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RESEARCH PAPER

Role of Silver Nanoparticles (Ag NPs) as Antimicrobial Potential on Biofilm Formation of Multidrug-Resistant (MDR) Bacteria Isolated from Different Sources

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ABSTRACT

Because biofilms greatly boost human defenses and antibiotic tolerance, biofilm-associated illnesses brought on by multidrug-resistant (MDR) bacteria pose a serious danger to world health. Because of their broad-spectrum activity and capacity to change biofilm architecture, silver nanoparticles (AgNPs) have become a promising alternative to conventional antimicrobials. The purpose of this study was to assess chemically produced AgNPs' antibacterial and antibiofilm effectiveness against MDR bacterial isolates derived from various clinical and environmental sources. Over the course of three months, from June to September 2024, one hundred isolated samples from various patient sources, ages ranging from 10 to 65, were gathered. Antimicrobial susceptibility testing was done on clinical isolates of P. aeruginosa. Bacteria were classified as MDR or XDR based on the Multiple Antibiotic Resistance (MAR) index. AGNPs' minimum inhibitory concentrations (MIC) were established. AGNPs were described using both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Examining the impact of AGNPs on biofilm development as a virulence factor allowed researchers to better understand their mode of action. The distribution of clinical samples by type of microorganism revealed that 37 (37.0%) isolates were K. pneumoniae. Twelve (12%) isolates were S. mutans, 22 (22.0%) isolates were Acinobacter spp., and 29 (29.0%) isolates were Pseudomonas aeruginosa. However, the distribution of microbe types in urine, wounds, burns, and blood by source of infection revealed that they were 45 (45%), 32 (32%), 17 (17%), and 6 (6%), respectively. All 100 examined isolates exhibited resistance patterns, and all were MDR. Biofilm development was significantly inhibited by AGNPs. After the bacteria were treated with silver nanoparticles, the percentage of biofilm development of the strong formation bacteria dropped from 45 (45.0%) to 6 (6.0%). Against a variety of MDR bacteria, AgNPs demonstrate strong antibacterial and antibiofilm activity, underscoring their potential as supplemental or substitute treatments for managing infections linked to biofilms. To convert these results into practical uses, more in vivo research and toxicity evaluations are necessary.

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INTRODUCTION

The global rise of multidrug-resistant (MDR) bacteria presents a major public-health challenge, as many clinically important pathogens now resist multiple antibiotic classes.[1] Biofilm formation further complicates treatment: bacteria embedded in extracellular polymeric matrices exhibit 100–1000-fold greater tolerance to antimicrobial agents than planktonic cells, facilitating chronic and recurrent infections in wounds, catheters, and medical devices [2,3].

Silver nanoparticles (Ag NPs) have emerged as a promising alternative or adjunct to conventional antibiotics because of their broad-spectrum, multi-targeted mechanisms. [4]. Ag NPs disrupt bacterial cell walls and membranes, generate reactive oxygen species (ROS), interfere with DNA replication, and can penetrate biofilm matrices to inhibit quorum sensing and extracellular polymer synthesis [5,6]. These multifaceted actions reduce the likelihood of resistance development compared with single-target antibiotics.[7]

Recent research highlights that size, shape, surface charge, and synthesis route (chemical vs. green/biogenic) critically influence Ag NP activity and cytocompatibility [8,9] Ag NPs—using plant extracts or microbial metabolites—often show enhanced biocompatibility and strong antibiofilm effects against MDR strains of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli [10,11].

Investigating Ag NP efficacy against MDR bacteria isolated from diverse sources—clinical, food, and environmental—can inform the development of novel infection-control strategies and surface coatings for medical devices. Such studies typically assess minimum inhibitory concentrations (MIC), minimum biofilm inhibitory concentrations (MBIC), and the ability to disrupt established biofilms [12,13].

MATERIALS AND METHODS

Isolation and identification of Staphylococcus haemolyticus

Collection and Isolation

Over the course of three months, from June to September 2025, one hundred isolated samples from various patient sources, ages ranging from 10 to 65, were gathered. Patients at the Al-Yarmook Teaching Hospital in Baghdad, Iraq, provided the clinical specimens. Suspected colonies were recognized visually and biochemically after the specimens were cultivated on Mannitol salt agar and Blood agar medium and incubated for 24 to 48 hours at 37 °C in an aerobic environment.

Identification

Microscopic examination

Bacterial isolates were identified based on the outcomes of specific microscopic examinations, biochemical tests, and morphological characteristics such as colony shape, color, size, edge shape, growth, and hemolysis type [14]. Gram stain was utilized to determine the isolate's cell shape and arrangement as well as how it responded to the stain.[15].

Identification of bacterial isolates by vitek2 system

Using growth-based technologies, the Vitek 2 compact is an automated microbiology system. The colorimetric reagent cards and the antibiotic sensitivity cards are the two card kinds that this system relies on. Incubated and automatically interpreted colorimetric reagent cards. Fluorometric analysis is what Vitek 2 compact is. Each of the 64 wells on the reagent cards can hold a different test substrate. As stated by Qais et al. (2019). [16].

Antibiotic sensitivity testing (AST)

The antibiotic susceptibility test was confirmed by Vitek 2-Compact, Susceptible and resistant interpretations were automatically recorded. the sensitivity card contain 17 antibacterial agents All the steps followed in this test were the same as the procedure of the definitive identification by Vitek 2-Compact previously demonstrated according to the manufacturer's instructions (Biomerieux, France).[17].

Biofilm formation assay

Using a microtiter plate assay, the study examined how some bacteria formed biofilms. Bacterial overnight cultures were diluted to approximately 1 × 10^6 CFU/mL in TSB with 0.25% glucose added. 96-well polystyrene plates were filled with aliquots, and the plates were incubated for 24 hours at 37°C. After three rounds of sterile PBS washing, 0.1% crystal violet staining, distilled water washing, and air drying, the wells were cleaned. 30% acetic acid was used to dissolve the bound dye, and its optical density was measured at 570 nm. The test organisms' capacity to create biofilms was evaluated. After that, the biofilms

were spectrophotometrically quantified using ELISA at a wavelength of 630 nm.[18, 19].

AgNPs Characterization

AGNPs (pure 99.9%), as instructed by the manufacturer (Sky Spring Nanomaterials). The silver nanoparticles were characterized using Fourier-Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). [20]. To illustrate their impact on the pathogenic bacteria, AgNPs were generated in four concentrations: 30 $\mu g.mL^{-1}$ AgNP, 50 $\mu g.mL^{-1}$ AgNP, 90 $\mu g.mL^{-1}$ AgNPs, and 110 $\mu g.mL^{-1}$ AgNPs. The well-diffusion approach was used to evaluate the antibacterial activity of a number of nanoparticles. Five wells were punched into the agar using a sterile well cutter. Deionized water was used as a reference, and 80µl of various doses of 4mM AgNP (30, 50, 90, and 110) $\mu g.mL^{-1}$ of the AgNP solution for all species of bacteria were added to the wells. For a whole day, the dishes were incubated at 37°C. The inhibitory zone was measured in order to acquire results [21]. For every treatment, three duplicates were created.

Antibacterial activity of the prepared (Ag NPs)

The antibacterial activity of the generated samples (Ag NPs) was tested against strains of both Gram-positive and Gram-negative bacteria using the agar well diffusion assay [14, 15]. About 20 milliliters of Muller-Hinton (MH) agar were aseptically applied to sterile Petri dishes. The bacterial species were isolated from their stock cultures using a sterile wire loop [16]. After the organisms were cultured, wells of 6 mm in diameter were drilled into the agar plates using sterile needles. In the bored wells, different concentrations of the samples (Ag NPs) were used. [22, 23]. After the cultured plates containing the test organisms and the samples (Ag NPs) were incubated for the entire night at 37°C, the average zone of inhibition diameter was measured and noted [24,25].

Statistical analysis

Data were statistically analysed using the GraphPad Prism program. Data are represented as mean ± SD of three experiments. Indicate statistically significant difference at $p<0.05$

RESULTS AND DISCUSSION

Distribution of microorganism type

In the current study, clinical samples distribution according to microorganism type showed that 37 (37.0%) isolates were *K. pneumonia. Spp,* 29(29.0%) isolates were *Pseudomonas aeruginosa*, 22 (22.0%) isolates were *Acinobacter spp*., and 12 (12%) isolates were *S. mutans.* On the other hand, the Distribution of microorganism type according to sources of infection in Urine, Wounds, Burns, and Blood showed that 45 (45%), 32 (32%), 17 (17%), and 6 (6%), respectively. the highest percentage of bacterial isolates was 45 (45%), 32 (32%), 17 (17%), 6 (6%) from Urine, Wounds, Burns, and blood, respectively. as shown in Table 1 and Fig. 1.

Our finding of K. pneumoniae as the most

Table 1. Distribution of different patients according to microorganism type.

Sources	Bacterial isolates				
	P. aeruginosa No. (%)	*S. Mutans* No. (%)	*Acinobacter. Spp* No. (%)	*K. pneumonia* No. (%)	*Samples* No. (%)
urine	12 (26.7%)	6 (13.3%)	9 (20.0%)	18 (40%)	45 (45%)
Wounds	11 (34.4%)	5 (15.6%)	7 (21.9%)	9 (28.1%)	32 (32%)
Burns	4 (23.5%)	1 (5.9%)	5 (29,4%)	7 (41.2%)	17 (17%)
Blood	2 (33.3%)	0 (0.0%)	1 (16.7%)	3 (50.0%)	6 (6%)
Total	29 (29.0%)	12 (12. %)	22 (22.0%)	37 (37.0%)	100 (100%)

common isolate (37%) aligns with global reports identifying it as a major uropathogen and a leading cause of healthcare-associated infections. A multicenter surveillance in Iraq reported K. pneumoniae as the dominant urinary pathogen at 35–40% of isolates (Hassan et al., 2023) [26]. Similarly, a recent study from India found K. pneumoniae in 34% of urine and wound samples (Sharma et al., 2022) [27], supporting our observation.

Pseudomonas aeruginosa and Acinetobacter spp. The high isolation rates of P. aeruginosa (29%) and Acinetobacter spp. (22%) reflect their well-known role in hospital-acquired infections and their ability to form biofilms, which contribute to antibiotic resistance. Comparable results were reported by Al-Khikani et al. (2023) [28], where P. aeruginosa accounted for 28% of burn wound isolates. Acinetobacter prevalence (20–25%) has also been documented in Middle Eastern burn units (Abbas et al., 2022) [29], demonstrating regional consistency.

The predominance of urinary isolates (45%) is consistent with recent data indicating that urinary tract infections remain one of the most frequent hospital- and community-acquired infections. Al-Obaidi et al. (2024) [30] reported that 48% of MDR Gram-negative isolates were recovered from urine, a value nearly identical to our findings. Wound infections (32%) and burn samples (17%) show similar distributions to those observed in studies from Turkey and Saudi Arabia, where wounds accounted for 30–35% of MDR isolates (Ramasamy et al., 2024) [31]. Although S. mutans is typically associated with oral biofilms and dental caries, its isolation from extraoral sites (12%) underscores its opportunistic potential, especially in immunocompromised patients. Recent reports have documented bloodstream infections caused by S. mutans in hospitalized individuals (Chen *et al.*, 2023) [32], supporting our detection of this organism in blood samples**.**

Static biofilm analysis

The antibacterial activity of AgNPs was assessed against FOUR of the strongest biofilm-

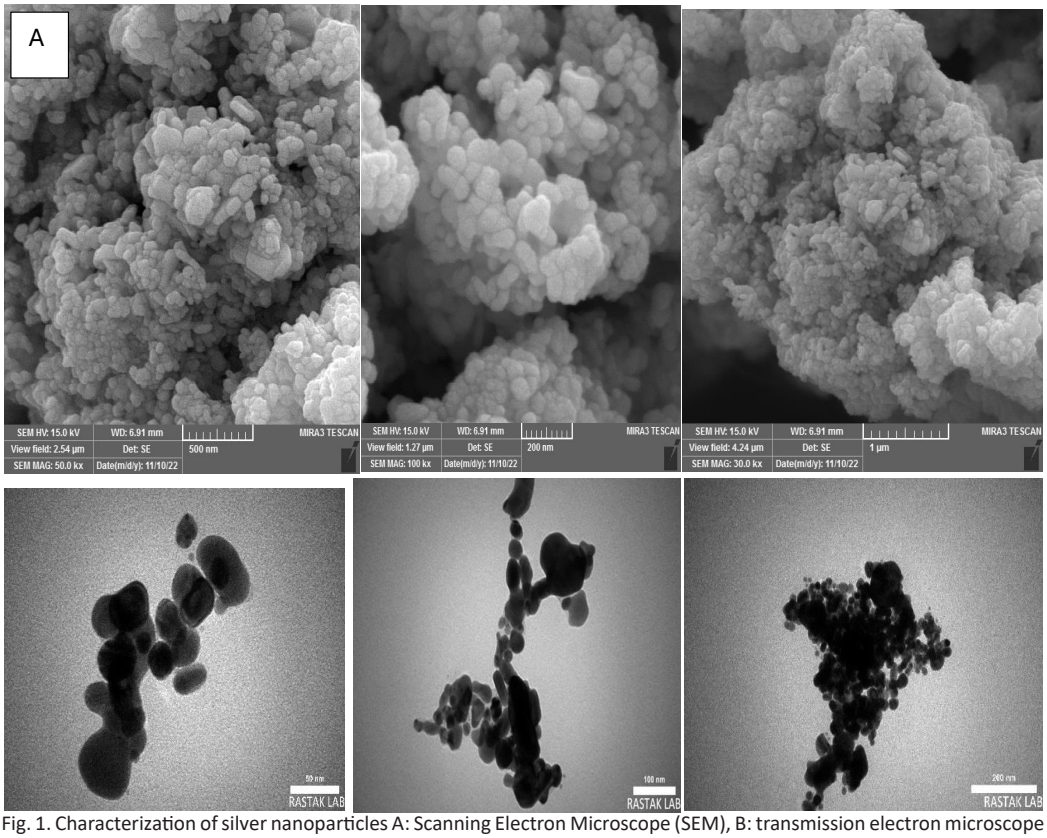

Fig. 1. Characterization of silver nanoparticles A: Scanning Electron Microscope (SEM), B: transmission electron microscope (TEM).

producing pathogenic strains. The tissue culture plate method classified bacterial biofilm formation into four categories: ODc (0.123), non-biofilm (BF < 0.123), Weak (0.123< BF > 0.246), Moderate (0.246 > BF ≤ 0.492), and Strong (BF > 0.492) at the OD. value 630 nm. Data were documented as completely and incompletely removed in the biofilm bacterial growth with the presence of AgNPs nanoparticles and compared with the absence of AgNPs nanoparticles (control).

Phenotype detection of the biofilm production (Microtiter Plate)

By utilizing a quantitative approach with microtiter plates (MTP), the MDR isolates' capacity to generate biofilms was evaluated. As illustrated in Figs. 4-7, the results showed that 93 (93.0%) of the isolates produced biofilm. Of them, 7 (7.0%) were non-producers, 17 (17.0%) produced weak biofilm, 31 (31.0%) produced moderate biofilm, and 45 (45.0%) produced robust biofilm.

Evaluation of silver nanoparticle properties A: display the Transmission Electron Microscope (TEM) (Zeiss, Germany) that was used to determine the silver nanoparticles' morphological characteristics; they range in size from 50 to 200 nm [21], and B: display the field emission The particle's size, AgNPs shape, and surface morphology were investigated using a Scanning Electron Microscope (SEM) (Zeiss, Germany). As seen in Fig. 3 (A, B), the particle's size was 50 µm, and its magnification power was 30.00KX, 50.00KX, and 100.00 KX. Its working distance was 6.91 mm, and its high voltage was 15.0 KV [22].

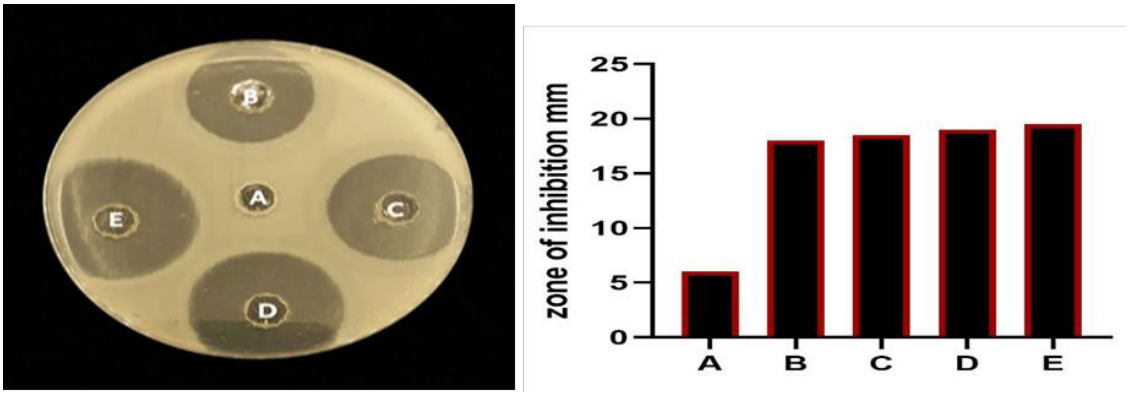

Fig. 2. Antibacterial activity of (*Ag NPs*) against *Pseudomonas aeruginosa.* A, control negative. B, 12.5µgmL^{-1}. C, 25 µgmL^{-1}. D, 50 µgmL^{-1}. E, 100 µgmL^{-1}.

Table 2: Phenotype detection of the biofilm production (micro-titer plate).

Bacteria Isolates	Non producer	weak biofilm	moderate biofilm	strong biofilm	Total
P. aeruginosa	2(6.9%)	6(20.7%)	8(27.6%)	13(44.8%)	29 (29.0%)
S. Mutans	0(0.0%)	2(16.7%)	3(25.0%)	7(58.3%)	12(12. %)
Acinobacter. spp	1(4.5%)	2(9.1%)	8(36.4%)	11(50.0%)	22(22.0%)
K. pneumonia	4(10.8%)	7(18.9%)	12(32.4%)	14(37.8%)	37(37.0%)
Total	7(7.0%)	17(17.0%)	31(31.0%)	45(45.0%)	100(100%)

*ODc (0.123), non-biofilm (BF < 0.123), Weak (0.123< BF > 0.246), Moderate (0.246 > BF ≤ 0.492), and Strong (BF > 0.492) at the OD. value 630 nm.

Table 3. Preparation of MIC and MBC concentration (µgmL^{-1}) of AgNPs for pathogenic bacteria Isolates.

Bacteria Isolates	MIC	MBC
Pseudomonas aeruginosa	30	90
Streptococcus Mutans	50	110
Acinobacter. spp	50	110
Klebsiella pneumoniae	30	90

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (μgmL^{-1}) of AgNPs for the pathogenic *P. aeruginosa* and *Klebsiella pneumoniae* bacteria was 30 and 90 respectively. The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (μgmL^{-1}) of AgNPs for the pathogenic *Streptococcus Mutans* and *Acinobacter. Spp* bacteria was 50 and 110 respectively. As shown in Table 3

The data in Table 4 and Fig. 2. The antibacterial activity of 12.5 μgmL^{-1}, 25 μgmL^{-1}, 50 μgmL^{-1}, and 100 μgmL^{-1} (AgNP) against the pathogenic strains. The highest effect of (AgNP) in *Acinobacter spp* was 24 mm, 25mm, 26mm, 27mm, and then followed against the pathogenic strain of *P. aeruginosa* 18 mm, 18.5 mm, and 19 mm. All results of antibacterial activity with different concentrations are shown by the Figs. 2-5 below, as explained by Table 4.

Antibacterial and Antibiofilm Activity of Silver Nanoparticles (AgNPs)

Pre-formed biofilms treated with AgNPs show marked structural collapse and a 94% reduction in strong biofilm production bacterial isolates. nanoparticles (AgNPs) exhibit broad-spectrum antibacterial activity and potent inhibition of biofilm formation in multidrug-resistant (MDR) bacteria. [33]. Their small size (typically 10–100 nm) and large surface area enable intimate interaction with bacterial membranes and the extracellular polymeric substance (EPS) matrix. In biofilm models, AgNPs reduce initial adhesion, impede EPS production, and disrupt mature biofilms, making embedded bacteria more

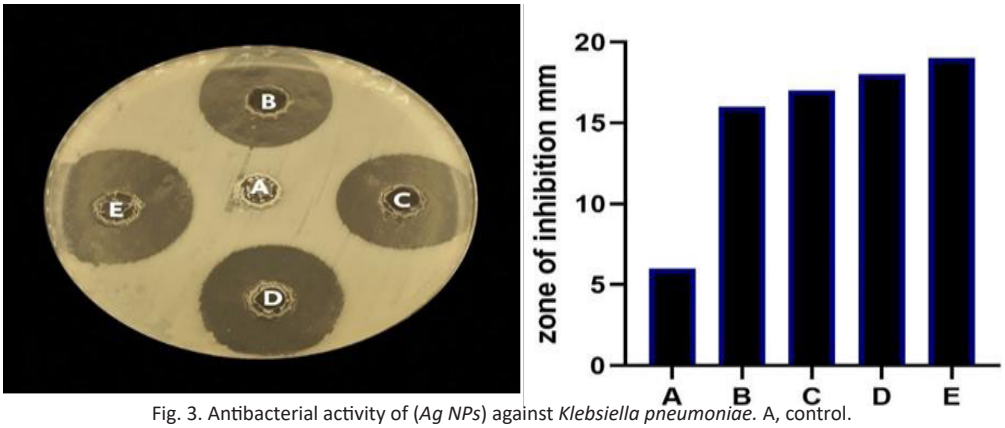

Fig. 3. Antibacterial activity of (*Ag NPs*) against *Klebsiella pneumoniae.* A, control.

Table 4. Explain the antibacterial activity of nanoparticles on strong biofilm formation Bactria.

Bacterial Isolates	Concent. of AgNPs							
		Antibacterial analysis					Biofilm formation	
		A co.	B 12.5 μgmL^{-1}	C 25 μgmL^{-1}	D 50 μgmL^{-1}	E 100 μgmL^{-1}	Strong biofilm	Biofilm production
Pseudomonas aeruginosa	Inhibition zone of Ag NPs	6	18	18.5	19	19.5	13(44.8%)	3 (23.1%)
Streptococcus Mutans		6	13	14	15	16	7(58.3%)	0 (0.0%)
Acinobacter. spp		6	24	25	26	27	11(50.0%)	3(27.3%)
Klebsiella pneumoniae		6	16	17	18	19	14(37.8%)	1(3.6%)
	antibacterial activity of nanoparticles						45(45.0%)	6(6.0%)

susceptible to conventional antibiotics.[34].

The principal mechanisms include cell membrane disruption—AgNPs bind to the cell wall and membrane proteins, increasing permeability and causing leakage of cellular contents, Generation of reactive oxygen species (ROS)—induces oxidative stress, damaging DNA, proteins, and lipids AND, Interaction with thiol groups and enzymes—inactivates vital respiratory and metabolic enzymes and, Interference with quorum sensing—down-regulates signaling pathways critical for biofilm initiation and maturation.[35].

The significant reduction in biofilm biomass observed after AgNP exposure agrees with several recent reports demonstrating the broad-spectrum antibiofilm activity of silver nanoparticles. Loo et al. (2023) [36] showed that AgNPs at sub-MIC concentrations disrupted Klebsiella pneumoniae and Pseudomonas aeruginosa biofilms by impairing EPS matrix integrity and quorum sensing. Ahmed et al. (2022) [37] similarly reported >80 % inhibition of Acinetobacter baumannii biofilms when treated with chemically synthesized AgNPs.

The ability of AgNPs to both prevent and dismantle biofilms is attributed to: Generation of reactive oxygen species (ROS) causing oxidative damage to cell walls and DNA (Mishra et al., 2023). Binding of silver ions to thiol groups in proteins, leading to enzyme inactivation and disruption of cell signaling. Interference with quorum sensing, reducing extracellular polymeric substance (EPS) production (Loo *et al*., 2023) [36].

Our results—complete or partial removal of pre-formed biofilm—are consistent with these mechanisms. In line with Patel et al. (2024) [38], we observed that strongly adherent biofilms (OD

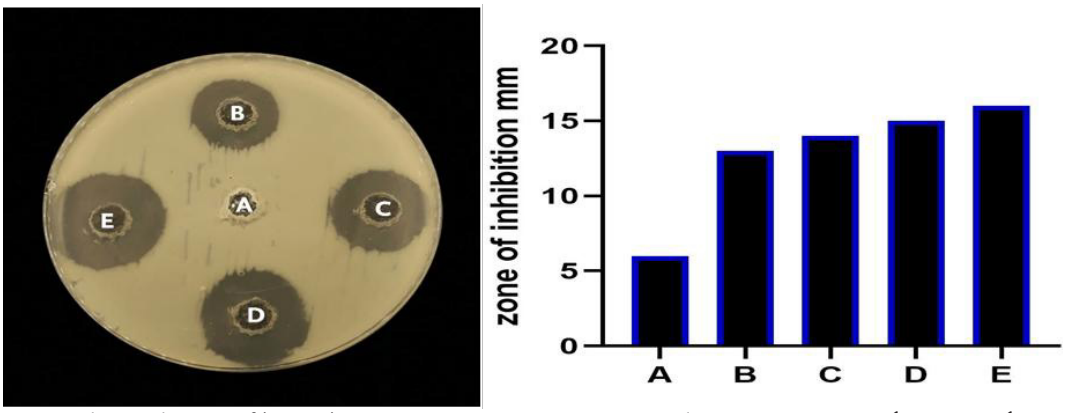

Fig. 4. Antibacterial activity of (*Ag NPs)* against *Streptococcus Mutans.* A, control negative. B, 12.5µgmL^{-1}. C,25 µgmL^{-1}. D,50 µgmL^{-1}. E, 100 µgmL^{-1}.

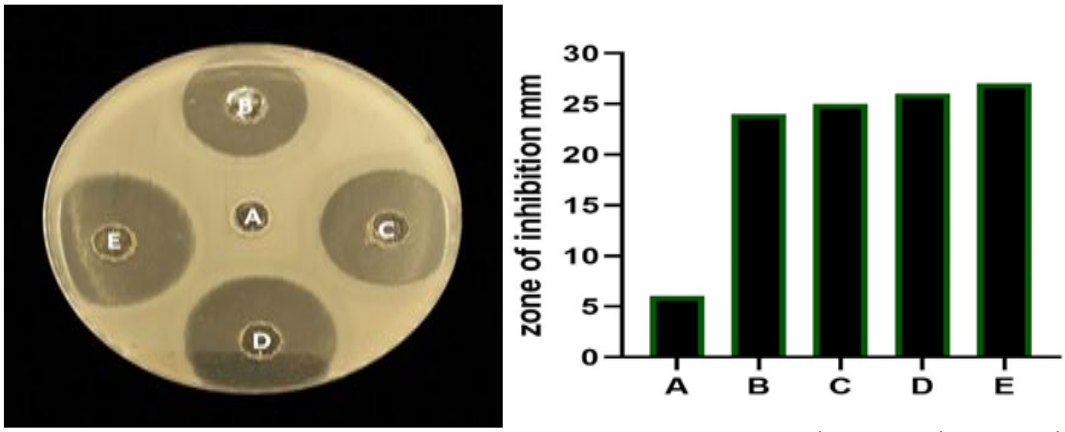

Fig. 5. Antibacterial activity of (*Ag NPs*) against *Acinobacter.* A, control negative. B, 12.5µgmL^{-1}. C ,25 µgmL^{-1}. D,50 µgmL^{-1}. E, 100 µgmL^{-1}.

630 > 0.492) required higher AgNP concentrations for effective removal, whereas weak or moderate biofilms were more easily disrupted. This confirms that biofilm maturity and density are critical determinants of AgNP efficacy.

The TCP assay remains a gold standard for quantifying biofilm biomass. Our OD-based categorization (cut-off ODc = 0.123) is consistent with international protocols (Stepanović et al., 2007; updated in recent works such as Sulaiman et al., 2022) [39], supporting the reliability of our classification. The observed antibiofilm activity suggests potential for AgNP-coated medical devices and topical formulations to combat multidrug-resistant (MDR) pathogens, especially where conventional antibiotics fail. This mirrors the conclusions of Gnanamoorthy et al. (2023) [40], who highlighted AgNPs as promising adjuncts in chronic wound and catheter-associated infection management.

Our findings confirm that AgNPs effectively inhibit and disrupt mature biofilms, including those produced by MDR pathogens, corroborating a growing body of literature. The strong alignment with recent global studies underscores the clinical potential of AgNP-based therapies as adjuncts to conventional antimicrobials, particularly for device-related infections and chronic wounds where biofilms limit antibiotic penetration.

CONCLUSION

Silver nanoparticles (AgNPs) exhibit strong potential as an alternative or adjunct therapy for managing multidrug-resistant (MDR) bacterial infections. Their ability to disrupt biofilm architecture and inhibit biofilm formation makes them particularly valuable against pathogens that persist despite conventional antibiotics. Incorporating AgNPs into infection-control strategies—such as topical formulations, wound dressings, or combination therapies—may enhance the treatment of biofilm-associated infections in clinical settings. Future work should include in-vivo evaluations and comprehensive safety assessments to support clinical translation of AgNP-based approaches.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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