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Anti Biofilm of Biosynthesized Silver Nanoparticles Mediated by Lactobacillus AcidophilusAgainst *klebsiellapneumoniae*

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Abstract. The isolate of *lactobacillus acidophilus*was obtained from the dairy products. These isolates were cultured on De man rogosa sharpe broth and agar for 24 h at 37°C.Based on the biochemical tests, these isolates identified as *Lactobacillus acidophilus*. And the fermentation test for carbohydrates distinguished the *Lactobacillus acidophils*, this isolate was diagnosed by biochemical tests and using the Vitek2 system as *Lactobacillus acidophilus*, the method of biosynthesis of nanoparticles is gaining an extremely significant field due to its economic and environmental advantages compared to the chemical and physical methods of synthesis.The goal of the current study is to use *Lactobacillusacidophils* to biosynthesize silver nanoparticles. Ag NPs were biosynthesized by adding silver nitrate (AgNO3) to the cell-free supernatant per *Lactobacillus acidophilus* at a concentration (5 mM) to be used as a precursor for the synthesis of Ag NPs. The color shift of AgNPs from yellow to reddish-brown was the first indication of biosynthesis. UV-visible spectrophotometry was used to characterize biosynthetic nanoparticles, and the maximum absorption peak of the nanoparticles (410 nm) was observed. The size and distribution of nanoparticles were determined using scanning electron microscopy (SEM), and the shape was circular, homogeneous, and the size varied from (30-100nm). The occurrence of Ag was investigated using Energy Dispersive(x-ray) Spectroscopy (EDS). Antibacterial activity of biosynthesized AgNPs against multidrug-resistant bacteria (MDR) of both gram-positive and gram-negative bacteria, as well as biofilm formation (Between September 2020 and March 2021, a total of (30)*Klebsiella pneumoniae* isolates were collected from clinical samples of urinary tract infection. The identification of *Klebsiella pneumoniae* isolates was established using morphological, cultural, and biochemical tests, and then confirmed using the Vitek-2 system. The numbers of obtained bacterial isolates according to the site of infection where (30) isolates urinary tract infection. regarding antibiotic susceptibility testing the present study demonstrate that all isolates (100%) were resistant to Ceftazidime, and Ceefpime (100%) respectively, whereas the lowest minocycline 26,67% due to misuse of antibiotics. The present study demonstrated the occurrence of some virulence factors such as capsule in all isolates (100%), biofilm formation was recorded in 10 isolates,.

INTRODUCTION

Nanotechnology is the analysis and modification of matter at the atomic and molecular scales on a scale of one billionth of a meter (i.e., 10-9m=1nm). The size of a nano product can vary from 1 to 100 nanometers [1]. Silver nanoparticles (AgNPs) have gotten a lot of recognition because of their activity of antimicrobial and ability to resist the formation of the biofilm, just as well their special

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physical, chemical, and biological properties and applications in electronics, optics, and medicine [2]. For synthesizing different kinds of nanoparticles, there are physical, mechanical, biological, and hybrid approaches, as well as more advanced, energy-intensive, and potentially dangerous to the environment physical and chemical methods [3].

The most crucial element in expanding nanoparticles' biomedical applications is to develop effective, non-toxic, and environmentally friendly methods for synthesis. The use of microorganisms to synthesize nanoparticles is one option for achieving this aim [4]. The extracellular synthesis of silver nanoparticles utilizing *Lactobacillus* bacteria, as well as the discovery of new efficient antimicrobial agents to overcome several tolerances ofmicroorganisms to antibiotics, tend to be low-cost and environmentally secure [5,6].

In 1933, Henrici made the first known discovery about biofilm when he noticed have been water bacteria do not free-floating but grow on submerged surfaces [7]. Biofilm is made up of multifaceted cell clusters embedded in an extracellular polysaccharide matrix that allows microorganisms to adhere to biomedical surfaces while also shielding them from the immune system and antimicrobial therapy [8]. The expression of a polysaccharide intracellular adhesive regulates biofilm formation. Biofilms are notoriously difficult to extract, and they are frequently resistant to systemic antibiotic treatment, necessitating the disposal of contaminated devices [9,10]. Biofilm organisms have built-in antibiotic resistance.

Nanotechnology can provide a way to infiltrate such biofilms and minimize the formation of the biofilm by using "neofunctionalization surface techniques."

Nanotechnology has the potential to prevent life-threatening biofilms from forming over medical devices. Silver was once known to be an antimicrobial. AgNPs hydrogel hybrids of various sizes of AgNPs have recently been demonstrated to be effective antibacterial agents [11]. Saxena *et* al. discovered have AgNPs immobilized in propylene-based sutures have antibacterial activity against *Klebsiella pneumoniae.*

Infections caused by microbial biofilm formation continue to be a significant concern to patients all over the world. Wound infections are especially troublesome [12], with chronic wounds like the foot, leg, and pressure ulcers being especially susceptible to infections of the biofilm [13]. To destroy or remove biofilms, antimicrobials should infiltrate the polysaccharide matrix and enter the microbial cells. The advancement of AgNPs as a new generation of antimicrobial agents might be an appealing and cost-effective strategy for combating Gram-negative bacteria drug resistance. Using the *Klebsiellapneumoniae* tube system and the Congo red agar (CRA) method, the researchers investigated the anti-biofilm capacity of AgNP biofilms.

MATERIALS AND METHODS

Culture of Lactobacillus

The isolates of *Lactobacillus acidophilus* were obtained from the dairy products which were stored in the Advanced Microbiology Laboratory /University of Babylon. This isolate was diagnosed by using biochemical tests and the Vitek2 system (*Lactobacillus acidophilus*) which was utilized as a source of *Lactobacillus*. De Man Rogase and Sharp broth (MRS broth) were inoculated with

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Lactobacillus acidophilus and incubated under anaerobic conditions using an anaerobic jar with the anaerobic gas back system at 37°C for 48 hrs. Colonies were captured and affirmed as *Lactobacillus* based on morphological and biochemical assays [14]. The second activation was worked from the first activation at 37°C for 24 hrs [15].

Ag Nanoparticles Biosynthesis by *Lactobacillus acidophilus*

The pure culture of *Lactobacillus acidophilus* (5 mL) was inoculated in a flask that contains De man Ragosa Sharpe broth (MRS) and incubated at 37 ° C for 24 hours at 100 rpm. After the incubation period, the centrifugation was done at 5,000 rpm for 25 minutes. Then the supernatant was taken. The pH of the supernatant was regulated by 0.4 M NaOH to delay the transformation process (the pH of the supernatant was acidic 4.4 to be neutral, NaOH was added to reach a pH of 7 to eliminate the effect of organic acids). Aqueous (Ag NPs.7H2O) 0.85 g. 0.005 M dissolved in 1000 ml of distilled water was added to 250 ml of the supernatant and then heated by a water bath at 85 ° C for 5-10 minutes. The transition mechanism is shown by the black deposition at the bottom of the flask. The flask was then incubated for 12 hours at 37 degrees Celsius. The ions clump together at the bottom of the flask. The product was centrifuged at 10,000 rpm for 20 minutes to extract the black precipitate. It was washed three times with deionized water to extract pure materials, then dried for four hours at 60 degrees Celsius in an oven with hot air, as shown in Figure (1) [16].

CHARACTERIZATION OF SILVER NANOPARTICLES

Analysis by Scanning Electron Microscope (SEM)

In the unit of the electron microscope, the Faculty of the Science / University of Kufa, a scanning electron microscope (S50; FEI assay) was used to characterize the morphology and size of the nanoparticles. Prepare the slides by placing a small drop of nanoparticle suspension for biosynthesis on the left slide, drying it, and then analyzing it with a scanning electron microscope (SEM). The microscope operates with a 5-10 kV accelerating voltage, various magnifications, low vacuum, spot size 4, and working distances of 5-10mm.

The analysis of the Energy Dispersive x-ray Spectroscopy (EDS)

The electron microscope unit at the Faculty of Science/ Kufa University used a Bruker EDS attached to an SEM to perform an elemental study of single particles. This study was conducted with a 10kV accelerating voltage, a spot size of 5, and working distances of 10mm to detect the existence of element Nanoparticles [17].

RESULTS

Biosynthesis of Silver Nanoparticles

Lactobacillus acidophilus demonstrated its ability in extracellular biosynthesis of Silver Nanoparticles (AgNPs) utilizing cell-free supernatant and silver nitrate (AgNO3) as a precursor to the synthesis of AgNPs (5M) added to the cell-free supernatant of *Lactobacillusacidophilus* by shaking the 24h incubation at 37 ° Celsius, *Lactobacillusacidophilus* possesses the ability to change the color of the reaction mixture from yellow to reddish-brown (Figuer.1) that is an indicator of the biosynthesis of AgNPs by *Lactobacillusacidophilus*

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FIGURE 1. Biosynthesis of Ag NPs by *Lactobacillus acidophilus*. (a) Culture suspension of *L. acidophilus* on MRS broth (24 h incubation). (b) Addition of AgNPs to the suspension then 24 h incubation

The X-Ray Diffraction (XRD)

Figure (2) demonstrates X-ray diffraction patterns of Ag NPs composite with *L.acidophilus*. XRD (hexagonal phase) analysis shows have the synthesized Nanoparticles were crystalline and pure in nature. The peaks at 2θ =32.05,38.39,46.82,65.02 and 78.11 were consecutive lines of spherical Ag-O nanoparticles, respectively.

The average particle size of Ag NPs was determined by applying Scherrer's equation, as shown in Equation (2) for peak reflection 1 at theta using full-width half-maximum (FWHM) results reported by Ashokkumar and Muthukumaran, and Chang et al. [18,19].

$$D = 0.9k/\Delta(2\theta).\ co(\theta),\ k = 0.9,\ \lambda = 0.15418nm,\ 2\theta = 38 \quad (1)$$

$$\text{Cos } 19 = 0945\text{D } k\lambda\text{D} = 8.4278 \text{ nm} \quad (2)$$

$$B cos\theta \quad (3)$$

The maximum measured diameter of a particle is 8.4278 nm, which is a line extension of the diffraction peaks, suggesting that the composites are nanometer-sized. The obtained nanoparticles have a very low synthesis; this might be due to the biological synthesis process used in the preparation of the nanoparticle**.**

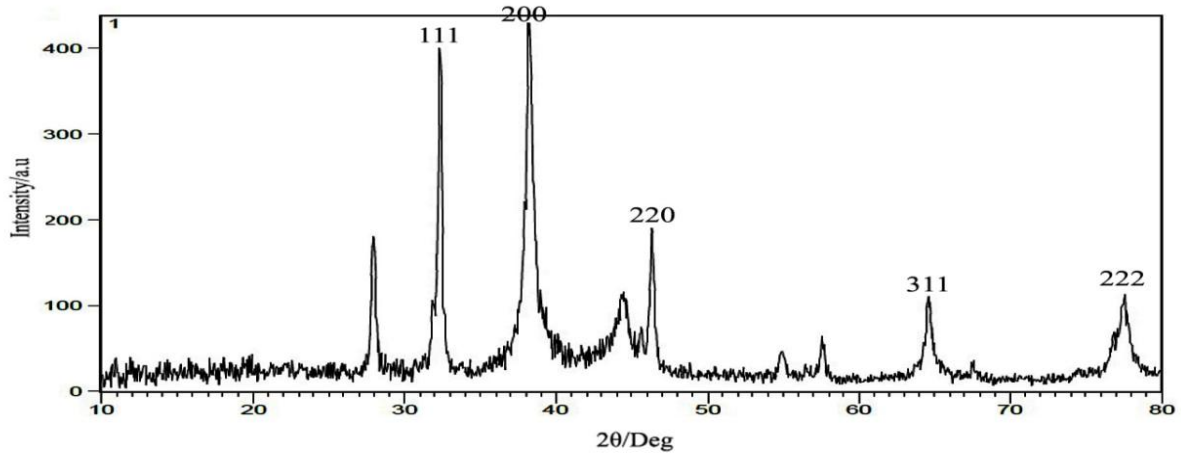

FIGURE 2.XRD analysis for Ag NPs biosynthesized by *L. acidophilus*

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Atomic Force Microscope (AFM)

Atomic Force Microscope analysis of synthesized AgNPs was carried out to assess their morphology (outer surface) and size range,as in figure (3) two-dimensional photo of a section of the surface of AgNPs with large molecular clusters up to 20 nm of AgNPs. The 2- D and 3-D images of AFM, figures (3, 4), showed that most of the nanoparticles are sphericalin shape and some of the agglomerations were present in the background of the nanoparticles. AFM analyses reached that obtained nanoparticles were in a hexagonal, polydispersed, nearly spherical in shape, these results were compatible with the study of reference [20]. AgNPs on freshlycleaned Nanoparticles ' lateral sizes differed from image to image due to the varying shape of the tips. Moreover,0-56.99 nm has been found to be relatively constant in height, this result was compatible with the study done by Monica *et.al*. [21]. Si substrates were imaged by AFM (Figure 4).

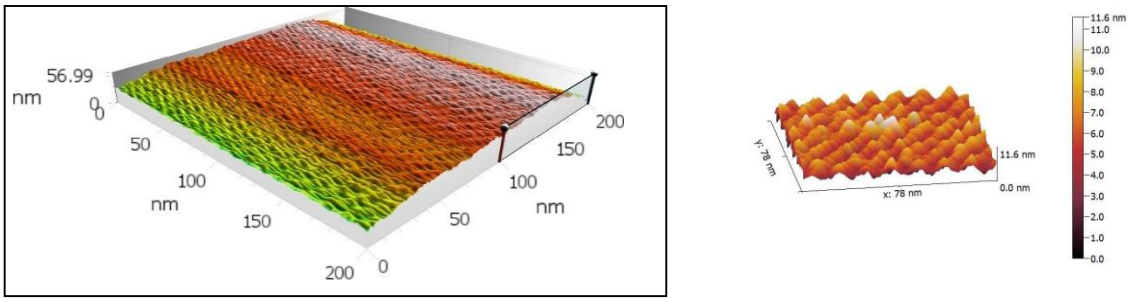

FIGURE 3. Two-dimensional image of Ag NPs biosynthesized by Lactobacillus acidophilus.

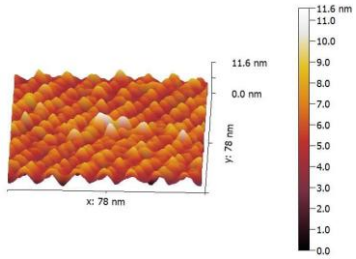

FIGURE 4. Three-dimensional image of Ag NPs biosynthesized by*Lactobacillus acidophilus.*

Field EmissionScanningElectron Microscopy(FESEM)

TheFESEM ofAgNPsphotographswere preparedusingthe biological methodas showninfigure (5).The FESEMcharacteristics ofAgNPs putonGallium Nitride(GaN)andSilicon(Si)substrateswere calculatedona two-parallelplate.Figure(5) showsthediametersofAgNPs(15.96 nm,23.94nm,25.24nm,28.22nm30.00nm),indicatingthediametersofnanoparticleswereaccurate and appropriate asAgNPs. The FieldEmissionScanningelectronmicroscope wasused to determine the size,location,andshape ofthe AgNPs. Thisimage showedthatAgNPs are sphericalclusters(hexonal)inshape,and theirsizesare lessthan70 nm this resultwascompatible withthe studydoneby Raut*et.al*.[22]

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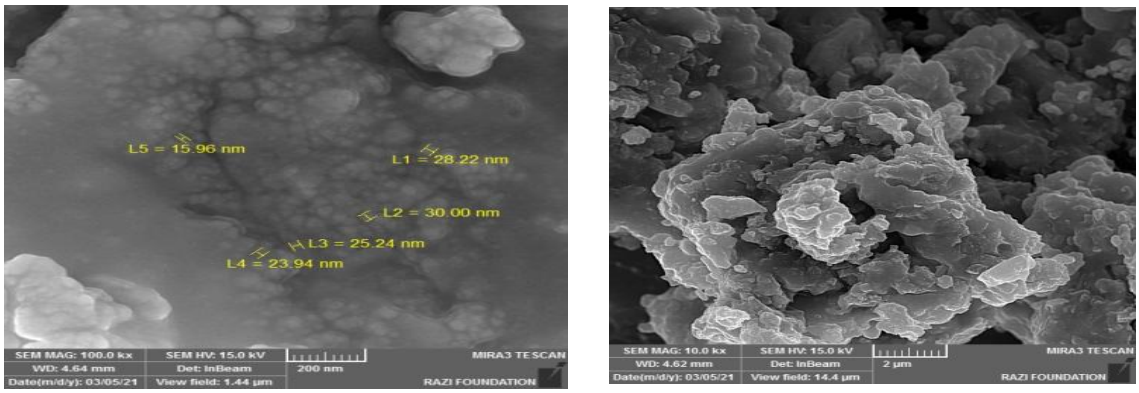

FIGURE 5. FE SEMimage of AgNPs biosynthesizedby*L.Acidophilus*

EDS ANALYSIS OF NANOPARTICLES

The element occurrence was quantified by energy-dispersive X-ray spectroscopy (EDS) by observing the optical absorption peaks of the silver and titanium elements. The presence of the silver element indicates a decrease in silver ions in the reaction mixture by the Lactobacillus supernatant. In the spot profile mode, the EDS spectrum showed strong signals from Ag atoms, intermediate signals from oxygen, and weaker signals from other atoms. The weight percentage of primary components of AgNPs formed by *Lactobacillus* was45.18%silver, O24.39% 20.68% carbon, 4.72% chlorine, S1.12%, Na2.78% and Ca1.12%, (Figuer.6)(Table.1). A peak of AgNPs was detected at 3Kev and is an atypical uptake of metallic AgNPs. Depending on the characterization of nanoparticles by SEM and EDS. The shape, size, distribution, and presence of metallic nanoparticles were determined and as a result AgNO3 (5 mmol) was used for further study.

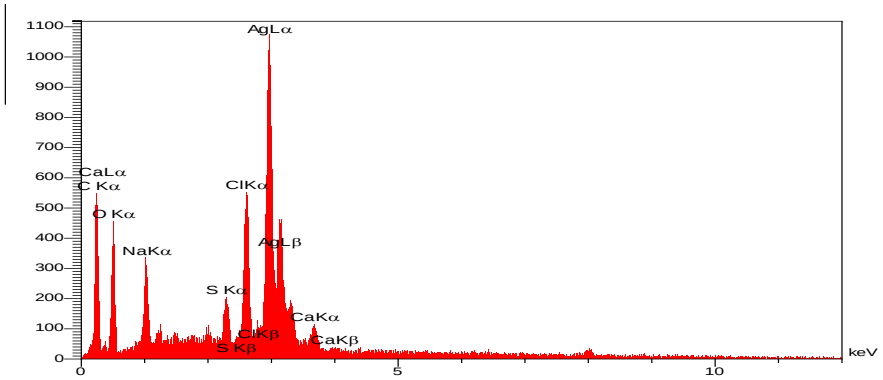

FIGURE 6. Point EDS analysis for Silver NanoparticleBiosynthes

TABLE 1. EDS

EIt	W%
C	20.68
O	24.39
Na	2.78
S	1.12
Cl	4.72
Ca	1.12

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Ag	45.18
	100.00

Clinical isolate of*Klesiellapneumoniae*

A total of (30) clinical isolates of bacteria. were collected from different sites of urinary tract infections and from both gender (male and female) that attended the hospitals in Babylon (Merjan Medical City, Al-Hilla teaching hospital, and Public health) during a period from September 2020 to March 2021. All isolates were taken under the supervision of subsptilities physician in the hospital. All samples were streaked on MacConkey's agar and Blood agar then incubated at 37C° under aerobic conditions for 24 hrs. Culture results were interpreted as being lactose fermenting and non-fermenting bacteria. Lactose fermenting isolates were sub-cultured, incubated for an additional overnight. All isolates were examined for colonial morphology, thenGram's staining was used to analyze them microscopically. Biochemical analyses have been used to identify suspected bacterial isolates to the level of species or subspecies [23,24], and confirmatory detection was done using the Vitek-2 system according to the instructions of themanufacturer.

The Congo Red Method uses a particular kind of formulated solid medium brain heart infusion broth (BHI) combined with 5% sucrose and Congo red for biofilm-forming screening. 37 g/l BHI, 50 g/l sucrose, 10 g/l agar No. 1, and 0.8 g/l Congo red stain were used in the medium. Congo red was prepared as a condensed aqueous solution that was autoclaved separately from the other medium constituents at 121 °C for 15 minutes. The concentrated solution was applied to cooled agar at 55 °C before autoclaving. At 37 °C, the inoculated plates were incubated aerobically for 24–48 hours. Black colonies with a dry crystalline consistency indicated a promising result. For the most part, weak slime producers remained pink, but the colonies' centers darkened. In the lack of a dry crystalline colonial morphology, the colonies darkened, indicating an indeterminate outcome [25]. In BHI agar supplemented with Congo red, the organisms' ability to form biofilms was checked. Exopolysaccharide formation by *Klebsiella pneumoniae* is shown by the presence of black crystallinecolonies.

Biofilm Formation by *Klebsiella pneumoniae*

Biofilm formation by*K.pneumoniae isolates* was investigated and the results showed that 25 isolates were strong, 5 isolates had moderate capacity of biofilmformation

RESULTS

The Activity of Antibiofilm of AgNPs through method of the tube

We analyzed how different concentrations of AgNPs affected biofilm formation in *Klebsiella pneumoniae, E. coli, Salmonella typhi, Enterobacter faecalis, and Pseudomonas aeruginosa*. After 24 hours of treatment, it was discovered that silver nanoparticles inhibit biofilm formation as robust biofilms (+++), resulting in moderate biofilm production at both 12.5 and 25 g / mL AgNPs concentrations, weak (+) production at both 50 and 75 g /mL AgNPs concentrations, and fully prevented the formation of the biofilm at 100 g / mL AgNPs by Escherichia coli, *Klebsiella pneumoniae, Salmonella typhi, and Pseudomonas aeruginosa*.. At doses of 12.5 mcg / mL AgNPs, no

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biofilm inhibition was observed, indicating a robust biofilm product (+++), but biofilm inhibition was observed at 25 mcg / mL, indicating a mild biofilm product (+++) from a strong biofilm produced (+++). For Salmonella typhi, moderate and weak biofilms were produced at 50 and 75 g/mL AgNPs, respectively, and 100 g/mL AgNPs fully inhibited biofilm formation. The formation of the biofilm was uniformly inhibited for all strains when treated with 100 g/mL AgNPs for up to 24 hours, and stable formation of the biofilm was observed in positive test tubes when treated without AgNPs (0 g/L), also no negative biofilm for all strains. With increasing concentrations of AgNPs, biofilm formation was slowly inhibited (Figure-7)(Table-2).

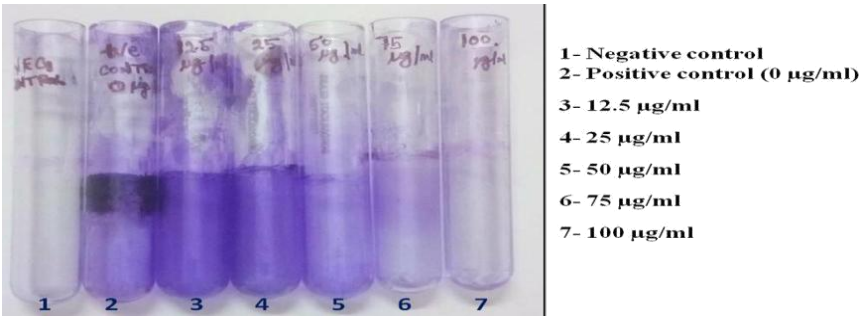

FIGURE 7. The activity of Anti-biofilm of Silver Nanoparticles with different concentrations by the method of the tube

Characterization of the Activity of Anti-biofilm of AgNPs over CRA Plates

Several species that contain exogenous polysaccharides have been shown to form biofilms. Microorganisms bind to a surface that is coated in dirt, an exopolysaccharide membrane that defends bacteria from detrimental environmental factors. *Escherichia coli*,*Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and fecal intestinal strains were grown in Brain Heart Infusion Agar to see whether they could form biofilms. After 24 hours of incubation, additive Congo Red (BHIC) with and without silver nanoparticles are tested. Colonies grown in the absence of AgNPs in the medium appeared as dry, crystalline black colonies, signaling the synthesis of exopolysaccharides, a prerequisite for biofilm creation.

When the species in BHIC were grown with AgNPs, the findings were different. The organisms tended to evolve in the existence of low concentrations of AgNPs (12.5 g/mL), but AgNP treatment prevented glycocalyx matrix synthesis, suggesting that dry black crystal colonies were not present. At high concentrations of AgNPs, bacterial growth was observed to be inhibited by more than 90%. The organism is unable to form a biofilm while the replication of the glycocalyx matrix is halted. Also, it was discovered that 25 g/ml AgNPs greatly slowed biofilm formation without influencing viability, while 100 g/ml AgNPs stopped biofilm formation entirely and inhibited organism development (Figure-8) Biofilm formation was slightly delayed without impacting viability, while biofilm formation was totally stopped and the organism's growth was inhibited at 100 g/ml [Figure-8],(Table-2)

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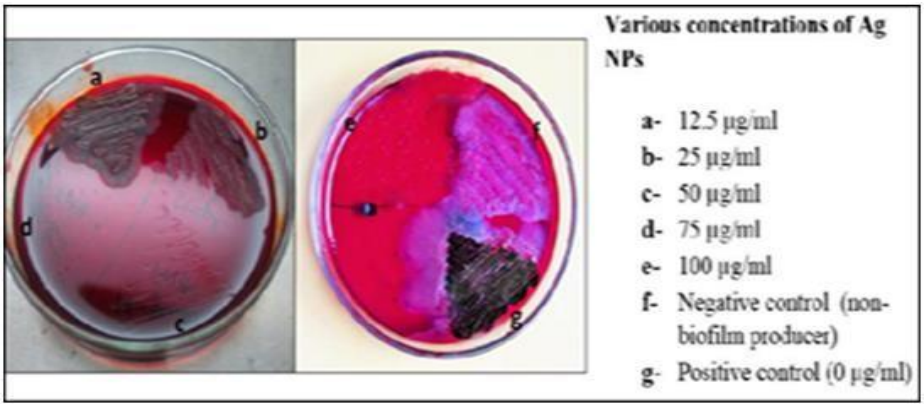

FIGURE 8. The activity of anti-biofilm of silver nanoparticles at various concentrations by the Congored agar method.

TABLE.2. Antibiofilm activity of AgNPs by tube and congo red agar method.

				Tube method					Congo red agar method				
S. No	SBP-MDR strains	Negative control	Positive control (0 µg/ml)	Different concentration of biosynthesized AgNPs in µg/ml									
				12.5	25	50	75	100	12.5	25	50	75	100
		Robust multidrug-resistant strains produced on biofilms											
	Salmonella typhi	–	+++	++	++	+	+	--	B	B	AB	R	--
	P.aeruginosa	–	+++	++	++	+	+	--	B	B	AB	R	--
	K. pneumoniae	–	+++	++	++	+	+	--	B	B	AB	R	--
	E. coli	–	+++	++	++	+	+	--	B	B	AB	R	--
	Enterobacterfaecalis	–	+++	+++	++	++	+	--	VB	B	B	R	--

MDR - multidrug-resistant strains; Mg/ml - micrograms per milliliter; AgNPs - silver nanoparticles; SBP - strong biofilm production; MDR - multidrug-resistant strains; Mg/ml - micrograms per milliliter; Mg/ml - micrograms per milliliter; Mg / m VB stands for very black colonies, while B stands for black colonies. R-red colonies; AB-almost black colonies. (+++/VB) indicates a solid biofilm product; (++/B) indicates a moderate biofilm product; (+/AB) indicates a weak biofilm product; (-/p) indicates a non-biofilmproduct.

DISCUSSIONS

Antimicrobials are becoming more immune to a wide range of chemical antimicrobial agents. As a result, a new approach for countering drug resistance in different microorganisms is critical. Due to their ability to inhibit microorganism growth, silver ions, and silver salts that utilized as antimicrobial agents in a number of fields for decades [27]. However, there are several risks of utilizing Ag ions or

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Ag salts as antimicrobial agents. One of the most possible reasons is the intervening effects of salts. This kind of restriction could be removed using silver in nano form. Biofilm inhibition declined in this analysis as the concentration of AgNPs in the sample increased. When the tubes were treated with AgNPs at concentrations of 12.5, 25, 50, and 75 g/ml, biofilm was formed [Figure-7], but none of the strains at 100 g/ml containing tubes formed biofilm [Figure-7].When Asaduzzaman et al. investigated the influence of AgNPs at different concentrations on the production of biofilm by *K. pneumoniae*, they observed similar results. At doses of 5, 10, and 20 g/ml of AgNPs, K. pneumoniae shaped biofilm, but no biofilm was detected at 40 and 80 g/ml of AgNPs. In the testtubethatdidnotcontainAgNPs,however,biofilmwasdetected[28].

However, Growing the organism on CRA supplemented with and without AgNPs was utilized to test the anti-biofilm efficacy of AgNPs. The organisms appeared as dry crystalline black colonies when grown without AgNPs, suggesting the production of exopolysaccharides (EPS), which is needed for the creation of biofilm Figure-8(g)]. The organisms that were cultivated with AgNPs, on the other hand, did not survive. The organisms continued to grow through treatment with lower concentrations of AgNPs (12.5 g/ml), but the AgNPs treatment prevented the synthesis of glycocalyx matrix, as shown by the lack of dry crystalline black colonies. At higher AgNP concentrations (100 g/ml), however, almost no growth was observed [Figure-8(c-e)]. As a result, when exopolysaccharide synthesis is halted, the organism is unable to form biofilm. Kalishwaralal et al. published similar findings against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* biofilms, finding that 100 nM of AgNPs decreased biofilms by 95-98 percent [29]. It has also been stated in the literature that providing an AgNPs coating on the surface of medical devices helps to prevent adhesion of the bacterial and eventual formation of the biofilm over the devices [30].

The findings are in agreement with the previous study findings on nanocrystalline silver's anti-biofilm behavior done by Kostenko *et* al. Nanocrystalline silver significantly reduced the number of viable cells in the biofilms analyzed. modifying the morphology of biofilms due to the roughness of the cell surface being damaged by 20 g/ml AgNPs. According to Ansari et al., the existence of water channels in the biofilm may explain the inhibitory influence of AgNPs on the current biofilm. AgNPs can diffuse directly through the exopolysaccharides layer through the pores and impart antimicrobial activity because all biofilms have water channels (pores) for nutrient transportation [31].

The relative surface area of a silver particle is increased when it is reduced to the nanoscale level, resulting in higher Ag+ release rates than for elemental silver particles [32].

Furthermore, nanoparticles have a greater ability to adhere to and infiltrate membranes of the bacterial, accumulating within cells and releasing silver ions continuously [31,33]). Ansari et al[31] said that Only a few techniques are available for detecting biofilms on medical equipment or structures. Staining both the bacteria and the glycocalyx is impossible, making bacterial biofilm demonstration difficult. According to Kostenko et al., Acticoat nanocrystalline silver has the best anti-biofilm efficacy as compared to Aquacel silver and Silverlon, and silver concentrationalonecannotaccountforsilverdressings'anti-biofilmefficacy [34].Ansari et al. found that when exopolysaccharide synthesis is blocked, *E. coli* and *K. pneumoniae* biofilms cannot shape on CRA medium and also in SEM observation by

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CONCLUSIONS

When assuming infections that take on the biofilm phenotype are notoriously difficult to treat. Antibiotic therapy may be ineffective against biofilm infections, or they may respond initially only to relapse weeks or months later. In those circumstances, invasive procedures like surgical removal and reconstruction of the contaminated tissue or system might be needed. As a result, screening for biofilm development is needed for the appropriate treatment of biofilm infections that cause clinical isolates. Just a few techniques can be used to detect the existence of biofilms over medical devices or surfaces. It's difficult to stain both the bacteria and the glycocalyx, which makes demonstrating bacterial biofilms difficult.

The nature of clinical specimens is associated with biofilm formation. Silver Nanoparticles with small particles have higher anti-biofilm activity, which is dependent on concentration. In the current study, Silver nanoparticles have a high anti-biofilm function. Silver Nanoparticles have a different anti-biofilm effect on different bacterial isolates, and *K. pneumonia* is more susceptible to nanoparticles. Silver nanoparticles may be utilized to prevent bacterial biofilms from forming, which can be useful in the treatment of infectious diseases caused by biofilms. We recommend that most research be done on this issue, especially in vivo and clinical trials to determine toxicity levels before using silver nanoparticles in the treatment of infections of the biofilm.

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