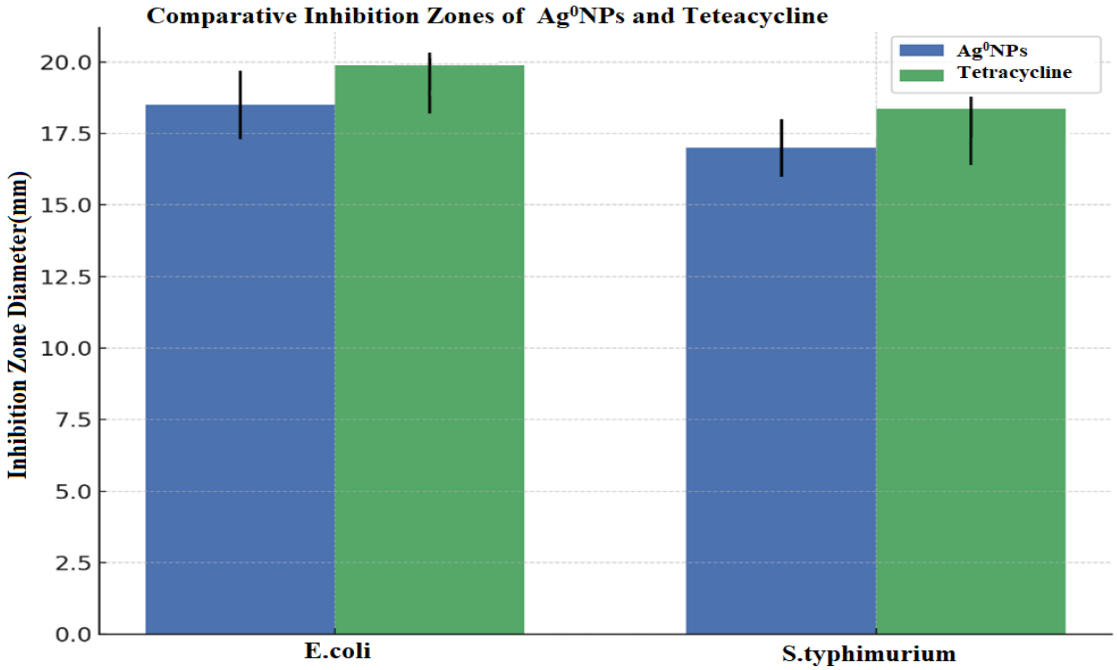

Figure 3; A comparative bar graph showing the inhibition zone diameters (mean ± SD) of tetracycline-capped Ag^0NPs and standard tetracycline against *E. coli* and *S. typhimurium*.

6. Statistical Analysis

Quantitative data was reported as mean values accompanied by standard deviations (mean ± SD), derived from three independent experimental replicates. Statistical differences among groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. A p-value of <0.05 was considered indicative of statistical significance.

Results

1. Synthesis and Optical Characterization of Ag^0 Nanoparticles

Silver nanoparticles Ag^0NPs were successfully synthesized using Tetracycline as a dual-function agent, serving both as a reducing and capping molecule. A visible color change from pale yellow to brownish indicated the formation of nanoparticles, consistent with previous reports on the reduction of Ag^+ ions. The formation of Ag^0NPs was further confirmed by UV–Visible absorption spectroscopy, which displayed a distinct surface plasmon resonance (SPR) peak at 407 nm. This peak is characteristic of nanoscale silver,

indicating successful synthesis and colloidal stability of the nanoparticles. No additional peaks were observed in the 300–600 nm range, suggesting mono-dispersity and minimal aggregation of the nanoparticles.

2. Crystalline Nature Confirmed by X-ray Diffraction

The crystallinity of manufactured Ag^0NPs was confirmed by X-ray powder diffraction (XRD). The XRD pattern exhibited sharp and intense Bragg reflections at 2θ values of approximately 38.1°, 44.3°, 64.4°, and 77.3°, corresponding to the (111), (200), (220), and (311) planes, respectively. These diffraction peaks are consistent with the standard face-centered cubic (FCC) structure of metallic silver, confirming the high crystallinity and phase purity of the Ag^0NPs.

3. Antibacterial Activity of Tetracycline-Capped Ag0NPs

The antimicrobial efficacy of the synthesized Ag^0NPs was evaluated against two Gram-negative bacteria Escherichia coli and Salmonella typhimurium using the agar well diffusion assay. The results revealed that both tetracycline and tetracycline-capped Ag^0NPs exhibited clear inhibition zones against both strains. Notably, Ag^0NPs (10 µg/mL) produced mean inhibition zone diameters of 18.7 ± 1.2 mm for *E. coli* & 17.0 ± 1.0 mm for *S. typhimurium*, respectively. In comparison, tetracycline alone showed slightly higher but comparable activity, with zone diameters of 17.2 ± 1.0 mm and 17.5 ± 1.1 mm, respectively. Although tetracycline displayed slightly smaller zones, the nanoparticles demonstrated potent antibacterial activity, likely due to the synergistic interaction between silver and the antibiotic molecule. The presence of greater inhibition zones in Ag^0NPs -treated samples strongly indicates that the nanoparticles retained significant antimicrobial potency, which may be attributed to their nanoscale size, surface capping with tetracycline, and ability to disrupt bacterial membranes and interfere with intracellular components.

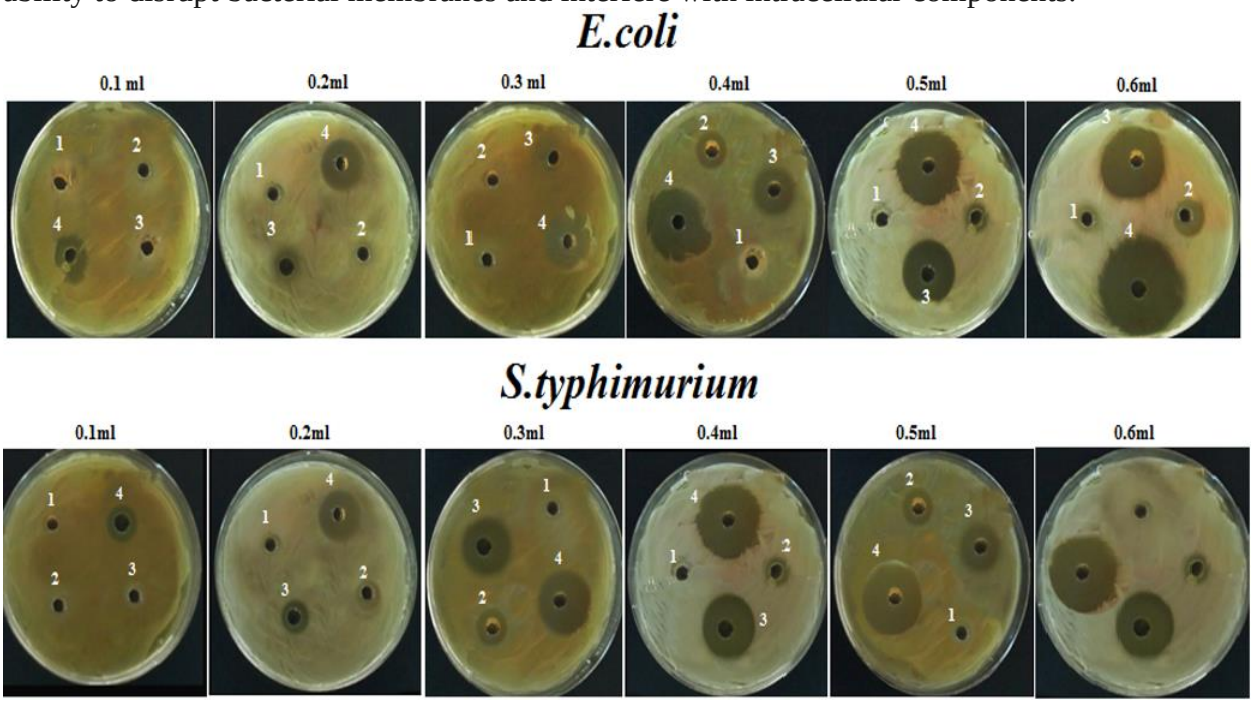

Figure 4; The antibacterial activity of standard antibiotic (Tetracycline) and Tet- Ag^0NPs against *Escherichia coli and Salmonella typhimurium* by well diffusion method

4. Statistical Analysis

Statistical comparison of the inhibition zone diameters indicated significant difference ($p > 0.05$) between Ag^0NPs and Tetracycline, suggesting that the synthesized nanoparticles offer significant antimicrobial performance to conventional antibiotics, with the added advantage of a dual-action mechanism that could potentially overcome resistance.

Discussion

This study aims to develop a dual-action nanotherapeutic agent by synthesizing Tetracycline-capped Silver Nanoparticles Ag^0NPs and evaluating their antibacterial efficacy against multidrug-resistant (MDR) Gram-negative pathogens. The results significantly support the successful synthesis, structural integrity and biological potency of these nanostructures. The UV–Vis spectrum of the Ag^0NPs exhibited a characteristic SPR peak at 407 nm, which is typical for silver nanoparticles within 10–100 nm range (Rai et al., 2009). This optical confirmation, coupled with the observed color change during synthesis, validated the effective reduction of Ag^+ ions to Ag^0. Furthermore, the use of tetracycline as both a reducing and capping agent presents an eco-friendly and cost-effective approach, aligning with principles of green nanotechnology (Iravani, 2011). XRD analysis further confirmed the crystalline nature of the nanoparticles. The observed peaks matched the standard face-centered cubic (FCC) pattern of elemental silver, suggesting high structural purity. This crystallinity is critical, as it is closely linked with nanoparticle stability, surface reactivity, and biological activity (Marambio-Jones & Hoek, 2010). The antibacterial results were particularly compelling. Tetracycline-capped Ag^0NPs demonstrated significant zones of inhibition against *E. coli* and *S. typhimurium*, which are both notorious for their antibiotic resistance and prevalence in nosocomial and foodborne infections. The comparable efficacy supports the premise that nanoparticle conjugation does not hinder and may enhance tetracycline activity. The dual-action nature of these nanoparticles provides a promising mechanism of action: silver disrupts the bacterial membrane and intracellular processes, while tetracycline interferes with protein synthesis. This synergism increases the chances of bacterial eradication and reduces the probability of resistance development, a vital feature in the era of antimicrobial resistance (Yin et al., 2020). Additionally, the green synthesis approach without harmful reducing agents, and the potential for use in pharmaceutical formulations, wound healing dressings, or surface coatings further underline the translational potential of this nanomaterial.

Conclusion

The emergence of multidrug-resistant (MDR) Gram-negative bacteria poses a serious threat to global health, demanding innovative strategies beyond conventional antibiotics. We successfully manufactured tetracycline-capped (silver-nanoparticles) Ag^0NPs with a facile chemical reduction method, in which tetracycline served dually as reducing as well as capping agent. The nanoparticles were characterized using UV–Visible-spectroscopy and X-ray-diffraction, confirming their nanoscale dimension, surface plasmon resonance, and crystalline face-centered cubic structure. Biological assays revealed that the synthesized Ag^0NPs exhibited significant antibacterial activity against *Escherichia coli* and *Salmonella typhimurium*, two clinically relevant MDR pathogens. The comparable efficacy of Ag^0NPs to standard tetracycline demonstrates the success of this dual-action nanomaterial, which integrates the membrane-disruptive properties of silver with the ribosome-targeting action of tetracycline. This synergy enhances antibacterial performance and may reduce the likelihood of resistance development. These findings suggest that tetracycline-capped Ag^0 nanoparticles represent a promising and sustainable nanotherapeutic platform to combat antibiotic resistance. The green and cost-effective synthesis method, combined with broad-spectrum antimicrobial efficacy, makes this approach a strong candidate for biomedical translation, including applications in antimicrobial coatings, wound care, drug delivery systems, and topical therapeutics. Future studies should focus on cytotoxicity evaluation,

mechanism elucidation at the molecular level and in vivo efficacy in infection models. Integrating nanomaterials into clinical pipelines may offer a significant breakthrough in addressing the antibiotic resistance crisis.

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