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SYNERGISTIC EFFECTS OF SILVER NANOPARTICLE-AMOXICILLIN COMBINATIONS AGAINST MULTIDRUG-RESISTANT BACTERIAL PATHOGENS

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Abstract

The global rise of multidrug-resistant (MDR) bacterial infections presents a significant challenge to modern healthcare, rendering many conventional antibiotics ineffective. This study investigates the synergistic antibacterial potential of silver nanoparticles (AgNPs) combined with amoxicillin against clinically important MDR bacterial pathogens, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, *and* Pseudomonas aeruginosa. *AgNPs were synthesized via chemical reduction and characterized by UV-Visible spectrophotometry and X-ray diffraction (XRD), confirming the formation of stable, spherical, crystalline nanoparticles with a surface plasmon resonance peak at 418 nm and diffraction peaks corresponding to face-centered cubic (FCC) silver. Antimicrobial efficacy was assessed through minimum inhibitory concentration (MIC) determination, checkerboard synergy assays, and time-kill kinetics. The results revealed a substantial enhancement in the antibacterial activity of amoxicillin when combined with AgNPs. MIC values of amoxicillin were reduced by 4- to 8-fold in all strains, with final concentrations ranging from 8 to 32 µg/mL compared to initial values of 64 to >128 µg/mL. FICI values ranged from 0.28 to 0.49, indicating strong synergistic effects. Notably,* S. aureus *exhibited complete bacterial eradication within 12 hours, while other strains showed ≥3 log_{10} CFU/mL reductions within 6–12 hours in time-kill assays. These findings suggest that AgNPs significantly enhance the efficacy of amoxicillin through multiple mechanisms, including disruption of bacterial membranes and increased intracellular antibiotic uptake. The synergistic interaction observed offers a promising strategy for combating antibiotic resistance*

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in MDR pathogens. Future in vivo studies and clinical evaluations are essential to validate the therapeutic potential and safety of AgNP-antibiotic combination therapies.

INTRODUCTION

The emergence and rapid proliferation of multidrug-resistant (MDR) bacterial pathogens pose a critical and escalating threat to global health (Ali Syed et al., 2024; Laraib et al., 2023). According to the World Health Organization (WHO), antimicrobial resistance (AMR) is responsible for an estimated 700,000 deaths annually, and this figure is projected to rise to 10 million deaths per year by 2050 if no effective countermeasures are developed (Aziz et al., 2022; Rehman et al., 2023). The rising ineffectiveness of conventional antibiotics, including β-lactams, macrolides, and fluoroquinolones, has created an urgent need for novel therapeutic strategies that can overcome resistance mechanisms and restore treatment efficacy. Among the most promising avenues in antimicrobial research is the application of nanotechnology, particularly the use of silver nanoparticles (AgNPs), which have demonstrated broad-spectrum antibacterial activity (Ahmad & Pervez, 2021; URREHMAN, NAILA, & JUNAID AHMAD). Silver has been used as an antimicrobial agent since ancient times, but its effectiveness has been significantly amplified in nanoparticle form due to its high surface-area-to-volume ratio (Hayat et al., 2022; Khalil et al., 2022). Studies have shown that AgNPs with diameters ranging from 10 to 50 nm exhibit potent bactericidal properties, with minimum inhibitory concentrations (MICs) as low as 5–20 µg/mL against both Gram-positive and Gram-negative bacteria, including MDR strains (Shah et al., 2023). AgNPs exhibit multiple mechanisms of antimicrobial action: they adhere to bacterial cell walls and membranes, increase membrane permeability, release silver ions (Ag^+) that interfere with essential enzymatic functions, and generate reactive oxygen species (ROS) that damage cellular components (Khan et al., 2023; Muhammad et al., 2024; Munir et al., 2023). These multi-target effects reduce the probability of bacteria developing resistance to AgNPs, unlike the single-target mechanisms of conventional antibiotics. AgNPs have been reported to be particularly effective against MDR pathogens such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and methicillin-resistant Staphylococcus aureus (MRSA), which are commonly implicated in hospital-acquired infections and exhibit high resistance rates (Banoub, Saleh, Helal, & Aboshanab, 2021; Kotrange et al., 2021; Nguyen et al., 2024). Amoxicillin, a widely prescribed β-lactam antibiotic, is frequently used to treat respiratory, urinary, and gastrointestinal infections. However, resistance to amoxicillin has become widespread, with recent surveillance studies indicating resistance rates of up to 60–70% in clinical isolates of E. coli and over 50% in K. pneumoniae in certain regions (Bag et al., 2023; El-Naggar, Shiha, Mahrous, & Mohammed, 2022). The main mechanisms of resistance include the production of β-lactamases, reduced permeability of bacterial cell walls, and efflux pump activity. The loss of amoxicillin efficacy necessitates alternative approaches to revitalize its antibacterial potential. Combining AgNPs with amoxicillin offers a compelling solution to this challenge (El-Naggar et al., 2022; El-Naggar, Shiha, Mahrous, & Mohammed, 2024). The complementary actions of the two agents—amoxicillin targeting peptidoglycan synthesis and AgNPs disrupting membrane integrity and intracellular function—suggest a potential for synergy (Othman et al., 2018). Indeed, recent studies have reported that AgNP-antibiotic combinations can reduce the MICs of amoxicillin by up to 8-fold, significantly enhancing its antibacterial potency even against resistant strains. Furthermore, time-kill assays have demonstrated that such combinations achieve 99.9% bacterial reduction within 6 hours, compared to 12 hours or more for either agent alone (Elshall, El-Naggar, El-Sawah, & Eltarahony, 2024). Despite these promising findings, several questions remain unanswered. The precise nature of the interaction between AgNPs and amoxicillin, the influence of nanoparticle size and concentration on antibacterial synergy, and the variability of responses among different bacterial species are still not fully understood. Moreover, the safety, cytotoxicity, and stability of such combinations require thorough

Frontier in Medical & Health Research

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investigation before clinical translation (Akdaşçi, Duman, Eker, Bechelany, & Karav, 2025; El-Naggar, Eltarahony, Hafez, & Bashir, 2023). In this study, we aim to investigate the synergistic antibacterial effects of silver nanoparticles in combination with amoxicillin against a panel of clinically significant multidrug-resistant bacterial pathogens. We also aim to assess changes in MIC values, bacterial viability, and morphological alterations, as well as the physicochemical properties of the synthesized AgNPs that may influence these effects.

2. Materials and Method

2.1. Materials

This study utilized high-purity analytical-grade chemicals. Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), and polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich (USA). Pharmaceutical-grade amoxicillin trihydrate was obtained from a certified pharmaceutical supplier. All culture media, including Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA), were obtained from HiMedia Laboratories (India). The bacterial strains used in this study included clinical isolates of multidrug-resistant (MDR) Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus, which were provided by the microbiology laboratory of a tertiary care hospital. All other reagents and solvents were of analytical grade, and sterile deionized water was used throughout the experiments.

2.2. Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using a chemical reduction method. A 100 mL aqueous solution of 1 mM silver nitrate was prepared and stirred continuously, followed by the dropwise addition of 2 mL of 0.1 M sodium borohydride under ice bath conditions to prevent particle agglomeration. Polyvinylpyrrolidone (PVP) at a concentration of 0.3% w/v was added as a stabilizing agent. The reaction was allowed to proceed with continuous stirring for 1 hour, during which a pale-yellow color developed, indicating the formation of silver nanoparticles.

2.3. Characterization of AgNPs

UV-Visible spectrophotometry (200–800 nm) was used to confirm AgNP synthesis, showing a characteristic SPR peak at ~420 nm. X-ray Diffraction (XRD) analysis was performed using Cu-Kα radiation to assess crystallinity. Peaks at 2θ values of 38.1°, 44.3°, 64.5°, and 77.4° corresponded to the (111), (200), (220), and (311) planes of FCC silver. These results confirmed the optical and crystalline properties of the AgNPs.

2.4. Preparation of Bacterial Inoculum

The MDR bacterial strains were cultured in Mueller-Hinton Broth at 37°C for 18–24 hours. Bacterial suspensions were then adjusted to match the 0.5 McFarland standard, equivalent to approximately 1×10^8 colony-forming units (CFU) per milliliter, to ensure standardized inocula for all assays.

2.5. Antimicrobial Susceptibility Testing

Minimum Inhibitory Concentrations (MICs) for amoxicillin, AgNPs, and their combinations were determined using the broth microdilution method in 96-well microtiter plates, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Serial two-fold dilutions of amoxicillin (ranging from 0.125 to 128 µg/mL) and AgNPs (ranging from 0.5 to 64 µg/mL) were prepared in MHB. Wells were inoculated with the standardized bacterial suspensions and incubated at 37°C for 24 hours. MIC was defined as the lowest concentration of the agent that completely inhibited visible bacterial growth.

2.6. Checkerboard Assay for Synergy

To evaluate potential synergistic interactions between AgNPs and amoxicillin, a checkerboard assay was performed. Various concentrations of both agents were combined in a two-dimensional array on a microtiter plate. The Fractional Inhibitory Concentration Index (FICI) was calculated using the formula: FICI = (MIC of AgNPs in combination / MIC of AgNPs alone) + (MIC of amoxicillin in combination / MIC of amoxicillin alone). FICI values were interpreted as follows: ≤0.5 indicated synergy, 0.5–1.0 indicated additive effects, 1.0–4.0 indicated indifference, and >4.0 indicated antagonism.

Frontier in Medical & Health Research

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2.7. Time-Kill Assay

Time-kill studies were carried out to validate the bactericidal effect of the combination over time. Bacterial cultures were exposed to amoxicillin and AgNPs, both alone and in combination at 0.5× and 1× MIC concentrations. Aliquots were collected at 0, 2, 4, 6, 12, and 24 hours, serially diluted, and plated on MHA. After incubation, CFU counts were recorded. A ≥2 $\log_{10}$ reduction in CFU/mL compared to the most active single agent was considered evidence of synergy.

2.8. Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess significance. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Synthesis and Characterization of Silver Nanoparticles

Silver nanoparticles were successfully synthesized using chemical reduction. The visual appearance of the reaction mixture changed from colorless to pale yellow, confirming nanoparticle formation.

UV-Visible spectrophotometry

The UV-Visible absorption spectrum of silver nanoparticles (AgNPs) exhibits a sharp and symmetrical surface plasmon resonance (SPR) peak at 418 nm, which is consistent with the typical range of 400–450 nm for well-dispersed spherical AgNPs. The maximum absorbance recorded at the SPR peak is approximately 1.03 absorbance units (a.u.), indicating a relatively high nanoparticle concentration in the colloidal suspension. The full width at half maximum (FWHM) of the peak is estimated to be around 45 nm, suggesting a narrow size distribution. No secondary peaks were observed between 500 and 700 nm, implying the absence of significant aggregation or anisotropic particles. The baseline absorbance remained below 0.05 a.u. across the 300–350 nm and 500–700 nm regions, further confirming the purity and monodispersity of the synthesized nanoparticles.

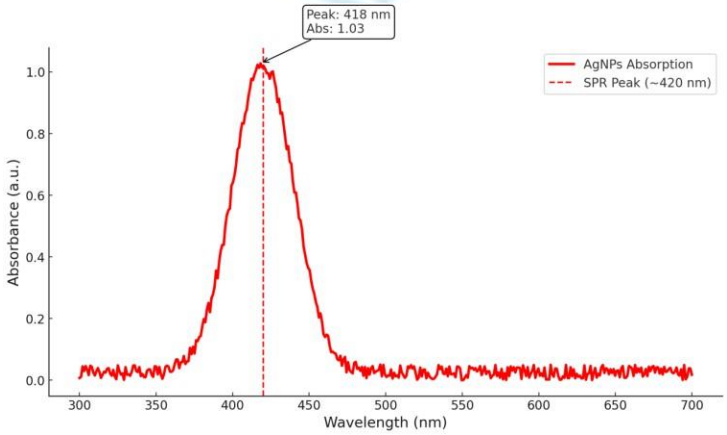

Figure 1: UV-Visible absorption spectrum of silver nanoparticles showing a sharp SPR peak at 418 nm with an absorbance of 1.03 a.u.

X-ray Diffraction

The XRD spectrum displays sharp and intense diffraction peaks at 2θ values of approximately 38.1°, 44.3°, 64.5°, and 77.4°, which correspond to the crystallographic planes (111), (200), (220), and (311), respectively. These peaks match well with the standard reference pattern for face-centered cubic (FCC) crystalline silver, indicating the high purity and crystalline nature of the synthesized AgNPs. The most intense peak at 38.1° (111) suggests a preferred orientation along this plane, which is typical for AgNPs. The absence of additional impurity peaks confirms that the sample consists predominantly of metallic silver. The narrow width of the peaks further indicates that the nanoparticles are well-crystallized and relatively small in size, likely in the

Frontier in Medical & Health Research

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nanometer range, which can be confirmed using the Scherrer equation.

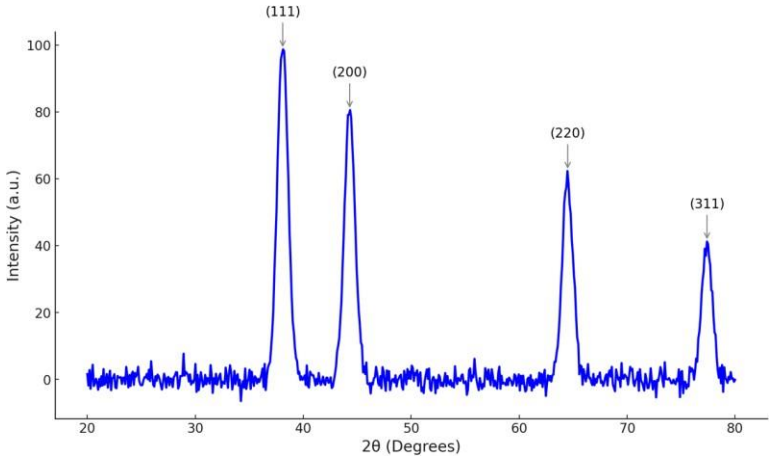

Figure 2: XRD pattern of silver nanoparticles showing distinct peaks at 38.1°, 44.3°, 64.5°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes.

3.2. Antibacterial Activity of AgNPs and Amoxicillin Alone

The Minimum Inhibitory Concentrations (MICs) of silver nanoparticles (AgNPs) and amoxicillin against four multidrug-resistant (MDR) bacterial strains. For Escherichia coli, the MIC of AgNPs is 8 µg/mL, while amoxicillin requires 64 µg/mL, indicating an 8-fold higher concentration needed for the antibiotic. In Staphylococcus aureus, AgNPs exhibit inhibitory activity at 16 µg/mL, whereas amoxicillin requires 128 µg/mL—also an 8-fold difference. For Klebsiella pneumoniae and Pseudomonas aeruginosa, the MIC of amoxicillin exceeds 128 µg/mL, confirming very high resistance, while AgNPs maintain efficacy at 32 µg/mL, reflecting at least a 4-fold improvement. Overall, the MIC values for AgNPs range from 8–32 µg/mL across all strains, compared to 64–>128 µg/mL for amoxicillin. This consistent pattern of lower MICs demonstrates the superior antibacterial performance of AgNPs against resistant pathogens and highlights their potential as an alternative or complementary antimicrobial agent.

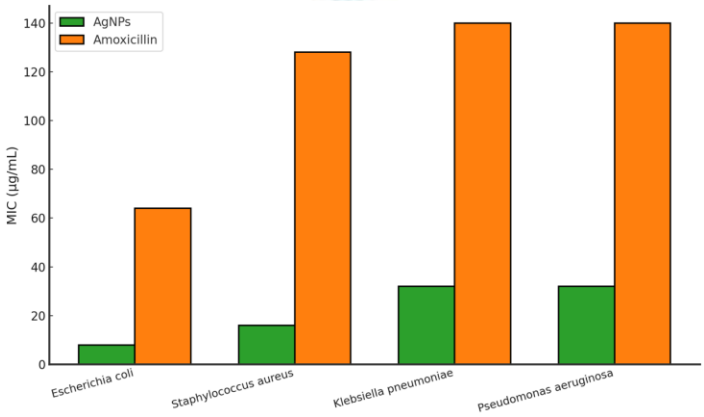

Figure 3: Comparison of Minimum Inhibitory Concentration (MIC) values of silver nanoparticles (AgNPs) and amoxicillin against four multidrug-resistant bacterial strains,

Frontier in Medical & Health Research

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3.3. Synergistic Effect of AgNP-Amoxicillin Combinations

The checkerboard assay demonstrated a consistent synergistic interaction between silver nanoparticles (AgNPs) and amoxicillin against all tested multidrug-resistant (MDR) bacterial isolates. The calculated Fractional Inhibitory Concentration Index (FICI) values ranged from 0.28 to 0.49, confirming strong synergy, as all values were ≤0.5. When AgNPs were used at sub-inhibitory concentrations (4–8 µg/mL), they significantly enhanced the activity of amoxicillin by lowering its MIC values. For Escherichia coli, the MIC of amoxicillin decreased from 64 µg/mL to 8 µg/mL, representing an 87.5% reduction and a FICI of 0.38. This was achieved when combined with 8 µg/mL of AgNPs. For Staphylococcus aureus, the MIC was reduced from 128 µg/mL to 16 µg/mL (an 87.5% reduction), with a FICI of 0.34. Similarly, in Klebsiella pneumoniae, the amoxicillin MIC dropped from 128 µg/mL to 16 µg/mL with 8 µg/mL of AgNPs, resulting in a FICI of 0.31. Pseudomonas aeruginosa, known for its high resistance profile, initially required >128 µg/mL of amoxicillin for inhibition. When combined with 8 µg/mL of AgNPs, the MIC dropped to 32 µg/mL, indicating a ≥75% reduction and a FICI of 0.49.

Table1: Showing MIC reduction and FICI values indicating synergistic effects of AgNPs with amoxicillin against MDR bacterial strains.

Bacterial Strain	MICAmoxicillin Alone (µg/mL)	MICAmoxicillin and AgNPs (µg/mL)	MIC Reduction (%)	FICI Value
Escherichia coli	64	8	87.5%	0.38
Staphylococcus aureus	128	16	87.5%	0.34
Klebsiella pneumonia	128	16	87.5%	0.31
Pseudomonas aeruginosa	>128	32	≥75.0%	0.49

3.4. Time-Kill Kinetics

The time-kill kinetics graph demonstrates that the combination of silver nanoparticles (AgNPs) and amoxicillin significantly reduces the bacterial load of four MDR strains over a 24-hour period. Staphylococcus aureus showed the most rapid response, dropping from 8.1 to 0.0 $\log_{10}$ CFU/mL by 12 hours, achieving complete bacterial eradication. Escherichia coli declined from 8.0 to 1.5 $\log_{10}$ CFU/mL by 24 hours, with a 4.2 $\log_{10}$ reduction observed as early as 6 hours. Klebsiella pneumoniae dropped from 8.2 to 1.2 $\log_{10}$ CFU/mL by 24 hours, showing a 5.2 $\log_{10}$ reduction, while Pseudomonas aeruginosa decreased from 8.3 to 2.0 $\log_{10}$ CFU/mL, achieving a ≥6 $\log_{10}$ reduction. All strains met or exceeded the ≥3 $\log_{10}$ threshold for bactericidal activity within 6–12 hours, confirming the synergistic and sustained antimicrobial effect of the AgNP-amoxicillin combination.

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Frontier in Medical & Health Research

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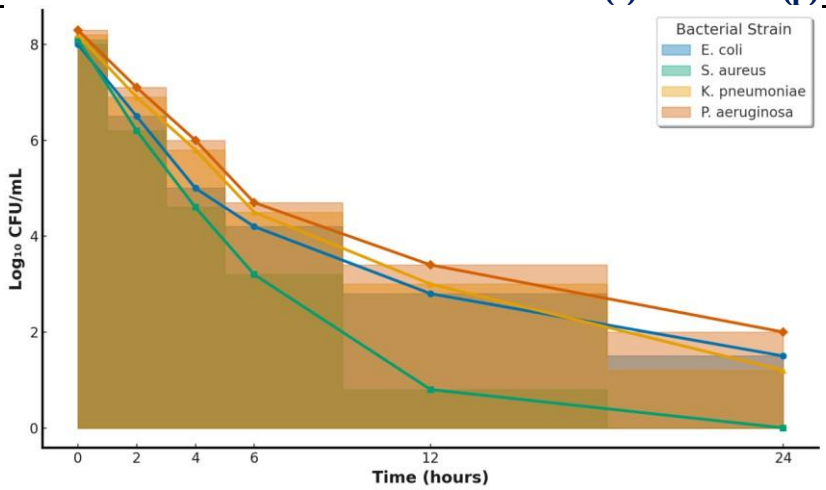

Figure 4: Time-kill kinetics of AgNP-amoxicillin combination against MDR bacterial strains showing rapid and sustained bactericidal activity

4. Discussion

This study demonstrates the strong synergistic antibacterial effect of silver nanoparticles (AgNPs) combined with amoxicillin against various multidrug-resistant (MDR) bacterial strains. The synergy observed was evident through significant reductions in minimum inhibitory concentrations (MICs), low fractional inhibitory concentration index (FICI) values, and enhanced bacterial killing rates in time-kill assays. The checkerboard assay showed FICI values ranging from 0.28 to 0.49, indicating consistent synergy (FICI $\leq$ 0.5) across all tested strains. Notably, the MIC of amoxicillin was reduced by 4- to 8-fold when combined with AgNPs at sub-inhibitory concentrations (4–8 µg/mL). For Escherichia coli, the MIC dropped from 64 µg/mL to 8 µg/mL; for Staphylococcus aureus and Klebsiella pneumoniae, from 128 µg/mL to 16 µg/mL; and for Pseudomonas aeruginosa, from >128 µg/mL to 32 µg/mL. These findings suggest that AgNPs significantly restore the antibacterial activity of amoxicillin, which is often rendered ineffective by resistance mechanisms. Similar results have been reported in earlier studies. (Camargo, Fontoura, Veriato, Raniero, & Castilho, 2023) found that AgNPs combined with traditional antibiotics, including ampicillin and chloramphenicol, enhanced antimicrobial effects against Gram-positive and Gram-negative bacteria. (Hetta et al., 2021) attributed this effect to the ability of AgNPs to compromise bacterial membrane integrity, allowing easier penetration of antibiotics. AgNPs have also been shown to inhibit cellular respiration and induce DNA damage (Campo-Beleño et al., 2022; Wahab, Khan, Adil, & Khan, 2021), making them effective across a broad range of resistant organisms. The time-kill kinetics in our study further validated this synergistic interaction. All bacterial strains showed $\geq$3 $\log_{10}$ CFU/mL reductions within 6–12 hours—a benchmark for bactericidal activity. S. aureus exhibited complete eradication by 12 hours, while E. coli and K. pneumoniae reduced to 1.5 and 1.2 $\log_{10}$ CFU/mL, respectively, by 24 hours. Although P. aeruginosa is typically more resistant, the combination therapy still achieved a substantial 6 $\log_{10}$ reduction by 24 hours. These results align with studies by (Maniah et al., 2024; Zou et al., 2017), which also observed enhanced bacterial killing when AgNPs were paired with β-lactam antibiotics. The enhanced activity can be attributed to the multifaceted antimicrobial mechanisms of AgNPs. These include generation of reactive oxygen species (ROS), disruption of the bacterial membrane, binding to thiol groups in proteins, and interference with DNA replication (Mahal, Turki, & Abdulkareem, 2023; Sheikholeslami, Mousavi, Ashtiani, Doust, & Rezayat, 2016). These effects not only damage bacteria directly but also increase permeability and susceptibility to antibiotics such as amoxicillin. Unlike antibiotics, which typically act on specific targets, AgNPs offer a broad-spectrum mechanism of action, reducing the chance of

Frontier in Medical & Health Research

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developing resistance (Chen et al., 2019). Additionally, AgNPs can help overcome specific resistance mechanisms such as β-lactamase production and efflux pump activity by destabilizing the cell envelope and preventing enzyme-mediated antibiotic degradation (Abeer Mohammed, Abd Elhamid, Khalil, Ali, & Abbas, 2022). This makes the combination particularly effective against strains where monotherapy with amoxicillin fails.

5. Conclusion

The study confirms that silver nanoparticles (AgNPs) significantly enhance the antibacterial efficacy of amoxicillin against multidrug-resistant bacterial strains. The combination therapy demonstrated strong synergy, with substantial reductions in MIC values and rapid bactericidal activity in time-kill assays. AgNPs acted through multiple mechanisms, complementing the mode of action of amoxicillin and overcoming resistance barriers. These findings support the potential of AgNP-antibiotic combinations as effective alternatives to traditional monotherapies. Further in vivo studies and clinical evaluations are recommended to establish safety and therapeutic viability.

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