

Antibacterial Effectivity of Silver Nanoparticles Biosynthesized from Oral Pathogenic *Staphylococcus aureus* Isolate Against Other Gram Positive Isolates

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Abstract

In recent years, staphylococcal infections, caused mostly by multiple-drug-resistant strains, have become particularly prevalent in clinical settings. Due to the development of antimicrobial drugs, the breakout and resistance of infectious diseases caused by resistant *Staph aureus*, pharmaceutical companies and researchers are now researching and developing novel unconventional antimicrobials. In this field, nanotechnology illustrates an innovative and unequalled strategy for developing novel metallic nanoparticle-based architectures with potential antibacterial properties. In this study, cell extract of MRSA strain was used for combination of silver nanoparticles. The synthesized MRSA-AgNPs were identified and described utilizing UV-visible spectroscopy, XRD, and SEM. Their antibacterial potential was evaluated on MRSA and MSSA strains which have been genotypically diagnosed through the disclosure of 533 base per *mecA* gene. The minimal inhibitory concentration (MIC) using micro dilution assay, and minimal bactericidal concentration (MBC) of Ag-NPs across methicillin sensitive *Staph. aureus* (MSSA), and methicillin resistant *Staph. aureus* (MRSA) were inspected in this investigation. Our outcomes indicated that MIC & MBC values of Ag-NPs to MRSA was 7.8 µg/ml and 31.3 µg/ml, separately, whilst MIC & MBC values toward MSSA were 3.9 µg/ml & 15.6 µg/ml, separately. The accomplished outcomes proposed that Ag-NPs display great effect against two significant clinical tested isolates in any case of their drug resistant developing mechanisms

Keywords: MRSA, Ag-NPs, *mecA* gene, XRD, surface plasmon resonance.

INTRODUCTION

Diseases caused via drug-resistant bacteria result in a substantial rise in mortality, costs, & morbidity associated with long-term therapy. MRSA is one of the multidrug-resistant strains; it is any strain of *Staph aureus* that is resistant to all -lactam antimicrobials. Methicillin resistance is caused by the *mecA* gene, which codes for an alternative penicillin-binding protein, PBP 2a.1.

S. aureus is the most common nosocomial infection in the world and one of the most significant opportunistic agents in humans.

By obtaining the *mecA* gene, *S. aureus* may develop into MRSA, which has a poor affinity for all beta-lactam antibiotics, including cephalosporins, in addition to methicillin, oxacillin, and nafcillin., carbapenems, and monobactams; consequently, treatment options for diseases caused by MRSA are restricted. 2.

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Consequently, research into the discovery of novel effective antibacterial drugs against MRSA strains is urgently required. Infections with MRSA have resulted in growing rates of morbidity and death in recent years; thus, research into managing these infections is urgently necessary. 3.

Due of its potential value in the struggle against organisms that are multidrug resistant, nanobiotechnology is a sizable field of research that deserves our undivided attention. By altering their size to change their impact, nanobiotechnology offers the chance to reevaluate the biological characteristics of previously established antibacterial compositions.. 4.

Among these nanomaterials, AgNPs have shown very outstanding physiochemical properties as well as several biological functions. AgNPs feature elevated thermoelectric conductivity, a vast surface region for scattering, catalytic effectiveness, non-linear behavior, and prominent antibacterial capabilities as a consequence of their expansive surface area to size ratio. The diameters of silver nanoparticles vary from 1 nm to 100 nm, and there are several silver precursors (or silver oxides) like silver nitrate, silver citrate, and silver acetate. Silverware's antimicrobial properties have been known since 1000 B.C., when it was used to preserve water. Ag-NPs, which are gradually being employed as antibacterial agents, may increase their antimicrobial application to MRSA, the leading due to nosocomial infection and repository of multi-resistant genes globally. 5.

Ultimately the MIC & MBC values of AgNPs which are prepared from MRSA strain against the same strain (MRSA) and compared with a further strain of methicillin – sensitive *S. aureus* (MSSA). with another strain of methicillin – sensitive *S. aureus* (MSSA).

Materials and methods

1. Bacterial strains

1.1. Phenotypic Characterization of bacterial isolates

Pathogenic strains which were phenotypically diagnosed as MRSA and MSSA were obtained from Dr. Zainab Nashaat Al-Saadi, college of science/ university of Wasit, and kept on mind heart imbuement (BHI) agar inclines (Oxoid, UK). Before performing any experiments, the both two isolates were re-identified biochemically by using API 20 Staph kit (biomerieux/ France). This was done to ensure purity of the bacterial strains as they were stored for months in the refrigerator. Isolates were recognized genotypically as MRSA and MSSA by PCR for *mecA* as portrayed below.

1.2. Genetic Confirmation of MRSA and MSSA

1.2.1. DNA extraction

DNA extraction was done by using FavorPrep™ Blood-Cultured Cell Genomic DNA Extraction MiniKit (FAVORGEN/ Taiwan), following the steps which

mentioned by.

1.2.2. Detection of *mecA* Gene

Both two *Staph aureus* insulate were examined for the existence of the 533bp PCR output of *mecA* gene, utilizing the following specific primers:

forward AAA ATC GAT GGT AAA GGT TGG C

and reverse AGT TCT GCA GTA CCG GAT TTT GC.

Amplification was done according to 6.

2. Antibiotic Susceptibility and Determination of MIC value Testing

Automated testing with the VITEK-2 AST-P592 card (biomerieux) was implemented agreeing to the manufacturer's enlightenin.

3. MRSA Nanoparticle Biosynthesis and Characterization

3.1. Preparation of Bacterial Extract

To prepare extract, the fast-growing MRSA and MSSA strains were directly inoculated on MHB separately and brooded for the time being at 37°C. The supernatant from the centrifuging of the two societies at 12,000 rpm for five minutes was then used for the biogenesis of silver nanoparticles. 7. Bacterial extract was filtrated by using Whitman filter paper, No.1 to get rid of impurities . The centrifugation was performed for bacterial extracts at 10,000 cycles per minute for 10 minutes, at that point supernatants were isolated to be ready to prepare the silver nanoparticles.

3.2. Preparation of AgNPs

To prepare AgNPs by bacterial extract, 10 ml of purified extract has been added to the solution of 2 mM of AgNO₃ to achieve an ultimate volume of 100 ml. pH was adjusted by ammonia to 11. The admixtures were agitated and wormed using thermal magnetic stirrer for a half hour at 80 °C. A brownish-red solution is developed as an indication of silver nanoparticles formation with final concentration 250 mg/L. This method according 8 with some modifications.

3.3. Characterization of AgNPs

The AgNPs were dominated by many techniques to investigate and confirm the identity of the synthesized nanomaterials as of AgNPs. This part of study work has done at AlFadhil of Scientific Training Company, Babil, Iraq. Ultraviolet-visible (UV-VIS) spectroscopy Jenway spectrometer model 6800 was utilized at the range of 200-800 nm. 9. Powder method is one of the X-ray diffraction analysis (XRD) which is used for testing AgNPs by XRD device (DX-2700 SSC 40 k V/30 mA, USA) according to 9. Scanning electron microscope (SEM) analysis has used following the same reference. 9.

4. Antibacterial Effectivity of AgNPs

4.1 Initial Determination of Antimicrobial Activity.

The antimicrobial effectivity of synthesized AgNP solution has firstly tested on MRSA and MSSA isolates by well dispersion method. The pure slants of bacterial disconnects were subcultured on at 35°C on a circulating shaker at 200 rpm. Wells of 6-mm width were created on MHA plates by gel punch. Both isolates then subcultured separately plates utilizing sterile cotton swabs. 20 µL and 40 µL (5 and 10 µg) of AgNP nanoparticle solution was added into one or adjacent two wells of MHA plates. After hatching at 35°C for 18 hours, the zones of inhibition have been noticed 10.

4.2 Determination of the MIC of AgNPs

Firstly, resazurin reagent was produced and utilized according to method by 11.

The AgNPs bio-synthesized from MRSA strain were evaluated for antimicrobial effectivity against MRSA and MSSA; by resazurin microplate assay (REMA) outlined by CLSI 10. All experiment steps have don following the procedure done by 12.

4.3. Determination of MBC of AgNPs

The minimal bactericidal concentration (MBC) was direct specified by direct culturing the content on MHA of each well with higher concentrations than the MIC value. The MBC amount was specified when there was no province development showed for the straightforwardly plated substance of each well 11.

Results

The two studied isolates of Staph aureus identified phenotypically as MRSA and MSSA and then evidenced genotypically through identification of the 533 bp *mecA* gene. Figure 1.

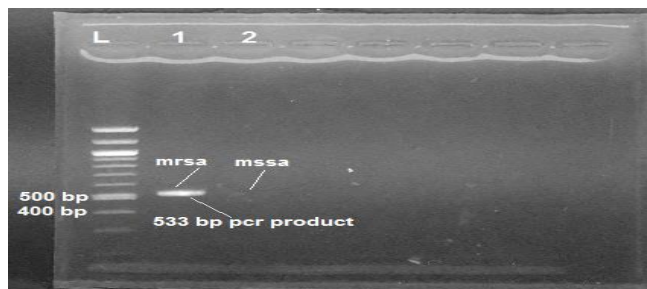


Figure 1. *mecA* gene detection the two Staph aureus isolates. Lane1 = *mecA* positive Staph aureus (MRSA); Lane 2= *mecA* negative Staph aureus (MSSA); Lane L= DNA molecular size marker (100 bp ladder).

The susceptibility profiles of tested MRSA AND MSSA to 15 antistaphylococcal antibiotics are offered in Figure 2 and Figure 3 individually. The MRSA isolate was multiple drug resistant, while MSSA isolate was not. In addition to

oxacillin MRSA was resistant to benzylpenicillin, imipenem, clindamycin, and vancomycin, whilst MSSA was sensitive to all tested antibiotics but benzylpenicillin and vancomycin.

The reducing of silver-ions into AgNPs has optically noticed after warming the hydrous solution consisting of silver-ions and MRSA essence, this prime solution was colorless, but with gradually adding more and more of the active ingredients to MRSA extract resulted in changing the color into brownish-red that reveals the forming of AgNPs. This appeared color belongs to the superficial plasmon resonance (SPR) of AgNPs, that is substantial trait of AgNPs which has found out by UV-visible-spectra as in Figure 4, that exhibited SPR summit of AgNPs settled around 444.5-451 nm and the same proclivity was spotted at diverse volumes of essence utilized after heating for 30 min.

The spotted XRD summit is established to be entirely connecting the different crystallographic planes, and the XRD style of our AgNPs was in accord with the reflection summit features of silver nanoparticles. Figure 5 shows the XRD pattern of the produced AgNPs, along with four distinct summits at $2\theta = 37.9^\circ, 44.4^\circ, 64.8^\circ,$ and 77.5° , which correspond to the (111), (200), (220), and (311) crystallographic planes of AgNPs (ICDD 04-0783).

Out of three balances (2, 1 and 0.5 m), AgNPs were visualized by SEM, and the image is given in Figure 6. The new AgNPs arrangement was poured on aluminum foil, after which it was allowed to dissolve the dissolvable and leave AgNPs. Consensus of the AgNPs has a circular form, as shown in the SEM image. The geology shows that there are circular AgNP molecules that wish to cluster together, like circles with a width of 41–45 nm, allocated on an AgNP agglomeration lattice. The antibacterial activity AgNPs was investigated against MRSA and MSSA using well-diffusion method. The inhibition zones MSSA isolate was higher than those of MRSA producing various but non-huge ($P > 0.05$). Figs. 7.

Minimum inhibitory concentration (MIC) of AgNPs was seen to evoke development inhibitory impact on the tried MRSA and MSSA two detaches at a fixation scope of 7.8 µg/mL and 3.9 µg/mL individually, creating extraordinary however non-noteworthy ($P > 0.05$) MICs., while complete growth elimination effect on the two tested isolates at a fixation scope of 31.3 µg/mL and 15.5 µg/mL individually creating extraordinary yet non-noteworthy ($P > 0.05$) MBCs. The variations seen in AgNPs MIC & MBC delivery against these two distinct strains of Staph aureus were generally unremarkable ($P > 0.05$). Figure 8.

Susceptibility Information			Analysis Time: 11.25 hours		Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation	
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S	
Cefoxitin Screen	POS	+	Clindamycin	<= 0.25	*R	
Benzylpenicillin	>= 0.5	R	Linezolid	2	S	
Ampicillin			Teicoplanin	<= 0.5	S	
Oxacillin	>= 4	R	Vancocmycin	>= 32	R	
Imipenem	<= 1	*R	Tetracycline	2	S	
Gentamicin High Level (synergy)			Tigecycline	<= 0.12	S	
Streptomycin High Level (synergy)			Fosfomycin			
Gentamicin	<= 0.5	S	Fusidic Acid			
Ciprofloxacin	<= 0.5	S	Rifampicin	<= 0.5	S	
Moxifloxacin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S	
Inducible Clindamycin Resistance	POS	+				

+= Deduced drug * = AES modified ** = User modified

Figure 2. VITEK-2 chart of antibiotic susceptibility of MRSA isolate.

Susceptibility Information			Analysis Time: 11.25 hours		Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation	
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S	
Cefoxitin Screen	NEG	-	Clindamycin	<= 0.25	S	
Benzylpenicillin	0.06	*R	Linezolid	2	S	
Ampicillin			Teicoplanin	<= 0.5	S	
Oxacillin	<= 0.25	S	Vancocmycin	>= 32	R	
Imipenem	<= 1	S	Tetracycline	<= 1	S	
Gentamicin High Level (synergy)			Tigecycline	<= 0.12	S	
Streptomycin High Level (synergy)			Fosfomycin			
Gentamicin	<= 0.5	S	Fusidic Acid			
Ciprofloxacin	<= 0.5	S	Rifampicin	<= 0.5	S	
Moxifloxacin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S	
Inducible Clindamycin Resistance	NEG	-				

+= Deduced drug * = AES modified ** = User modified

Figure 3. VITEK-2 chart of antibiotic susceptibility of MSSA isolate.

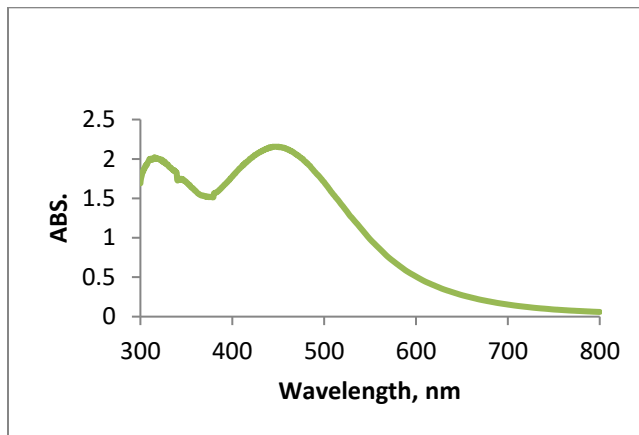


Figure 4. UV-visible spectrum of AgNPs synthesized by MRSA extract.

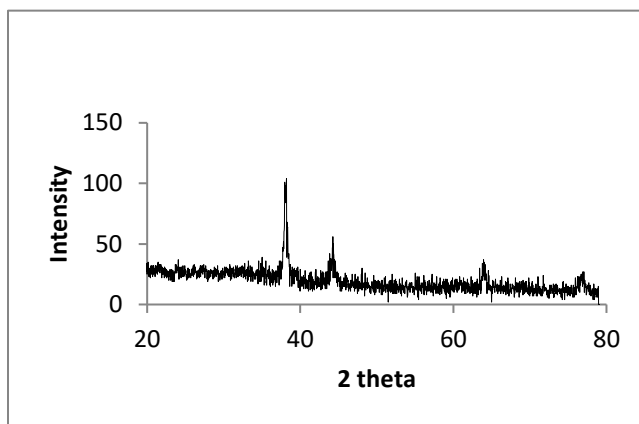


Figure 5. Powder XRD pattern of AgNPs.

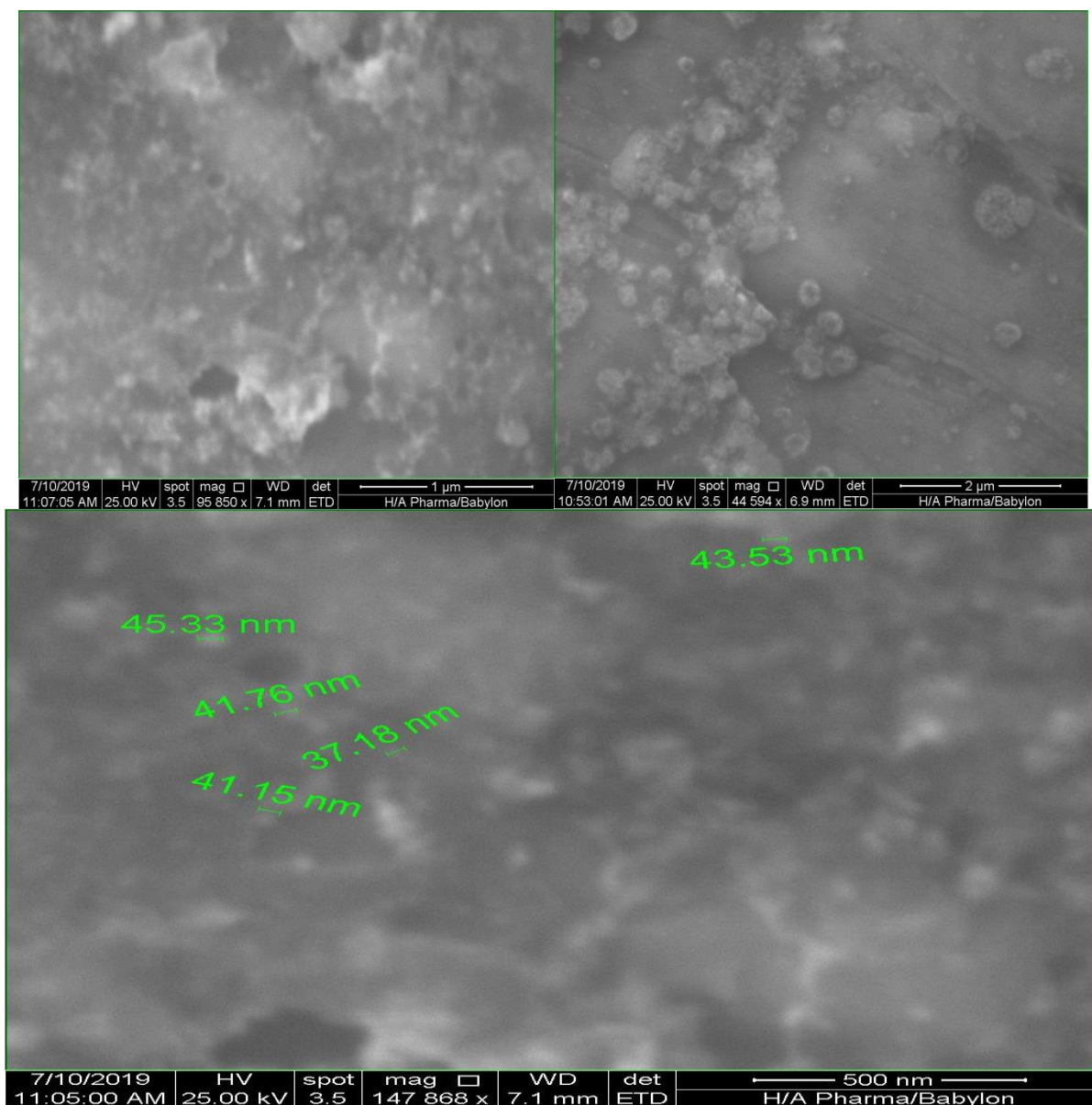


Figure 6. SEM picture of new combined AgNPs in 3 scales (1, 2 and 0.5 μm) that are orchestrated via add 4 ml of concentrate to 0.001 M of Ag particle at 80° C.

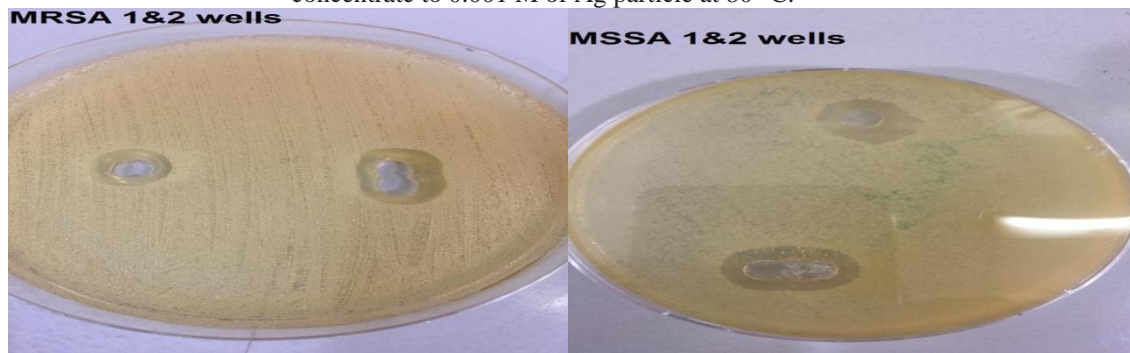


Figure 7. Inhibition zones of MRSA and MSSA isolates by well-diffusion method.

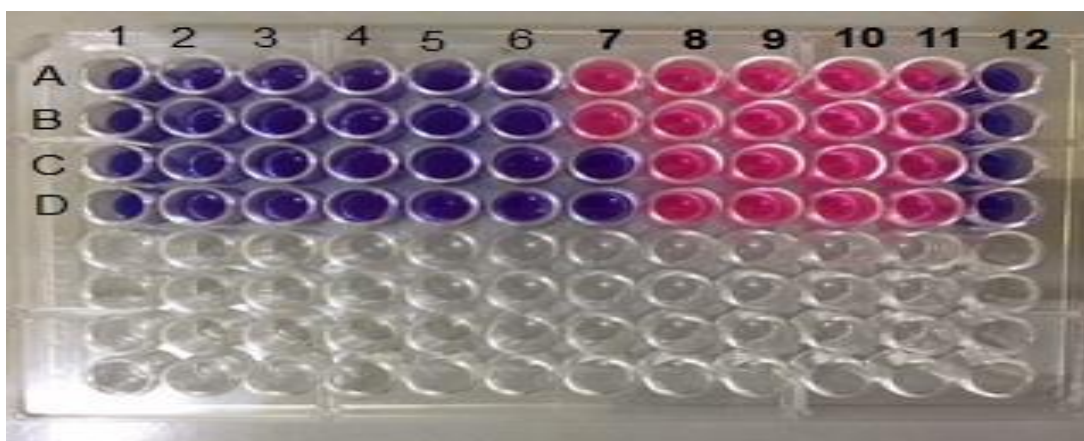


Figure 8. Microtiter plate demonstrating the microbroth dilution approach for MIC detection.

Discussion

The occurrence of MRSA, which is resistant to several drugs, and MSSA, which is very deadly, in medical clinics and network settings throughout many nations is the most severe worry about Staph aureus., including Iraq 13-17. MRSA detaches are generally resistant to beta-lactam medicines due to the transfer of the *mecA* quality 18,19. In this study, MRSA inspired 100 percent in vitro protection against all beta lactam anti-infection medicines tested: benzylpenicillin, oxacillin, and imipenem.

Our work is the first to assess the antibacterial activity of AgNPs generated from MRSA extracts against the same live strain as well as the MSSA strain.

The UV-Vis spectra recorded for the fluid silver nitrate-MRSA response medium as a component of time (Figure 4) show that the surface silver band is floured of AgNPs affixed around 444.5-451 nm, and a similar pattern was observed at various volumes of concentrates used following 30 minutes of warming. The surface plasmon band in the silver nanoparticles arrangement remains close to 444.5-451 nm throughout the response time frame, indicating that the particles are dispersed in the aqueous arrangement with no evidence of buildup. Following the completion of the reaction, the silver nanoparticles arrangement was examined for soundness. It was discovered that the silver nanoparticle arrangement was astonishingly balanced out for over a half year with no evidence of buildup even at the end of this length. The particles are therefore balanced out in arrangement by the covering specialist, which is most likely proteins released by the biomass. Comparable finds prompted investigations. 20,21.

The XRD example of the AgNPs, uncovers the mush focused cubic building with the determined grid boundaries, $a = b = c = 4.071 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$, which concur well with the past revealed amount 22.

The particles of AgNPs which was looked like a cloud, and this cloud was as an aftereffect of small AgNPs consolidated after dissolvable being vanished due to its high capacity,

large surface region, and fine size. Accordingly, there was a variation appropriation of size somewhere in the range of 20 and 50 nm, where the more modest amassed to frame a nebula or a bundles and another form is free and not converged with different particles, subsequently, they show to be plainly similar to a circle. Our outcomes was comparative to studies done by9,23, where AgNPs are portrayed as groups and packs [39].

The antibacterial activity AgNPs was investigated against MRSA and MSSA using well-diffusion method. The inhibition zones MSSA isolate was higher than those of MRSA creating unique however non-huge ($P > 0.05$). Figure 6.

The goal of the present study was to focus on the biosynthesis of AgNPs with the development of an extracellular cycle using MRSA, not only to make it simple, suitable, and inexpensive but also to generate stable nanoparticles. 24,25. Even while MRSA generates enough exoenzymes and a few toxins to be dangerous, we used the novel short-term culture in conjunction with nanoparticles to thwart the collaboration Silver plating. AgNP production from *Fusarium oxysporum* strains through extracellular cycle was shown by Duran et al. The downstream metal nanoparticle fuse method is simple and environmentally friendly. 26.

When MRSA supernatant was added to the AgNO₃, the Ag particle count was significantly reduced, and the shading changed from clear yellow to caramel red. The placement of AgNPs is indicated by the presence of a tanish red tone. 26. The surface vibration of AgNPs on the flour plate may have caused the median's earthy hue. 27. Since Roman times, silver has been used for its alleged general antibacterial properties. However, advances in the production of Ag-NPs have made the revival of the use of silver as a revolutionary bactericide possible. Due to their resistance to a broad variety of diverse anti-infection treatments, MRSA and MSSA were typically highlighted in the present assessment study. Sondi and Salosummit-Sondi demonstrated that AgNPs might be used as an antibacterial specialist by using *Escherichia coli* as a

model for gram-negative microscopic organisms. 28. Shahverdi et al additionally referenced that the AgNPs antimicrobially effect on Staph aureus and E. coli 29.

In our investigation, the estimates of the MIC & MBC of Ag-NPS against the two segregates of MSSA and MRSA were discovered in the range of 1.9-3.9 μ g/ml and 15.5-31.3 μ g/ml, respectively. The most conclusive evidence that MRSA and MSSA are susceptible to AgNPs may result from the plasmolysis of their cell dividers or the separation of cytoplasm from those dividers. 17. The effectiveness of bionanosilver antibacterial devices might vary depending on the kind of microscopic organisms and nanoparticle volume. The earlier work by Song et al., who suggested that the susceptibility of MRSA is caused by the obstruction of bacterial cell division union, has advanced the helplessness of MRSA to AgNPs. 30.

Since the mean of the MIC & MBC foci observed in our inquiry were never seen by any other examination up to this point, our conclusion may be unique. The results of Iwalokun et al., who discovered that AgNP evoked development inhibitory effects against Staph aureus through a focus range of 2.5-10 μ g/mL with typical MICs of 4.7 μ g/mL and 4.9 μ g/mL, respectively, against together MRSA and MSSA, provided the closest MIC & MBC values to our investigation. 31. While Fernandez et al indicated that MIC & MBC estimations of Ag-NPs of 12.5 μ g/ml and 25 μ g/ml, separately for Staph aureus 32. Martinez et al revealed that Ag-NPs were inhibitory at convergence of 16.67 μ g/ml against Staph aureus 33. Then again our outcomes demonstrated extremely high antibacterial action when contrasted with before action of Ayala-Nunez et al have revealed MIC & MBC estimations of Ag-NPs 1800 μ g/ml and 2700 μ g/ml, individually 34.

Conclusions

During the course of our research, the extracellular union of bioactive AgNPs became much more concentrated. Clearly, MRSA may be used to efficiently construct bioactive nanoparticles using less material in an eco-friendly and nontoxic setting. The interaction of bioactive nanoparticles with MRSA vaccination in AgNO₃ was seen in just a few long areas.

UV-vis spectrophotometry of Ag particles injected to this bacterial supernatant validated the transformation of Ag particles into Ag-NPs. The earthy red pattern of colloidal AgNPs was formed by combining AgNPs using MRSA disengage removal after heating. AgNPs display SPR with the highest retention at 444.5-451 nm.

Ag-NPs generated from MRSA shown 100% efficacy in contrast to a similar disengage. The impediment zone demonstrated antibacterial activity against MRSA and MSSA in the testing phase. The activity demonstrates that the silver nanostructures produced by this cycle are suitable for application in nanomedicine.

In the long run. Due of its ability to battle multidrug-resistant bacteria, nanobiotechnology is a promising and significant field of research. To increase the efficacy of Ag-NPs as a bactericidal agent for in-vivo use, it is necessary to do more study on the adverse effects and features of human cells and bacteria.

Conflict of Interests

The writers publicize that there is no genuine or expected irreconcilable circumstance corresponding to this article.

REFERENCES

- Ventola, C.L. The Antibiotic Resistance Crisis. P T., 2015; 40(4): 277-83.
- Stapleton, P.D, Taylor P.W. Methicillin resistance in Staphylococcus aureus mechanisms and modulation. Sci Prog. 2002; 85(1): 57-72.
- Narayanan N., Adams C.D., Kubiak D.W., et al. Evaluation of treatment options for methicillin-resistant Staphylococcus aureus infections in the obese patient. Infect Drug Resist. 2019; 17; 12: 877-891.
- Rangari I A.A., Sharma I.N.K., Goyal M.R. et al. Evaluation and Efficacy of In-Vitro Antibacterial Activity of Silver Nano Particles Against Multidrug Resistant Bacterial Isolates from Skin Infections of Patients at a Tertiary Care Hospital in Western Uttar Pradesh of India. Int J Curr Microbiol App Sci, 2015; 4(10): 764-773.
- Parades D., Ortiz C., Torres R. et al. Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against Escherichia coli O157:H7 and methicillin-resistant Staphylococcus aureus (MRSA). Int. J Nanomed, 2014; 9: 1717-1729.
- Omar N.Y., Ali H.A.S., Reem Abdel Hameed Harfoush R.A., El Khayat E.H. Molecular Typing of Methicillin Resistant Staphylococcus aureus Clinical Isolates on the Basis of Protein A and Coagulase Gene Polymorphisms. Int J Microbiol, 2014; 650328.
- Nanda A., Saravanan M. Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomed Nanotech Bio Med, 2009; 5(4): 452-456
- Hashim R., Oda A.M. and Fadhil I. Green synthesis of Silver Nanoparticles by Beta vulgaris(Chard) Extract: Characterization and Antibacterial Activity. As J Chem, 2019; 31(8):1881-1884
- Oda A.M., Abdulkadhim H., Jabuk S.I.A., Hashim R. et al. Green synthesis of silver nanoparticle by cauliflower extract: characterization and antibacterial activity against storage. IET Nanobiotechnol, 2019; 13(5): 530-535.
- Clinical and laboratory standard institute. Performance standards for antimicrobial susceptibility. Twenty-fourth information supplement, 2016; CLSI document M100-S26. CLSI, Wayne, PA.
- Elshikh M., Ahmed S., Funston S. et al. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett, 2016., 38: 1015-1019.
- Coban A.Y. Rapid Determination of Methicillin Resistance among Staphylococcus aureus Clinical Isolates by Colorimetric Methods. J Clin Micro, 2012; 50(7): 2191-2193.
- Tong S.Y.C., Davis J.S., Eichenberger E., Holland T.L., Fowler V.G. Staph aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbiol. Rev, 2015; 2: 603-661.
- Diekema D.J., Pfaller M.A., Schmitz F. J., Smayevsky J., Bell J., Jones R.N., Beach, M., SENTRY Participants Group. Survey of Infections Due to Staphylococcus Species: Frequency of Occurrence and Antimicrobial Susceptibility of Isolates Collected in the United States, Canada, Latin America, Europe, and the Western Pacific Region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin. Infect. Dis, 2001; 32(2): S114-32.
- Laupland K.B., Church D. L. Population-Based Epidemiology and Microbiology of Community-Onset Bloodstream Infections. Clin. Microbiol. Rev, 2014; 27: 647-664.
- Ali M.A., Al-Mathkhury H.J.F. Incidence of Ciprofloxacin-Resistant of

- Methicillin Resistant *Staphylococcus aureus* isolated from Iraqi patients. *Iraqi J Sci*, 2018; 59(3A): 1225-1230.
17. Jassim F.A. Bacteriological Evaluation of Diabetic Foot Infections of Patients in Al- Salam Hospital in Mosul City/ Iraq and Antibiotic Sensitivity Pattern. *IJAR*, 2014; 2(5): 614-623.
 18. Abdulgader S.M., Shittu A.O., Nicol M.P., Kaba M. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus Aureus* in Africa: A Systematic Review. *Front Microbiol*, 2015; 6: 348.
 19. Liu Y., Zhang J., Ji Y. PCR-based Approaches for the Detection of Clinical Methicillin-Resistant *Staphylococcus Aureus*. *Open Microbiol J*, 2016; 10: 45–56.
 20. Kolya H., Maiti P., Pandey A., Tripathy T. Green synthesis of silver nanoparticles with antimicrobial and azo dye (Congo red) degradation properties using *Amaranthus gangeticus* Linn leaf extract. *J Anal Sci Technol*, 2015; 6(33): 1-7.
 21. Dubey S.P., Lahtinen M., Särkkä H., Sillanpää M. Bioprospective of *Sorbus aucuparia* leaf extract in development of silver and gold nanocolloids. *Colloids Surf. B: Biointerfaces*, 2010; 80(1): 26-33.
 22. Anuratha M., Jawahar A., Umadevi M., Sathe V.G., Milton Franklin Benial A. Adsorption of N-(1-(2-bromophenyl)-2-(2-nitrophenyl)ethyl)-4-methylbenzenesulfonamide on silver nanoparticles: SERS investigation. *Acta A*, 2015; 138: 234-240.
 23. Muhammad A.R., Zakia K., Anum R., et al. Size- and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes', *Nanomat*, 2016; 6: 74.
 24. Mukherjee P., Senapati S., Mandal D., Ahmad A., Khan M.I., Kumar R., et al. Extra cellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. *Chem Biochem*, 2002; 3: 461-3.
 25. Ahmad A., Mukherjee P., Senapati S., Mandal D., Khan M.I., Kumar R., et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B*, 2003; 28: 313-8.
 26. Sastry M., Ahmad A., Khan M.I., Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycetes. *Curr Sci*, 2003; 85: 162-70.
 27. Ahmad A., Senapati S., Khan M.I., Kumar R., Sastry M. Extra-/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, *Trichothecium* sp. *J Biomed Nanotechnol*, 2005; 1:47-53.
 28. Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloids Interface Sci*, 2004; 275: 177-82.
 29. Shahverdi A.R., Fakhimi A., Shahverdi H.R., Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine*, 2007; 3: 168-71.
 30. Song H.Y., Ko K.K., Oh I.H., Lee B.T. Fabrication of silver nanoparticles and their antimicrobial mechanisms. *Eur Cells Mater*, 2006; 11:58.
 31. Iwalokun B.A., Akinloye O., Udoh B.E., Akinyemi K. O.. Efficacy of silver nanoparticles against multidrug resistant clinical *Staphylococcus aureus* isolates from Nigeria. *J Immuno Immunoch*, 2019; 40(2): 1-23.
 32. Fernandez E.J., Garcia-Barrasa J., Laguna A., Lopezde Luzuriaga J.M., Monge M., Torres C. The preparation of highly active antimicrobial silver nanoparticles by an organometallic approach. *Nanotech*, 2008; 19:1-6.
 33. Martinez-Castanon G.A., Nino-Martinez N., Martinez Gutierrez F., Martinez-Mendoza J.R., Ruiz F. Synthesis and antibacterial activity of silver nanoparticles with different sizes. *J Nano Res*, 2008; 10:1343-1348.
 34. Ayala-Nunez N.V., Lara H.H., Turrent Liliana del C.I., Padilla C.R. Silver nanoparticles toxicity and bactericidal effect against Methicillin-resistant *Staphylococcus aureus*: Nanoscale does matter. *Nanobiotech*, 2009; 5(1): 2-9.