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The Antibacterial Activity of Silver Nanoparticles and Detection of the *Fnba* Gene in *S. Aureus* Isolated from Burn Patients

Reyam Majeed Hassan*

Zainab Dakhil Degaim*

* Department of Microbiology, College of Medicine, University of Thi-Qar, 64001, Iraq

Email: riam.m.msc@utq.edu.iq; zainab-d@utq.edu.iq

Abstract: The purpose of the recent study was to profile of biofilm formation gene *(fnbA*) in *S. aureus* which isolated from burn patients, and to detect the antibacterial activity and MICs of silver nanoparticles against pathogenic *S. aureus* isolated from burn wounds. One hundred and sixty samples from burn patients were collected from August to October 2023 in a burn center in the Thi-Qar province of Iraq. The *S. aureus* was only identified in (35; 21.90%) isolates. Microscopical examination, morphological characterization, diverse biochemical testes, were used to recognize *S. aureus* isolates. The PCR technique results recorded that 42.8% of S. aureus isolates had the *fnbA* gene. The Ag NPs had antibacterial activity against completely *S. aureus* isolates.

Keywords: *S. aureus*, burn, Ag NPs, MIC, *fnbA* gene, PCR.

Introduction : Annually, about one million individuals worldwide suffer from burns, making it one of the most prevalent injuries globally (1). Exposure to heat, radiation, electricity, or chemicals can cause damage to the skin, leading to a burn. Severe burns can result in significant complications, including sepsis caused by a bacterial infection, shock due to low blood volume, or the formation of scar tissue due to improper wound healing. Skin injury leads to the death of skin cells, resulting in a significant depletion of body fluids, dehydration, disruption of electrolyte balance, renal failure, and subsequent circulatory collapse. An infection presents a significant risk to the lives of individuals who have sustained burns. As a result of the lack of protection offered by intact layers of the skin, burned skin is very vulnerable to pathogens and other illnesses (1).

The *S. aureus*, was one of the causative agents of infections in burn. It has the ability to gradually and indefinitely establish itself in the nasal cavity of healthy individuals. However, in cases of compromised immune function, the body is susceptible to infecting nearby skin and soft tissues and can even invade deeper tissues and the bloodstream, resulting in systemic infections (2). *Staphylococci* are often detected as pathogens in chronic wounds (3). In these types of wounds, cellular aggregates typically form and are surrounded by a self-produced extracellular matrix (4).

S. aureus infection involves the presence of several virulence factors that contribute to the processes of colonisation, dissemination, and immune evasion. *Staphylococci* are responsible for more than 80% of bacterial infections in persons (5). Biofilms are responsible for 60% of burn-related deaths, making them a significant concern in burn cases (6). *S. aureus* produces two separates yet closely related multifunctional cell wall-anchored (CWA) proteins that specifically attach to the host glycoprotein fibronectin. The fibronectin-binding proteins (*FnBPA* and *FnBPB*) consist of two separate domains (7). One of the first proteins of Gram-positive bacteria to be defined was the fibronectin binding proteins (*FnBP*s) (7). *Fnb*A gene facilitate the colonization and infection of the host by *S. aureus* via adhesion to Fn present in the extracellular matrix of the tissues. Following adhesion, *S. aureus* is able to invade a variety of non-professional phagocytic mammalian cells, such as epithelial cells, fibroblasts, endothelial cells, osteoblasts (8).

Nanotechnology includes the study and application of science, engineering, and technology at a size of 1 to 100 nanometers. Silver nanoparticles have become a prominent research topic in recent years, among the several types of nanoparticles. (9). Ag NP have great antibacterial characteristics due to their large surface-to-volume ratio, allowing them to effectively combat bacteria even at low concentrations (10). Additionally, they have low costs and have shown minimal harm to cells. Silver nanoparticles have many potentials uses in the field of biomedicine (11). Silver compounds are commonly utilized in medicine to treat burns, wounds, and many infectious diseases (12). Ag+ is combined with microbial DNA and RNA to cause damage, Ag+ inhibits protein translation by destructing 30S ribosomal subunit. And the cell wall synthesis in gram-positive bacteria (13, 14). These nanoparticles exhibit antibacterial properties by adhering to the cell membrane surface, causing disruption of cellular permeability and respiration processes, and inactivating membrane proteins (15). The purpose of this study was to profile of *fnbA* gene in *S. aureus* isolated from burn patients; also, to determine the antibacterial effects and MIC of Ag nanoparticles against *S. aureus.*

Material and methods

Samples Collection and bacterial identification

One hundred and sixty swabs were collected from burn patients, whom were admitted to burn center in the Nasiriyah Hospital during the period from August to October 2023. The specimens were transported in a sterile, leak-proof container to the laboratory, and cultured on blood agar, mannitol salt agar (Biolab, Hungary), Chrom agar (HI media, India), then incubated aerobically for 24 h at 37 °C, the pure isolated colonies of S. aureus were subsequently transferred onto BHI agar medium for the purpose of preservation and conducting additional biochemical tests, which helped confirm the identification of the isolates.

Antibacterial activity & MIC analysis of Ag nanoparticles against *S. aureus* The antimicrobial activity of Ag NPs was assessed by Agar well diffusion method against *S. aureus* obtained from burn infections. *S. aureus* suspended in D.W, the turbidity generated by the growth was adjusted to match the 0.5 McFarland standard (1.5 X 108 CFU/ml) requires an optical density. A sterile cotton swab was submerged in the suspension and the swab was dipped and used to evenly spread across the whole surface of a Mueller Hinton agar plate. Using a sterile cork-borer, pores with a diameter of 7 mm were formed and filled with 100 µl of Ag NPs with a concentration of (25µg/ml) and using the distal water as control. After 24 hours, the Petri plates were placed in an incubator set at 37°C. To determine the antibacterial activity, the growth inhibition zones were measured in millimetres in diameter (16).

To determination of the MICs of the Ag NPs was evaluated using the broth micro-dilution method in a 96-well microtiter plate following the criteria of the Clinical and Laboratory Standards Institute (17). In brief, Bacterial isolates were cultured overnight in BHIB media (Tm, India), then diluted to achieve a turbidity equivalent to 0.5 McFarland Standard using D.W. Fifty µL of diluted testing substances were added to the wells of a dilution plate, and 50 µL aliquots of the bacterial suspension were delivered into the wells of this plate. After being incubated at 37 °C overnight, the MIC of the tested agents that stop the observable growth of bacteria were determined and defined as the MIC values.

Extraction of *S. aureus* DNA and PCR technique

The Presto™ Mini gDNA Bacteria Kit was utilized to extract the DNA from all isolates of *S. aureus*. The pairs of specific primer of *fnb*A gene as following: forward: 5'- ATC AGC AGA TGT AGC GGA AG -3' and reverse: 5'- TTT AGT ACC GCT CGT TGT CC -3' (18). The total volume of the PCR reaction was 20 µl, which consisted of the following components: 12.5µl of Master Mix (Geneaid/Taiwan), 3µ liters of bacterial DNA, 1µ liter of forward and reverse primer for the *fnbA* gene, and the remainder of the volume was filled with nuclease-free water. The program of PCR for *fnbA* gene: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min, extension at 72°C for 1min and final extension for 5min after the last cycle. Agarose gel electrophoresis of PCR product was carried out in 1.4% agarose gel and the presence of a 156bp band indicate a positive result for *fnb* A gene (18).

Ethical approval: The present investigation followed to the ethical standards outlined in the Declaration of Helsinki. The committee of researchers at the Thi-Qar Health Directorate (No. 2023/165 on 8/8/2023) has viewed and approved this study. and verbal consent was obtained from all patients after explaining the purpose of the study.

Statistical analysis: The data of the current study was statistically analysis by using SPSS based in using Chi-square, one-way ANOVA, LSD.

Results

Bacterial isolation and identification

Out of the 160 from the samples taken from burned individuals in the Thi-Qar province, only 35 (21.90%) of the isolates were able to ferment mannitol and appeared as yellow colonies on the MSA medium, and which grown as green colonies on chrom agar. these colonies were identified as *S. aureus,* as shown in Fig. (1). On the other hand, 53 out of the 160 isolates (33.10%) were identified as other bacterial species. There were 72 samples (45%) that did not show any growth, as depicted in Fig. (2). Completely isolates of *S. aureus* were identified using biochemical assays, including*:* Catalase, Coagulase and DNase tests which gave positive results for those tests. Statistically, there were a significant difference at (p.<0.001) among bacterial groups.

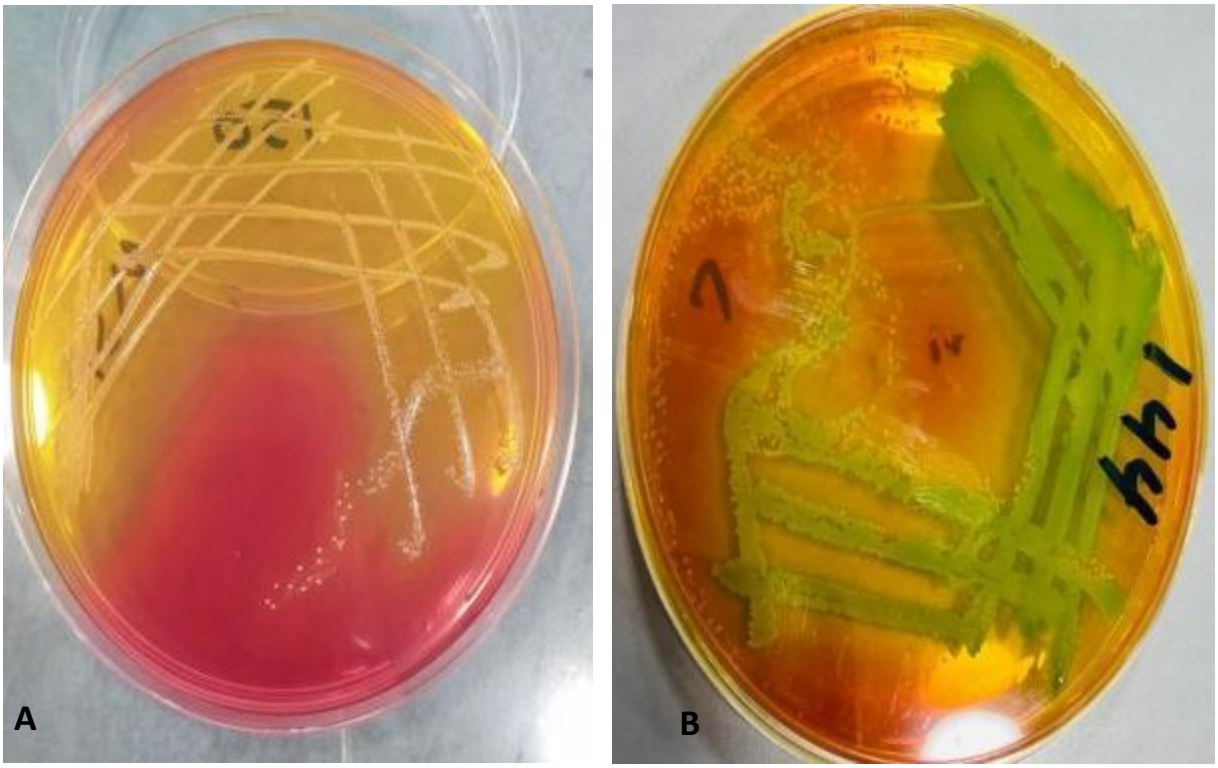
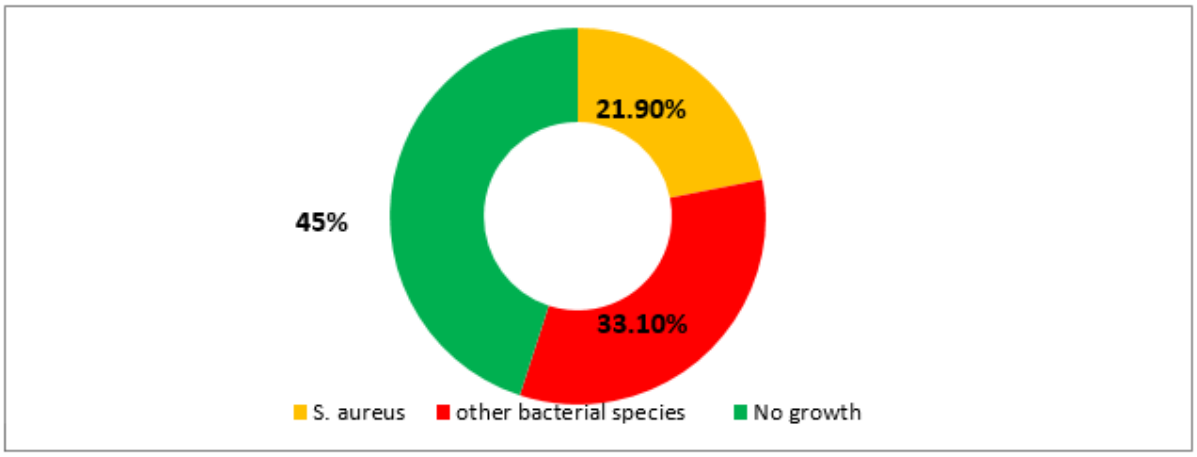


Figure (1):The appearance of *S. aureus* on A- MSA B - chrom agar.



CalX2= 431, TabX2=7.81, df=3, p. value < 0.001

Figure (2): Distribution of bacterial groups in burn patients.

The current results showed a significant difference at (p. < 0.05; 0.006) of the most isolated *S. aureus* in the patients with third degree of burn (45.71%), followed in the patients with second degree (34.29%), while the lowest isolated *S. aureus* in the patients with first degree of burn (20.0%), as presented in table (1).

Table (1): The distribution of *S. aureus* according to burn grade

Burn Grade	***S. Aureus***	
	No.	**%**
1st Grade	**7**	**20.0**
2nd Grade	**12**	**34.29**
3rd Grade	**16**	**45.71**
Total	**35**	**100**
Cal X^2= 10.1 Tab X^2= 5.99 D= 2 P. Value. 0.006		

Antibacterial activity and MIC of Ag NPs against *S. aureus* isolates

The effect of Ag NPs against completely S. *aureus* isolates recorded that this agent had antibacterial activity, as shown in table (2).

Table (2) : Antibacterial activity of Ag NPs against all isolates of *S. aureus*

Inhibition Zone (Mm)	No. Of Isolates	P. Value < 0.001
12-15	3	LSD= 1.76
16	8	
18-20	11	
22-26	9	
27-28	4	

The results of MIC test of Ag NPs against completely *S. aureus* isolates by microdilution method revealed that the turbidity was noticed in microtiter plate wells, 3.125 and 1.562 mg/ml containing Ag nanoparticles indicating the growth of bacteria, and the concentration (6.25 mg/ml) as MIC against *S. aureus* isolates, as revealed in figure (3).

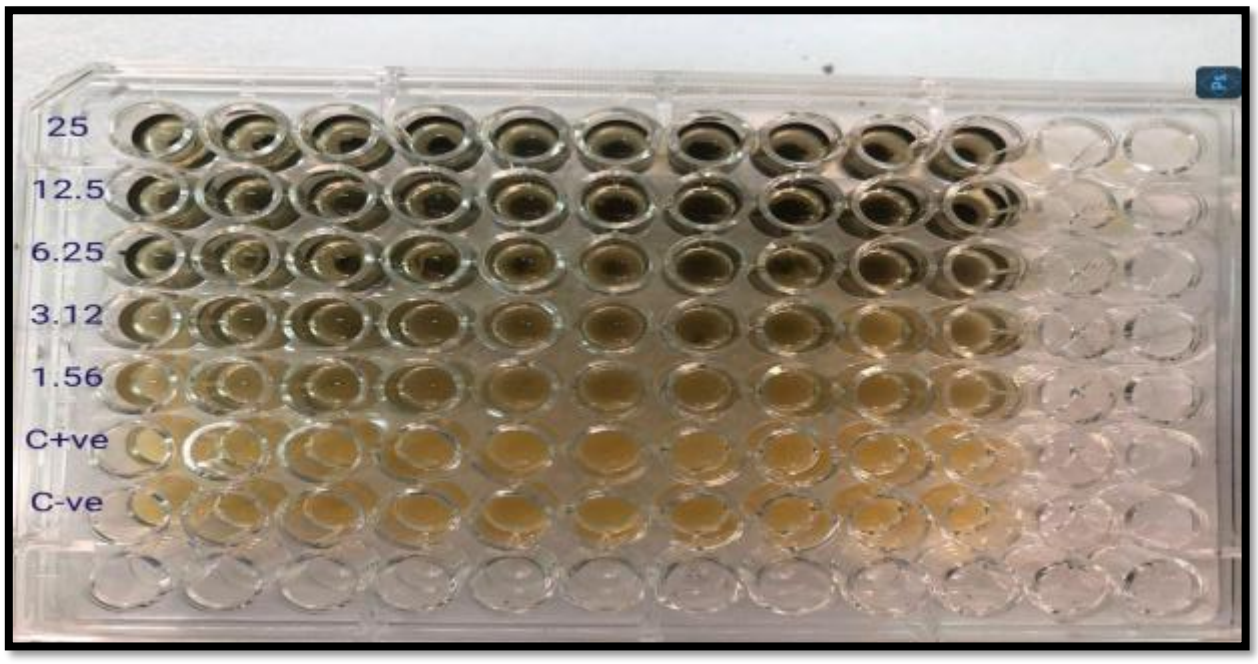


Figure (3): The MIC test of Ag NPs by microdilution method

Molecular diagnosis

The results of PCR technique showed (15/35; 43%) of *S. aureus* isolates giving positive results for *fnbA* gene. The bands of *fnbA* gene which determined the size of *fnbA* gene, nearly 156bp, as shown in Figure. (4)

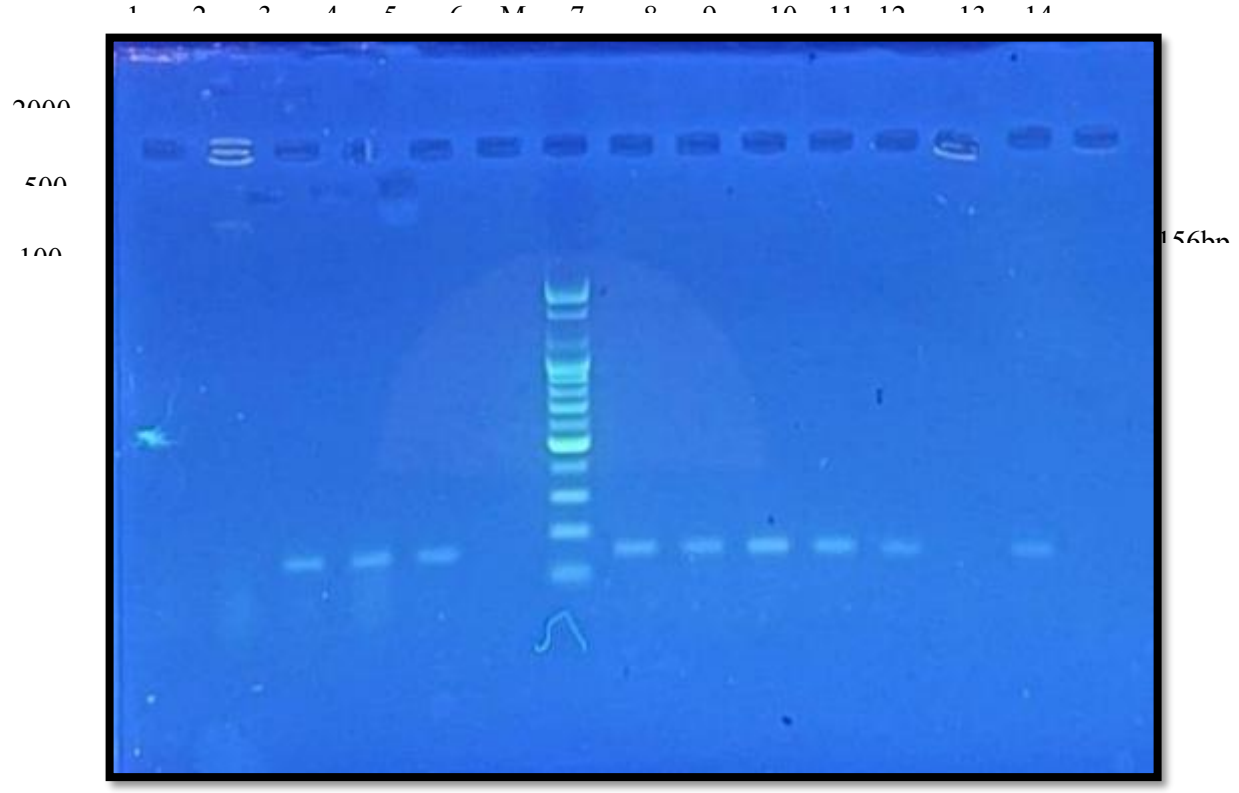


Figure (4): Agarose gel electrophoresis of *fnbA* gene amplification, wherever: M : ladder, 3,4,5, 7,8,9,10,11,13 positive results ; 1,2,12,14 negative results.

Discussion

From burn wound swabs from burn patients, approximately 35 (21.90%) exhibited mannitol fermentation on MSA. *S. aureus* is the commonest pathogenic bacteria found in different wound specimens. In recent decades, the prevalence of infections caused by *S. aureus*, namely MRSA strains, in burn centers has emerged as a significant global health concern (19). According to multiple publications, there is an increasing incidence of *S. aureus* infection among burn patients with severe wounds who are admitted to specialized burn centers. Consequently, significant focus has been given to this problem in recent years (20). Continuous surveillance of infections caused

by *S. aureus* in hospitalized patients is crucial for both preventing and managing these infections, as well as ensuring appropriate and effective treatment (21).

The present results were closely matched with local study conducted in Misan, which found that 18 out of 105 samples (17.14%) were identified as *S. aureus* indicated that *S. aureus* is the primary bacterium found in burn wounds (22).

Studies in the USA found that coagulase-negative *Staphylococci* and *S. aureus* are the most prevalent among their patient groups, with rates of 47.1% and 44.1%, respectively. Gram-negative bacteria (26.5%) have the lowest rate of growth (23).

The present results disagreed with several studies like: the locally study (24) documented that (85/276; 67.460%) identify as methicillin resistance *S. aureus* (MRSA) which collected from burn patients.

The results of PCR technique revealed 43% of the biofilm encoding gene establish in pathogenic *S. aureus* isolated from burn wounds. The examined gene (*fnbA*) gene may be related with increased pathogenicity of pathogenic *S. aureus*. Various microbial factors contribute to the attachment of *S. aureus* to various surfaces. *S. aureus* produces a range of microbial surface components recognizing adhesive matrix molecules that interact with extracellular ligands in the host. (25).

fnbA and *fnbB*, have a crucial impact on the pathogenicity of *S. aureus* due to their capacity to attach to fibronectin and fibrinogen and trigger the uptake of the bacterium by non-professional phagocytes such endothelial cells through integrin-mediated mechanisms (26). Given that biofilm formation enhances resistance to antimicrobial agents and antibiotic resistance, this finding has significant importance. other researcher (27), discovered that the ability of *S. aureus* isolates to respond to antimicrobial mediators generated by mast cells is linked to the increased production of α-hemolysin, fibronectin-binding protein A, and several regulatory mechanisms.

The current results were closely agreed with (28) noticed the results of the genetic analysis of adhesion and biofilm genes as: *fnbA* in (35/83; 42.1%). Also, the research conducted by (29) which found *fnb*A was present in 82.2% of MRSA strains and *fnb*B in 46.7%. Several studies were disagreed with recent results like: (30) revealed that the prevalence of *fnbA* gene was (4.9%); while the study done in Iran observed that the incidence of *fnbA* gene much higher than in the present data (75.7%) (31). Also, other study reported that (81%) of isolates had the *fnbA* gene (32); and (33) recorded that among the examined *S. aureus*, (19/32; 59%) harbored *fnbA* gene.

The results of antimicrobial activity and MIC of Ag nanoparticles against *S. aureus* recorded that the concentration of 25 mg/ml was the best antimicrobial activity due to efficient diffusion in the

agar medium, causing an inhibition zone, referring to the fact that Ag NPs might cause inhibition of bacterial growth.

The MIC of nanoparticles against examined *S. aureus* was (6.25mg/ml). The scientific community aims to develop a successful alternative to replace available antibiotics that are ineffective due to resistance. Additionally, it serves as an effective alternative to antibiotics due to the increasing challenge of Drug Resistance bacteria. Nanoparticles are being considered as a potential substitute for antibiotics for solving the issue of bacterial resistance (34). The results of study performed by (35,36) showed the MIC concentration against *S. aureus* was (0.039 mg/mL; 0.625 mg/ml), respectively; while, (37) revealed that Ag NPs had high activity with slightly concentration against *S. aureus* isolates as MIC (12.5 µg/mL).

Conclusion

The silver nanoparticles have potential bacterial effects against pathogenic *S. aureus*.

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