Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Silver Nanoparticles Synthesized from Acacia glauca Leaves: A Promising Agent Targeting Virulent Genes of Staphylococcus aureus

Reem Mahdi Saleh
University of Anbar
Omar Mohammed Hassan
sc.omerhasan@uoanbar.edu.iq
University of Anbar

Research Article

Keywords: silver nanoparticles, chitosan, quorum sensing, virulence, genes, S. aureus

Posted Date: April 25th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4282121/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Nowadays, antibiotics are the only therapeutic agents against *Staphylococcus aureus* infection. Widespread antibiotic resistance poses a threat to the world. Several studies have attempted to test whether alternative agents acting alone or in synergy with antibiotics can overcome this problem. For many years, silver nanoparticles have demonstrated multi-level action targeting the physiological activities of bacteria. The study aimed to synthesize silver nanoparticles by the green method from *Acacia glauca* leaves stabilized by chitosan (Ch-AgNPs) and evaluate their effect against the expression of a set of quorum sensing and virulent genes in MDR *S. aureus*. Ch-AgNPs were characterized by physicochemical tests including UV, X-ray, FTIR, TEM, FESEM, XRD, and zeta potential. Minimum inhibition concentration (MIC) was used to detect the antibacterial activity of Ch-AgNPs. After MIC treatment, the growth curve of *S. aureus* was plotted. The expression of genes (*gyrb*, *AgrA*, *AgrB*, *RNAIII*, *mecA*, *rot*, *spa*, *hla*, *coa*, and *psm*) was evaluated before and after exposure to Ch-AgNPs by quantitative real-time PCR. The dark brown color was the primary indicator of Ch-AgNPs formation. Ch-AgNPs showed absorption at 430 nm. The particles had a round and regular shape with an average size of 8 nm. The synthesized nanoparticles have a high degree of crystallinity, with thin peaks at 2θ° of 38.200 and 44.250. At +33 mV, the zeta potential confirmed high colloidal stability. The synthesized nanoparticles showed a high antibacterial effect with a MIC of 2.1 µg/mL, inhibiting the growth of *S. aureus*. All identified genes showed a significant decrease in their expression by RT-PCR after exposure to a subMIC of Ch-AgNPs, except for the *gyrb* gene, which is a housekeeping gene. Silver nanoparticles made from Acacia glauca leaves have the potential to be an effective antibacterial agent. They exert effects at a molecular level against the quorum sensing and virulence genes of MDR *S. aureus*.

Introduction

Today, the world is rapidly moving towards the development of natural product nanoparticles as a means to combat chemical hazards and gases. Biomolecules are favorable to nanotechnology for developing metal nanoparticles of biological molecules characterized by cost-effectiveness and authenticity (Kumara Swamy et al. 2015). Nanoparticles made of metals have drawn attention because of their wide usage in different biomedical fields (Obaid et al. 2023). Synthesis of AgNPs by plant extracts is considered an excellent tool because these extracts contain a wide range of metabolites such as gallic acid, ascorbic acid, quinones, and water-soluble flavones that cause quick reduction of silver compared to microbes and fungi (Al-Zahrani et al. 2021). Chitosan has grown in its application area; it acts as a stabilizing agent (Collado-González et al. 2017). Cytotoxicity and low stability are the main problems silver nanoparticle applications face. Some studies that search for effective approaches to reduce cytotoxicity and enhance solubility showed that AgNPs modifications with biopolymer coating may change their biological activity and toxicity (Sanyasi et al. 2016; Gherasim et al. 2020). From the point of view of an eco-friendly reducing and stability agent, chitosan deserves special attention in the synthesis of AgNPs (Fiorati et al. 2020; Jouyban and Rahimpour 2020).

Genus Acacia is considered the second largest in the Fabaceae family, with more than 1200 species. Different parts of Acacia (rot, leaves, pods, and bark) have been used for the isolation of a number of pharmacological molecules (Sanchez et al. 2018). Acacia leaves contain tannins, condensed tannins, and phytophenolic compounds in large amounts (Batiha et al. 2022). Different extracts from Acacia are documented for their antioxidant, cytotoxicity, and antimalarial effects (Sadiq et al. 2017). Also, they proved their role in scavenging superoxide anions and having inhibition ability against *Staphylococcus aureus* (Alam et al. 2017).

Staphylococcus aureus is considered one of the essential pathogens for humans, forming the major reason for bacteremia, skin and soft tissue infections, endocarditis, pleura pulmonary, and osteoarticular disease (Tong et al. 2015). For many years, the ability of microorganisms to tolerate harsh conditions like temperature, acidity, nutrient diminishing, toxic materials, and ultraviolet has prompted researchers to get attention to study this phenomenon. The development of molecular biology contributes to understanding their survival strategies (Kim and Yeon 2018).

A quorum sensing system is a mechanism used by bacterial communities to detect and respond to bacterial density by secreting chemical signals that control gene expression and regulation (Goswami 2017). The emergence of new bacterial strains that are multidrug-resistant has drawn attention to the need to develop new treatment methods; thus, the obstruction of QS chemical signals has been researched as one of the possible controlled strategies (García-Contreras 2016; Santhakumari and Ravi 2019). In a direct molecular mechanism, RNAIII can inhibit surface proteins like protein A and block the translation of the Rot protein (Biosset et al. 2007). Transcription of toxins and exoproteins is blocked by Rot binding, which leads to up-regulation of alpha-toxin, enterotoxins, degradative exoenzymes, and down-regulation of surface proteins (Da et al. 2012). Also, recent studies found that the response regulator AgrA, by binding to the operon promoter sequence, can directly up-regulate Phenol-soluble modulins (PSMs) (Queck et al. 2008). AgNPs have confirmed their role as a promising alternative to compete with different microorganisms. Their unique antibacterial features can inhibit MDR strain growth (Shaikh et al. 2019). No previous information is available on the synthesis of silver nanoparticles from the plant species *Acacia glauca*. Therefore, this study aimed to biosynthesize Ch-AgNPs using *Acacia glauca* leaves and evaluate their effect on gene expression of quorum sensing and virulence factor genes in *S. aureus*.

Materials and methods

Preparation of *Acacia glauca* leaf extract

Fresh leaves of *Acacia glauca* were collected from a tree in the garden of the College of Science, University of Anbar, Iraq, in March 2023. The leaves were left to dry in the open air, then cleaned and ground using a pestle and mortar. Next, 10 g of *Acacia glauca* leaf powder was dissolved in 90 mL of distilled water on a magnetic stirrer for 3 hours. The resulting extract was filtered using Whatman filter paper (No. 1), then sealed in airtight vials and stored at 4°C.

Biosynthesis of chitosan-silver nanoparticles

Chitosan-coated silver nanoparticles (Ch-AgNPs) were synthesized following the method described in previous studies with some modifications (Hassan et al. 2020; Mutter and Hassan 2024). Briefly, 5 mL of previously prepared Acacia glauca leaf extract was added to 15 mL of 0.1% chitosan and 80 mL of 20 mM $AgNO_3$, followed by heating at 80 °C on a magnetic stirrer for 30 minutes. The initial formation of Ch-AgNPs was detected by a color change from yellow to dark brown.

Characterization of Ch-AgNPs

The optical properties of Ch-AgNPs were evaluated by UV-vis spectroscopy. The properties of the functional groups were detected by monitoring the spectral bands with FTIR. TEM estimated the morphological characteristics (size and shape). FESEM was used to capture the microstructure of the nanomaterials. XRD was used to estimate the crystal and molecular structure, particle size, and degree of crystallinity. Zeta potential was performed to determine the surface charge and understand the degree of nanoparticle stability.

Bacterial sample

Staphylococcus aureus isolates were obtained from various clinical sources at Ramadi Teaching Hospital, Iraq. Isolates were identified using routine microbiological and biochemical tests, and the diagnosis was confirmed using the VITEK-2 compact system. Isolates were subjected to molecular screening with conventional PCR using the specific primers listed in Table 1 to detect housekeeping genes (*16srRNA* and *gyrb*), quorum sensing genes (*RNAIII*, *AgrA*, and *AgrB*), and some virulence genes. (*mecA, coa, rot, spa, hla, and psm*). One isolate that contained all the tested genes was selected for the gene expression study, as shown in Figure 7.

Table 1. Primers used in this study**.**

Gene	Primers' Sequences (5'→3')	Size product (bp)	References
RNAIII -F	GCACTGAGTCCAAGGAAACTAAC	**82**	Jing et al. 2022
RNAIII -R	AAGCCATCCCAACTTAATAACC		Wang et al. 2023
agrA -F	TCCAGCAGAATTAAGAACTCG	**141**	Jing et al. 2022
agrA -R	ATATCATCATATTGAACATACACT		Wang et al. 2023
agrB -F	GCCCATTCCTGTGCGACTTA	**101**	Jing et al. 2022
agrB -R	GGGCAAATGGCTCTTTGATG		
psm -F	TATCAAAAGCTTAATCGAACAATTC	**176**	Jing et al. 2022
psm -R	CCCCTTCAAATAAGATGTTCATATC		
hla -F	AAAAAACTGCTAGTTATTAGAACGAAAGG	**95**	Jing et al. 2022
hla -R	GGCCAGGCTAAACCACTTTTG		Gao et al. 2022
spa -F	CAGCAAACCATGCAGATGCTA	**100**	Jing et al. 2022
spa -R	GCTAATGATAATCCACCAAATACAGTTG		Gao et al. 2022
coA -F	CACAACCAGTTGCACAACCATTA	**125**	Matias 2015
coA -R	GGGACCTTGAACGATTTCACC		
Rot -F	ATTTTGCAATTAGAAACACTTTTGG	**83**	Cheung et al. 2011
Rot -R	TCTTCTCTAGACATTTTGTATTCGCTTT		
mecA -F	TCCAGATTACAACTTCACCAGG	**162**	Cheung et al. 2014
mecA -R	CCACTTCATATCTTGTAACG		

Antibacterial activity of Ch-AgNPs

Minimum Inhibitory Concentration (MIC) of Ch-AgNPs

The Resazurin Microtiter-Plate Assay (REMA) was performed as described by Coban (2012), with some modifications as follows: 100 µl of Muller-Hinton broth was added to each of the 96 wells of the microtiter plate. 100 µl of silver nanoparticles was added to the first row, then serial dilution was done by pipetting and transferring 100 µl from the first well to the others, respectively, except for the last row (control) to make decreasing concentrations (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128). 20 µl of overnight culture bacterial suspension was added to each well; for the control row, only the first four wells had bacterial suspension, while the others remained without. The microtiter plates were covered with a lid and wrapped in parafilm, then incubated for 24 hours at 37 °C. After incubation, 10 µl of Resazurin solution was added to each well, then re-incubated for 2 hours, and a color change was observed (purple

and pink). The results were recorded by observing the color change; the MIC value was recorded for the well with the lowest concentration without changing the resazurin color.

Growth curve

The growth rate of *S. aureus* was measured according to Hall et al. (2013). In addition to the control, bacterial cultures were grown in flasks with nutrient broth and incubated overnight. The next day, two concentrations of silver nanoparticles (2.1 µg/mL and 1.05 µg/mL) were added to each flask except the control group and then incubated in a shaking incubator with adequate aeration for a specified time. After each interval, cultures were transferred to a sterile spectrophotometer cuvette to measure OD. The optical density was recorded over time and plotted to measure the growth rate.

Gene Expression by qRT-PCR

To study the effect of Ch-AgNPs on the expression of quorum sensing and virulence genes in *S. aureus*, the isolate was cultured on an LB medium with a sub-inhibitory concentration of Ch-AgNPs and incubated overnight at 37 °C.

RNA was extracted using the *TransZol* Up Kit (TRANS China). *The EasyScript® One-Step GDNA Removal and cDNA Synthesis Super-Mix (Trans/China) kit was used.* The reaction components of cDNA synthesis were 10µl of 2xEX reaction mix, 7µl of mRNA, 1µl RT enzyme, 1µl gDNA remover, 1µl random primer, 1µl Oligo (dT) primer, and 3µl RNase-free water. The PCR program for cDNA synthesis was at 25 °C for 10 min., 42 °C for 15 min., 85 °C for 5 sec., and 4 °C for holding, respectively.

The reaction components and volumes of RT-PCR were 10µl of 2xEasyScript PCR Super Mix, 2µl of cDNA, 2µl of (FandR) primers, and 6µl of Nuclease-free water. Each reaction for each gene was done with two replicates.

The program of Real-time PCR by the Rotor-Gene Q device was done in 35 cycles with three basic steps: firstly, denaturation at 94 °C for 10 sec., annealing at 58 °C for 15 sec., and eventually extension at 72 °C for 20 sec. The annealing temperature was set for each gene, as shown in Table 1. The housekeeping gene (*gyrb*) was used as an internal control. Relative changes in gene expression were analyzed using the gene expression fold ($2^{\Delta\Delta Ct}$) method described in Livak and Schmittgen (2001).

Statistical analysis

The data was presented as mean ± standard deviation. A one-way ANOVA test was performed, considering p-values less than 0.05 as statistically significant. The SPSS software was utilized for data analysis.

Results

Biosynthesis of Ch-AgNPs

The absorption spectra of silver nanoparticles coated with chitosan at a concentration of 0.1% (w/v) in water are shown in Figure 1. Characteristic absorption peaks for silver were obtained at 432 nm. The main function of the chitosan molecule during the synthesis of metal nanoparticles is to control the coating process to achieve effective stabilization of the nanoparticles.

To emphasize the synthesis and stabilization of silver nanoparticles resulting from capping agents, FTIR analysis was used. Figure (2) clarifies the functional groups at different stretches of bonds represented by different peaks. The spectral bands of chitosan at wave numbers 3324 cm^{-1} and 2930 cm^{-1} represent amine group bands (N-H). The thiol group (S-H) appeared at the peak at 2358 cm^{-1}, the two peaks at 1756 and 1665 cm^{-1} were assigned to the carbonyl group C=O, the band 1546 cm^{-1} was referred to as C=C, the band 1028 cm^{-1} was assigned to C-O, and the band at 663 cm^{-1} returned to C-S.

TEM provided the study with the morphological characteristics of the silver nanoparticles. Figure 3 depicts the morphology of the formed nanoparticles, which have a regular spherical shape and exhibit low levels of aggregation returns when coated with chitosan. Figure 4 shows that 8nm was the mean size obtained by TEM.

The crystal structure of Ch-AgNPs was determined using XRD technology (Figure 5). The distinct peaks at 38.1°, 44.25°, 64.45°, and 77.15° indicate the metallic silver reflections representing the face-centered cubic crystal, with Miller indices (111), (200), (220), and (311). This reflects the silver structure. A strong and sharp diffraction peak appeared at 38.1° and 44.25°, which can be indexed to the Miller (111) and (200) indices. The reflection shows the cubic shape of Ch-AgNPs, which has been previously reported by the Joint Committee on Energy Diffraction Standards (JCPDS pdf no. 89-3722). The XRD pattern of Ch-AgNPs showed the crystalline nature of the polymeric nanoparticles. Notably, no peaks were observed for impurities' other crystalline phases.

Antibacterial effect of Ch-AgNPs

The MIC of Ch-AgNPs on *S. aureus* isolates was evaluated using the REMA method. The findings showed that the MIC of Ch-AgNPs was 4.2 µg/mL; therefore, the sub-MIC concentration was 2.1 µg/mL. Hence, a concentration of 2.1 µg/mL was used to treat *S. aureus* isolate to assess the impact of Ch-AgNPs on the expression of quorum-sensing and virulence genes.

Growth curve analysis

The growth curve of *S. aureus* in Figure 6 showed the growth of bacteria over time after being treated with two concentrations of Ch-AgNPs, 2.1 and 1.05 µg/mL, respectively, in addition to the control. The graph shows the antibacterial effect of 2.1 µg Ch-AgNPs on the treated isolate, which inhibited bacterial growth during the first 3 hours, followed by a reduced level of growth compared to the untreated isolate and 1.05 µg/ml.

Molecular detection of *S. aureus* genes

Based on multiplex PCR results, the prevalence of quorum-sensing system genes *agrA*, agrB, and *RNAIII* in *S. aureus* isolates were 22 (96.65%), 21 (91.30%), and 22 (96.65%), respectively. Virulence Genes prevalence for *S. aureus* isolates (*mecA*, *hla*, *rot*, *coa*, *spa*, and *psm*) were 22 (96.65%), 21 (91.30%), 22 (96.65%), 23(100%), 22 (96.65%), and 23 (100%), respectively.

Gene expression by qRT-PCR

The expression of quorum sensing and virulence genes in *S. aureus* was decreased after treatment of the isolate with 2.1 µg/ml of biosynthesized Ch-AgNPs, as Figure 8 illustrates. On the other hand, the housekeeping gene (*gyrb*) showed stable expression with a $2^{-\Delta\Delta ct}$ equivalent to 1.

Discussion

The biosynthesis of Ch-AgNPs was primarily ensured by the color changing from transparent to pale yellow and then to reddish-brown. This is mainly due to the active plant components found in Acacia leaf extracts, including flavones, polysaccharides, proteins, and phenols, which were responsible for the reduction of Ag^{+} to Ag^{0} and consequently the formation of nanoparticles (Rather et al. 2022). This green method is described as an eco-friendly approach that is efficient, cost-effective, and energy-efficient. The formation of AgNPs was further detected by a group of physiochemical tests. The formed solution absorbance showed a sharp peak at 432 nm, which reflects the shape and size of AgNPs, which further confirms the formation of AgNPs (Dashora et al. 2022). The wide appearance of the peak exhibited in Figure 1 is because of its plasmon resonance nature (Desai et al. 2012; Ashraf et al. 2016). Chitosan is a protective agent that stabilizes and preserves the surface properties of particles, preventing them from agglomerating or losing their surface properties. Chitosan biopolymer has a strong affinity for metal ions due to the presence of many amino and hydroxyl groups, which play a crucial role in the release of metal ions (Nate et al. 2018).

FT-IR analysis revealed the presence of several amino groups in chitosan that act as binding and capping agents. This may indicate that specific amino groups are responsible for the interaction with the surface of metal nanoparticles, acting as AgNP anchoring sites (Kalaivani et al. 2018). Proteins in the plant extract can also bind to AgNPs by electrostatic attraction of negatively charged carboxyl groups (Abdulla et al. 2021). The transmission electron microscopy (TEM) images reveal that the silver nanoparticles (AgNPs) are incorporated into the chitosan nanocomposite matrix, exhibiting a spherical morphology. The polymer sample exhibited a homogeneous distribution of AgNPs. The chitosan-grafted silver nanoparticles exhibited a size range of 5 to 100 nm, with the majority of particles being smaller than 8 nm. Remarkably, it was discovered that the particles exhibited a high degree of uniformity in terms of their size. The increased size and varied shapes are a result of the adsorption of silver onto the surface of chitosan nanoparticles, which causes the creation of a chelated ring-like structure. Dara et al. (2020) found that the size and shape of silver nanoparticles are influenced by the concentration and composition of stabilizing and reducing agents. The contact between chitosan polymer molecules and AgNPs, whether through coordinate or ionic interactions, has been found to improve the dispersion of

silver nanoparticles without generating substantial alterations in morphological features (Mohammed et al. 2023).

In the investigation of the antibacterial action of nanoparticles, there is no specific method; previous published papers used different protocols. Loo et al. (2018), like our study, used resazurin dye in their investigation of the antibacterial action of nanoparticles. The lowest concentration of antibacterial agents that inhibits determined amounts of bacterial growth can be approximately defined by MIC (Kowalska-Krochmal et al. 2021). Using a resazurin microtiter assay (REMA) is an inexpensive, simple, fast, and efficient method. Active bacteria can reduce the blue dye of Resazurin to pink fluorescent dye (resorufin) by oxidoreductase, which directly quantifies the bacterial metabolic activity utilized to determine the minimum inhibition concentration (Chakansin et al. 2022). The MIC of the study Ch-AgNPs was 4.2 µg/ml, which provides strong evidence of the antibacterial effect of AgNPs. This result agrees with Yuan et al. (2017). This evidence of high inhibition is mainly due to the large surface area to volume ratio. Also, the different antibacterial mechanisms possessed by silver nanoparticles include disrupting membranes by ROS, perforating membranes by AgNPs, interfering with ATP production, denaturing ribosomes, and interfering with DNA replication (Yin et al. 2020). Many studies search for the anti-*Staphylococcus aureus* of AgNPs synthesized from different plants against *S. aureus*. Das et al. (2017) reported significant activity against MDR *S. aureus* by green synthesis AgNPs with 8µg/ml. MIC, Asghar et al. (2020) found that the MIC was 25µg/ml against *S. aureus* as a result of AgNPs fabricated from the leaves of *Syzygium cumini*. Extract from the seed of *Phoenix dactylifera* was reported by Ansari and Alzohairy (2018) to have antibacterial activity with a MIC 10.6 µg/ml against *S. aureus*. Also, Parvekar et al. (2022) illustrated in their study that silver nanoparticles exhibited anti-*S. aureus* action at MIC 0.625 mg/mL. On the other hand, Tyavambiza et al. (2021) found that the activity of silver nanoparticles synthesized by *Cotyledon orbiculate* showed activity towards gram-negative bacteria more than gram-positive bacteria, mainly because of the differences in the cell wall structure, with MIC values of 5 µg/ml for *P. aeruginosa* and 20 µg/ml for *S. aureus*. It is noteworthy that another study by Kim et al. (2011) reported that manufactured AgNPs inhibited both G^{+ve} (*S. aureus*) and G^{-ve} (*E. coli*) at the same concentration with a MIC of 100 µg/ml.

The growth curve graphed previously in Figure 6 showed that the lag phase of *S. aureus* without treatment continued for the first hour, while the exponential phase began and elevated gradually after one hour. 2.1µg/ml of the study Ch-AgNPs, as illustrated, appear to have complete inhibition of *S. aureus* growth for the first 3 hours of incubation, followed by low levels of growth in comparison to 1.05µg/ml and the control. The negative effect of AgNPs on bacterial growth and reproduction results from the induction of cellular stress in *S. aureus* by AgNPs, which affects metabolic activity through the modulation of ATP synthesis (Yuan et al. 2017). Alahmad et al. (2022) reported that AgNPs significantly reduced *S. aureus* growth with a 12µg/mL MIC. On the other hand, some other studies fabricated silver nanoparticles from other plant extracts and reported a high concentration of AgNPs needed to inhibit *S. aureus* growth in comparison to our study, like Qais et al. (2019) study, when they exhibited that MICs above 32µg/ml of AgNPs synthesized from *Murraya koenigii* showed ability to inhibit more than 90% of

S. aureus. AgNPs synthesized from bacteria are also reported to be effective against *S. aureus* growth. A study of Gomaa (2017) showed that AgNPs from soil bacteria inhibit *S. aureus* growth and reproduction when treated with 50 mg/mL of them. All this evidence by comparison enhances the strength of *Acaci glauca* extract chosen in the synthesis of an effective anti-bacterial nanoagent.

In the evaluation of gene expression by qPCR before and after treatment with an antibacterial agent, it is necessary to select highly stable expressed genes (HKGs) to compare and obtain reliable qPCR results (Bustin et al. 2009). The *Gyrb* gene was selected as one of the most stably expressed reference genes and considered an ideal choice for the normalization of qPCR (Sihto et al. 2014), which is consistent with this study where the *Gyrb* gene showed a highly stable expression with a fold change equal to 1 even after treatment with highly effective 2.1µg/ml of Ch-AgNPs, as shown in figure 8.

As a modern, safe, and promising strategy to overcomethe worldwide antibiotic resistance problem and a highly virulent strain of *S. aureus*, AgNPs were tested mainly for inhibition of quorum sensing genes, which regulate and administer a large group of other virulence genes (Masimen et al. 2022). AgrB is an endopeptidase located within a cytoplasmic membrane with hydrophobic and hydrophilic segments responsible for the maturation and export of AIP (Tan et al. 2018). The fold change $2^{-\Delta\Delta Ct}$ of AgrB was recorded as 0.776 after treatment with Ch-AgNPs, which confirms the ability of silver nanoparticles to down-regulate the virulence of *S. aureus*. AgrB has a highly conserved region in the first hydrophilic segment, which is completely conserved among *S. aureus* agr types (Thoendel et al. 2010). Any change in this first conserved segment leads to elimination of *AgrB* activity (Qiu et al. 2005), while the mutation in the second conserved transmembrane domain does not affect its activity (Thoendel et al. 2010). AgrA is a response regulator of the agr system that binds to a specific site of the RNAIII and RNAII promoters (P2 and P2) (Koenig et al. 2004). *AgrA* exhibited partial downregulation in its expression after being exposed to 2.1µg/ml of CH-Ag NPs with 0.856 2-ΔΔCt, as illustrated in Table 3. Koenig et al. (2004) demonstrated that AgrA binds strongly to the P2 promoter of RNAII more than RNAIII, and any mutation that occurs to it leads to a partial defect in *AgrA*, which leads to a significant delay in the activation of the Agr system. RNAIII, the main regulator of the Agr system, was the most affected by 2.1µg/ml Ch-AgNPs and exhibited a highly significant decrease in its expression with $2^{-\Delta\Delta Ct}$ 0.027. RNAIII is a post-transcriptional regulator, having C-rich and unpaired regions responsible for binding with many targets' mRNA (Bronesky et al. 2016). A study by Xiong et al. (2002) exhibited that a mutant or inhibition in the RNAIII leads to repressing (down-regulating) a group of low-molecular-weight proteins (toxin and exoenzymes).

All of the virulence factors of *S. aureus* detected previously in this study showed downregulation when treated with the MIC of the research biosynthesized Ch-AgNPs, as shown in Table 3. The *psm* gene was the most affected gene, with a fold change of 0.039., which confirms a significant decrease in gene expression after treatment with 2.1µg/ml as Table 3 showed. Phenol-soluble modulin is one of the pore-forming toxins (PFTs) which is the exceptional gene regulated by direct binding of Agr regulator response with the promoter region of *the psm* operon (Queck et al. 2008). Wang et al. (2007) demonstrated that, with dysfunctional Agr systems, PSMs were completely absent.

In the second rank of inhibition, the superantigen surface Ig-binding protein A encoded by the *spa* gene mainly acts by capturing IgG and preventing phagocytosis (Bear et al. 2023). The quantitative measurement by qPCR showed that *the spa* gene also exhibited a significant decrease after nano-exposed 2-Ct equal to 0.092. Recent modern computational research by Waseem et al. (2023) computationally at the molecular level exhibited a high binding affinity between AgNPs and amplified *spa* gene -7.19 kJ/mol, which explains and is consistent with the result of our study and may pave the way to using silver nanoparticles as an alternative tool against MDR.

The virulent strain of *S. aureus* resistant to beta-lactam antibiotics is mainly mediated by *mecA* gene expression, which suffers a significant decrease in expression after being exposed to 2.1 µg/ml of Ch-AgNPs with a fold change of 0.674. The result is consistent with the study of Rashid et al. (2020), who found that silver nanoparticles produced from ginger extract caused a significant decrease in the *mecA* gene of *S. aureus*. *The mecA* gene is considered one of the mobile genetic elements that bacterial stains gain it by horizontal transfer (Lakhundi and Zhang 2018). Zinc oxide nanoparticles ZNO-NPs also affect *the mecA* gene with a significant decrease in the relative gene expression (P 0.001) (Abdelraheem et al. 2021).

The global regulator repressor of toxin (*Rot*) with a fold change of 0.657 indicated a significant reduction in its expression because of the effect of Ch-AgNPs. *Rot* function but not transcription is also regulated by the Agr system accessory gene regulator; the upregulation of *Rot* occurs during the stationary phase (Hsieh et al. 2008). Ch-AgNPs previously blocked the activities of *S. aureus* in the exponential phase. Singh et al. (2019) also proved the down-regulation of rot by silver nanoparticles.

Alpha hemolysin is one of the S. *aureus* exotoxins encoded in the core genome belonging to the pore-forming cytotoxins (Chen et al. 2015). After being treated with Ch-AgNPs, the *hla* gene showed a significant decrease in expression, but it wasn't as much as other genes' expression, with a fold change of 0.804. Soleimani and Habibi-Pirkoohi (2017) results showed that silver nanoparticles fabricated from *Chlorella vulgaris* inhibit the expression of *the hla* gene even with a concentration less than the MIC. Nanoparticles synthesized from material other than silver nitrate also showed antibacterial activity, nanoparticles from Antimony Tin Oxide (ATONPs) reduce the expression of *the hla* gene and prevented hemolysin production (Park et al. 2023).

Conclusion

Chitosan stabilized silver nanoparticles were synthesized by green method from *Acacia glauca* leaves, which proved to have a high antibacterial effect with low MIC. It is effective in inhibiting quorum sensing genes as well as virulence genes in *S. aureus*, as it represents a promising agent that could potentially be used as an alternative agent to antibiotics in multidrug-resistant strains of *S. aureus*.

Declarations

Acknowledgements

The authors thank Dr. Muhannad Karim Anid, Iraqi Genetics Company, Iraq, for allowing them access to the company's laboratory.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study's conception and design. **Reem Mahdi Saleh** performed material preparation, data collection, and analysis. **Omar Mohammed Hassan** wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

References

1. Abdelraheem WM, Khairy RMM, Zaki AI, Zaki SH (2021) Effect of ZnO nanoparticles on methicillin, vancomycin, linezolid resistance and biofilm formation in Staphylococcus aureus isolates. Annals of Clinical Microbiology and Antimicrobials 20(1). https://doi.org/10.1186/s12941-021-00459-2
2. Abdulla NY, Ibraheem IJ, Hassan OM (2021) Green Synthesis of Eco-Friendly Silver Nanoparticles by Onion Peels Extract and Evolution of its Synergistic Bactericidal Activities with Antibiotics. Biochemical and Cellular Archives 21(2):4213–4217
3. Alahmad A, Al-Zereini WA, Hijazin TJ, Al-Madanat OY, Alghoraibi I, Al-Qaralleh O, Al-Qaraleh S, Feldhoff A, Walter J-G, Scheper T (2022) Green Synthesis of Silver Nanoparticles Using Hypericum perforatum L. Aqueous Extract with the Evaluation of Its Antibacterial Activity against Clinical and Food Pathogens. Pharmaceutics 14(5):1104. https://doi.org/10.3390/pharmaceutics14051104
4. Alam P, Alajmi MF, Arbab AH, Parvez MK, Siddiqui NA, Alqasoumi SI, Al-Rehaily AJ, Al-Dosari MS, Basudan OA (2017) Comparative study of antioxidant activity and validated RP-HPTLC analysis of rutin in the leaves of different Acacia species grown in Saudi Arabia. Saudi Pharmaceutical Journal 25(5):715–723. https://doi.org/10.1016/j.jsps.2016.10.010
5. Al-Zahrani S, Astudillo-Calderón S, Pintos B, Pérez-Urria E, Manzanera JA, Martín L, Gomez-Garay A (2021) Role of Synthetic Plant Extracts on the Production of Silver-Derived Nanoparticles. Plants 10(8):1671. https://doi.org/10.3390/plants10081671
6. Ansari MA, Alzohairy MA (2018) One-Pot Facile Green Synthesis of Silver Nanoparticles Using Seed Extract of Phoenix dactylifera and Their Bactericidal Potential against MRSA. Evidence-Based

Complementary and Alternative Medicine 2018:1–9. https://doi.org/10.1155/2018/1860280

7. Asghar MA, Yousuf RI, Shoaib MH, Asghar MA (2020) Antibacterial, anticoagulant and cytotoxic evaluation of biocompatible nanocomposite of chitosan loaded green synthesized bioinspired silver nanoparticles. International Journal of Biological Macromolecules 160:934–943. https://doi.org/10.1016/j.ijbiomac.2020.05.197
8. Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I (2016) Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques. Scientific Reports 6(1). https://doi.org/10.1038/srep20414
9. Batiha GE-S, Akhtar N, Alsayegh AA, Abusudah WF, Almohmadi NH, Shaheen HM, Singh TG, De Waard M (2022) Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Genus Acacia. Molecules 27(21):7340. https://doi.org/10.3390/molecules27217340
10. Bear A, Locke T, Rowland-Jones S, Pecetta S, Bagnoli F, Darton TC (2023) The immune evasion roles of Staphylococcus aureus protein A and impact on vaccine development. Frontiers in Cellular and Infection Microbiology 13. https://doi.org/10.3389/fcimb.2023.1242702
11. Boisset S, Geissmann T, Huntzinger E, Fechter P, Bendridi N, Possedko M, Chevalier C, Helfer AC, Benito Y, Jacquier A, Gaspin C, Vandenesch F, Romby P (2007) Staphylococcus aureus RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. Genes & Development 21(11):1353–1366. https://doi.org/10.1101/gad.423507
12. Bronesky D, Wu Z, Marzi S, Walter P, Geissmann T, Moreau K, Vandenesch F, Caldelari I, Romby P (2016) Staphylococcus aureus RNAIII and Its Regulon Link Quorum Sensing, Stress Responses, Metabolic Adaptation, and Regulation of Virulence Gene Expression. Annual Review of Microbiology 70(1):299–316. https://doi.org/10.1146/annurev-micro-102215-095708
13. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55(4):611–622. https://doi.org/10.1373/clinchem.2008.112797
14. Chakansin C, Yostaworakul J, Warin C, Kulthong K, Boonrungsiman S (2022) Resazurin rapid screening for antibacterial activities of organic and inorganic nanoparticles: Potential, limitations and precautions. Analytical Biochemistry 637:114449. https://doi.org/10.1016/j.ab.2021.114449
15. Chen Y, Yeh AJ, Cheung GYC, Villaruz AE, Tan VY, Joo H-S, Chatterjee SS, Yu Y, Otto M (2014) Basis of Virulence in a Panton-Valentine Leukocidin-Negative Community-Associated Methicillin-ResistantStaphylococcus aureusStrain. Journal of Infectious Diseases 211(3):472–480. https://doi.org/10.1093/infdis/jiu462
16. Cheung GYC, Villaruz AE, Joo H-S, Duong AC, Yeh AJ, Nguyen TH, Sturdevant DE, Queck SY, Otto M (2014) Genome-wide analysis of the regulatory function mediated by the small regulatory psm-mec RNA of methicillin-resistant Staphylococcus aureus. International Journal of Medical Microbiology 304(5–6):637–644. https://doi.org/10.1016/j.ijmm.2014.04.008

17. Cheung GYC, Wang R, Khan BA, Sturdevant DE, Otto M (2011) Role of the Accessory Gene Regulator agr in Community-Associated Methicillin-Resistant Staphylococcus aureus Pathogenesis. Infection and Immunity 79(5):1927–1935. https://doi.org/10.1128/iai.00046-11
18. Coban AY (2012) Rapid Determination of Methicillin Resistance among Staphylococcus aureus Clinical Isolates by Colorimetric Methods. Journal of Clinical Microbiology 50(7):2191–2193. https://doi.org/10.1128/jcm.00471-12
19. Collado-González M, Montalbán MG, Peña-García J, Pérez-Sánchez H, Víllora G, Díaz Baños FG (2017) Chitosan as stabilizing agent for negatively charged nanoparticles. Carbohydrate Polymers 161:63–70. https://doi.org/10.1016/j.carbpol.2016.12.043
20. Da F, Joo H-S, Cheung GYC, Villaruz AE, Rohde H, Luo X, Otto M (2017) Phenol-Soluble Modulin Toxins of Staphylococcus haemolyticus. Frontiers in Cellular and Infection Microbiology 7. https://doi.org/10.3389/fcimb.2017.00206
21. Dara PK, Mahadevan R, Digita PA, Visnuvinayagam S, Kumar LRG, Mathew S, Ravishankar CN, Anandan R (2020) Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): in vitro studies on antioxidant and antibacterial applications. SN Applied Sciences 2(4). https://doi.org/10.1007/s42452-020-2261-y
22. Das B, Dash SK, Mandal D, Ghosh T, Chattopadhyay S, Tripathy S, Das S, Dey SK, Das D, Roy S (2017) Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage. Arabian Journal of Chemistry 10(6):862–876. https://doi.org/10.1016/j.arabjc.2015.08.008
23. Dashora A, Rathore K, Raj S, Sharma K (2022) Synthesis of silver nanoparticles employing Polyalthia longifolia leaf extract and their in vitro antifungal activity against phytopathogen. Biochemistry and Biophysics Reports 31:101320. https://doi.org/10.1016/j.bbrep.2022.101320
24. Desai R, Mankad V, Gupta SanjeevK, Jha PrafullaK (2012) Size Distribution of Silver Nanoparticles: UV-Visible Spectroscopic Assessment. Nanoscience and Nanotechnology Letters 4(1):30–34. https://doi.org/10.1166/nnl.2012.1278
25. Fiorati A, Bellingeri A, Punta C, Corsi I, Venditti I (2020) Silver Nanoparticles for Water Pollution Monitoring and Treatments: Ecosafety Challenge and Cellulose-Based Hybrids Solution. Polymers 12(8):1635. https://doi.org/10.3390/polym12081635
26. Gao P, Wei Y, Wan RE, Wong KW, Iu HTV, Tai SSC, Li Y, Yam HCB, Halebeedu Prakash P, Chen JHK, Ho PL, Yuen KY, Davies J, Kao RYT (2022) Subinhibitory Concentrations of Antibiotics Exacerbate Staphylococcal Infection by Inducing Bacterial Virulence. Microbiology Spectrum 10(4). https://doi.org/10.1128/spectrum.00640-22
27. García-Contreras R (2016) Is Quorum Sensing Interference a Viable Alternative to Treat Pseudomonas aeruginosa Infections? Frontiers in Microbiology 7. https://doi.org/10.3389/fmicb.2016.01454
28. Gherasim O, Puiu RA, Bîrcă AC, Burduşel A-C, Grumezescu AM (2020) An Updated Review on Silver Nanoparticles in Biomedicine. Nanomaterials 10(11):2318. https://doi.org/10.3390/nano10112318

29. Gomaa EZ (2017) Silver nanoparticles as an antimicrobial agent: A case study on *Staphylococcus aureus* and *Escherichia coli* as models for Gram-positive and Gram-negative bacteria. The Journal of General and Applied Microbiology 63(1):36–43. https://doi.org/10.2323/jgam.2016.07.004
30. Goswami J (2017) Quorum Sensing by Super Bugs and their Resistance to Antibiotics, a Short Review. Global Journal of Pharmacy & Pharmaceutical Sciences 3(3). https://doi.org/10.19080/gjpps.2017.03.555614
31. Hall BG, Acar H, Nandipati A, Barlow M (2013) Growth Rates Made Easy. Molecular Biology and Evolution 31(1):232–238. https://doi.org/10.1093/molbev/mst187
32. Hassan OM, Ibraheem IJ, Adil BH, Obaid AS, Abdulqader Salih T (2020) Synthesis of Silver Nanoparticles by ecofriendly nvironmental method using Piper nigrum, Ziziphus spina-christi, and Eucalyptusglobulus extract. Journal of Physics: Conference Series 1530(1):012139. https://doi.org/10.1088/1742-6596/1530/1/012139
33. Himabindu (2009) Molecular Analysis of Coagulase Gene Polymorphism in Clinical Isolates of Methicilin Resistant Staphylococcus aureus by Restriction Fragment Length Polymorphism Based Genotyping. American Journal of Infectious Diseases 5(2):163–169. https://doi.org/10.3844/ajidsp.2009.163.169
34. Hsieh H-Y, Tseng CW, Stewart GC (2008) Regulation of Rot Expression in Staphylococcus aureus. Journal of Bacteriology 190(2):546–554. https://doi.org/10.1128/jb.00536-07
35. Jing S, Ren X, Wang L, Kong X, Wang X, Chang X, Guo X, Shi Y, Guan J, Wang T, Wang B, Song W, Zhao Y (2022) Nepetin reduces virulence factors expression by targeting ClpP against MRSA-induced pneumonia infection. Virulence 13(1):578–588. https://doi.org/10.1080/21505594.2022.2051313
36. Jouyban A, Rahimpour E (2020) Optical sensors based on silver nanoparticles for determination of pharmaceuticals: An overview of advances in the last decade. Talanta 217:121071. https://doi.org/10.1016/j.talanta.2020.121071
37. Kalaivani R, Maruthupandy M, Muneeswaran T, Hameedha Beevi A, Anand M, Ramakritinan CM, Kumaraguru AK (2018) Synthesis of chitosan mediated silver nanoparticles (Ag NPs) for potential antimicrobial applications. Frontiers in Laboratory Medicine 2(1):30–35. https://doi.org/10.1016/j.flm.2018.04.002
38. Kim S-H, Lee H-S, Ryu D-S, Choi S-J, Lee D-S (2011) Antibacterial Activity of Silver-Nanoparticles Against Staphylococcus Aureus and Escherichia Coli. Microbiology and Biotechnology Letters 39(1):77–85
39. Kim S-R, Yeon K-M (2018) Quorum Sensing as Language of Chemical Signals. Fundamentals of Quorum Sensing, Analytical Methods and Applications in Membrane Bioreactors :57–94. https://doi.org/10.1016/bs.coac.2018.03.010
40. Koenig RL, Ray JL, Maleki SJ, Smeltzer MS, Hurlburt BK (2004) Staphylococcus aureus AgrA Binding to the RNAIII- agr Regulatory Region. Journal of Bacteriology 186(22):7549–7555. https://doi.org/10.1128/jb.186.22.7549-7555.2004

41. Kowalska-Krochmal B, Dudek-Wicher R (2021) The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. Pathogens 10(2):165. https://doi.org/10.3390/pathogens10020165
42. Kumara Swamy M, Sudipta KM, Jayanta K, Balasubramanya S (2014) The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from Leptadenia reticulata leaf extract. Applied Nanoscience 5(1):73–81. https://doi.org/10.1007/s13204-014-0293-6
43. Lakhundi S, Zhang K (2018) Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clinical Microbiology Reviews 31(4). https://doi.org/10.1128/cmr.00020-18
44. Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001.1262
45. Loo YY, Rukayadi Y, Nor-Khaizura M-A-R, Kuan CH, Chieng BW, Nishibuchi M, Radu S (2018) In Vitro Antimicrobial Activity of Green Synthesized Silver Nanoparticles Against Selected Gram-negative Foodborne Pathogens. Frontiers in Microbiology 9. https://doi.org/10.3389/fmicb.2018.01555
46. Masimen MAA, Harun NA, Maulidiani M, Ismail WIW (2022) Overcoming Methicillin-Resistance Staphylococcus aureus (MRSA) Using Antimicrobial Peptides-Silver Nanoparticles. Antibiotics 11(7):951. https://doi.org/10.3390/antibiotics11070951
47. Matias CSS (2015) Quorum-sensing system of Staphylococcus aureus isolates from diabetic foot ulcers. Dissertation, University of Lisbon
48. Mohammed AM, Hassan KT, Hassan OM (2023) Assessment of antimicrobial activity of chitosan/silver nanoparticles hydrogel and cryogel microspheres. International Journal of Biological Macromolecules 233:123580. https://doi.org/10.1016/j.ijbiomac.2023.123580
49. Mutter TY, Hassan OM (2024) Insecticidal Activity of Green Synthesized Silver Nanoparticles from Pelargonium Citronellum against Citrus Mealybug, Planococcus Citri. Plant Protection 8(1):69–78. https://doi.org/10.33804/pp.008.01.5073
50. Nate Z, Moloto MJ, Mubiayi PK, Sibiya PN (2018) Green synthesis of chitosan capped silver nanoparticles and their antimicrobial activity. MRS Advances 3(42–43):2505–2517. https://doi.org/10.1557/adv.2018.368
51. Obaid AS, Hassan KT, Hassan OM, Ali HH, Ibraheem IJ, Salih TA, Adil BH, Almoneef MM (2023) In-vitro antibacterial, cytotoxicity, and anti-prostate cancer effects of gold nanoparticles synthesized using extract of desert truffles (Tirmania nivea). Materials Chemistry and Physics 301:127673. https://doi.org/10.1016/j.matchemphys.2023.127673
52. Park I, Jailani A, Lee J-H, Ahmed B, Lee J (2023) The Antibiofilm Effects of Antimony Tin Oxide Nanoparticles against Polymicrobial Biofilms of Uropathogenic Escherichia coli and Staphylococcus aureus. Pharmaceutics 15(6):1679. https://doi.org/10.3390/pharmaceutics15061679

53. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S (2020) The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomaterial Investigations in Dentistry 7(1):105–109. https://doi.org/10.1080/26415275.2020.1796674
54. Qais FA, Shafiq A, Khan HM, Husain FM, Khan RA, Alenazi B, Alsalme A, Ahmad I (2019) Antibacterial Effect of Silver Nanoparticles Synthesized Using Murraya koenigii (L.) against Multidrug-Resistant Pathogens. Bioinorganic Chemistry and Applications 2019:1–11. https://doi.org/10.1155/2019/4649506
55. Qiu R, Pei W, Zhang L, Lin J, Ji G (2005) Identification of the Putative Staphylococcal AgrB Catalytic Residues Involving the Proteolytic Cleavage of AgrD to Generate Autoinducing Peptide. Journal of Biological Chemistry 280(17):16695–16704. https://doi.org/10.1074/jbc.m411372200
56. Queck SY, Jameson-Lee M, Villaruz AE, Bach T-HL, Khan BA, Sturdevant DE, Ricklefs SM, Li M, Otto M (2008) RNAIII-Independent Target Gene Control by the agr Quorum-Sensing System: Insight into the Evolution of Virulence Regulation in Staphylococcus aureus. Molecular Cell 32(1):150–158. https://doi.org/10.1016/j.molcel.2008.08.005
57. Rashid A, Molavi F, Mahmoudzadeh H (2020) The effect of silver nanoparticles on mecA gene expression in methicillin-resistant samples of Staphylococcus aureus. New Cellular and Molecular Biotechnology Journal 11(41):67–82
58. Rather MA, Deori PJ, Gupta K, Daimary N, Deka D, Qureshi A, Dutta TK, Joardar SN, Mandal M (2022) Ecofriendly phytofabrication of silver nanoparticles using aqueous extract of Cuphea carthagenensis and their antioxidant potential and antibacterial activity against clinically important human pathogens. Chemosphere 300:134497. https://doi.org/10.1016/j.chemosphere.2022.134497
59. Sadiq MB, Tharaphan P, Chotivanich K, Tarning J, Anal AK (2017) In vitro antioxidant and antimalarial activities of leaves, pods and bark extracts of Acacia nilotica (L.) Del. BMC Complementary and Alternative Medicine 17(1). https://doi.org/10.1186/s12906-017-1878-x
60. Sanchez C, Nigen M, Mejia Tamayo V, Doco T, Williams P, Amine C, Renard D (2018) Acacia gum: History of the future. Food Hydrocolloids 78:140–160. https://doi.org/10.1016/j.foodhyd.2017.04.008
61. Santhakumari S, Ravi AV (2019) Targeting quorum sensing mechanism: An alternative anti-virulent strategy for the treatment of bacterial infections. South African Journal of Botany 120:81–86. https://doi.org/10.1016/j.sajb.2018.09.028
62. Sanyasi S, Majhi RK, Kumar S, Mishra M, Ghosh A, Suar M, Satyam PV, Mohapatra H, Goswami C, Goswami L (2016) Polysaccharide-capped silver Nanoparticles inhibit biofilm formation and eliminate multi-drug-resistant bacteria by disrupting bacterial cytoskeleton with reduced cytotoxicity towards mammalian cells. Scientific Reports 6(1). https://doi.org/10.1038/srep24929
63. Shaikh S, Nazam N, Rizvi SMD, Ahmad K, Baig MH, Lee EJ, Choi I (2019) Mechanistic Insights into the Antimicrobial Actions of Metallic Nanoparticles and Their Implications for Multidrug Resistance. International Journal of Molecular Sciences 20(10):2468. https://doi.org/10.3390/ijms20102468

64. Sihto H-M, Tasara T, Stephan R, Johler S (2014) Validation of reference genes for normalization of qPCR mRNA expression levels inStaphylococcus aureusexposed to osmotic and lactic acid stress conditions encountered during food production and preservation. FEMS Microbiology Letters 356(1):134–140. https://doi.org/10.1111/1574-6968.12491
65. Singh N, Rajwade J, Paknikar KM (2019) Transcriptome analysis of silver nanoparticles treated Staphylococcus aureus reveals potential targets for biofilm inhibition. Colloids and Surfaces B: Biointerfaces 175:487–497. https://doi.org/10.1016/j.colsurfb.2018.12.032
66. Soleimani M, Habibi-Pirkoohi M (2017) Biosynthesis of Silver Nanoparticles using Chlorella vulgaris and Evaluation of the Antibacterial Efficacy Against Staphylococcus aureus. Avicenna journal of medical biotechnology 9(3):120–125
67. Tan L, Li SR, Jiang B, Hu XM, Li S (2018) Therapeutic Targeting of the Staphylococcus aureus Accessory Gene Regulator (agr) System. Frontiers in Microbiology 9. https://doi.org/10.3389/fmicb.2018.00055
68. Thoendel M, Kavanaugh JS, Flack CE, Horswill AR (2010) Peptide Signaling in the Staphylococci. Chemical Reviews 111(1):117–151. https://doi.org/10.1021/cr100370n
69. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG (2015) Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clinical Microbiology Reviews 28(3):603–661. https://doi.org/10.1128/cmr.00134-14
70. Tyavambiza C, Elbagory AM, Madiehe AM, Meyer M, Meyer S (2021) The Antimicrobial and Anti-Inflammatory Effects of Silver Nanoparticles Synthesised from Cotyledon orbiculata Aqueous Extract. Nanomaterials 11(5):1343. https://doi.org/10.3390/nano11051343
71. Wang R, Braughton KR, Kretschmer D, Bach T-HL, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nature Medicine 13(12):1510–1514. https://doi.org/10.1038/nm1656
72. Wang X, Wu X, Shen L, Rao L, Wang B, Zhao H, Zhang J, Xiao Y, Guo Y, Xu Y, Chen L, Yu F (2023) Phylogenetic Analysis and Virulence Characteristics of Methicillin-Resistant Staphylococcus aureus ST45 in China: a Hyper-Virulent Clone Associated with Bloodstream Infections. mSystems 8(2). https://doi.org/10.1128/msystems.00029-23
73. Waseem M, Naveed M, Rehman S ur, Makhdoom SI, Aziz T, Alharbi M, Alsahammari A, Alasmari AF (2023) Molecular Characterization of spa, hld, fmhA, and lukD Genes and Computational Modeling the Multidrug Resistance of Staphylococcus Species through Callindra harrisii Silver Nanoparticles. ACS Omega 8(23):20920–20936. https://doi.org/10.1021/acsomega.3c01597
74. Xiong Y, Van Wamel W, Nast CC, Yeaman MR, Cheung AL, Bayer AS (2002) Activation and Transcriptional Interaction between agr RNAII and RNAIII in Staphylococcus aureus In Vitro and in an Experimental Endocarditis Model. The Journal of Infectious Diseases 186(5):668–677. https://doi.org/10.1086/342046

75. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH (2020) The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. International Journal of Nanomedicine Volume 15:2555–2562. https://doi.org/10.2147/ijn.s246764
76. Yuan Y-G, Peng Q-L, Gurunathan S (2017) Effects of Silver Nanoparticles on Multiple Drug-Resistant Strains of Staphylococcus aureus and Pseudomonas aeruginosa from Mastitis-Infected Goats: An Alternative Approach for Antimicrobial Therapy. International Journal of Molecular Sciences 18(3):569. https://doi.org/10.3390/ijms18030569

Table

Table 3 is not available with this version.

Figures

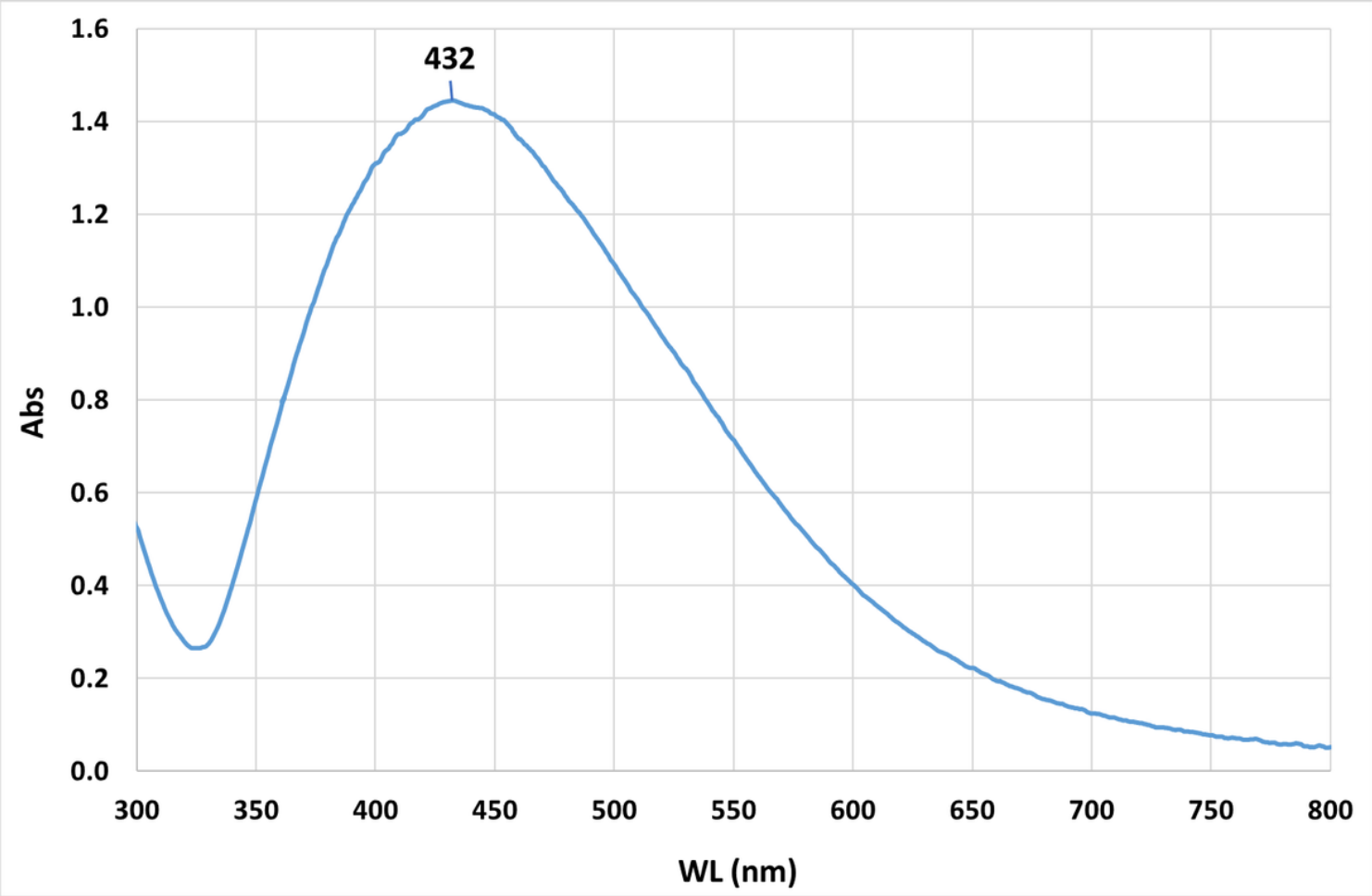

Figure 1

UV-vis spectra of the biosynthesized Ch-AgNPs.

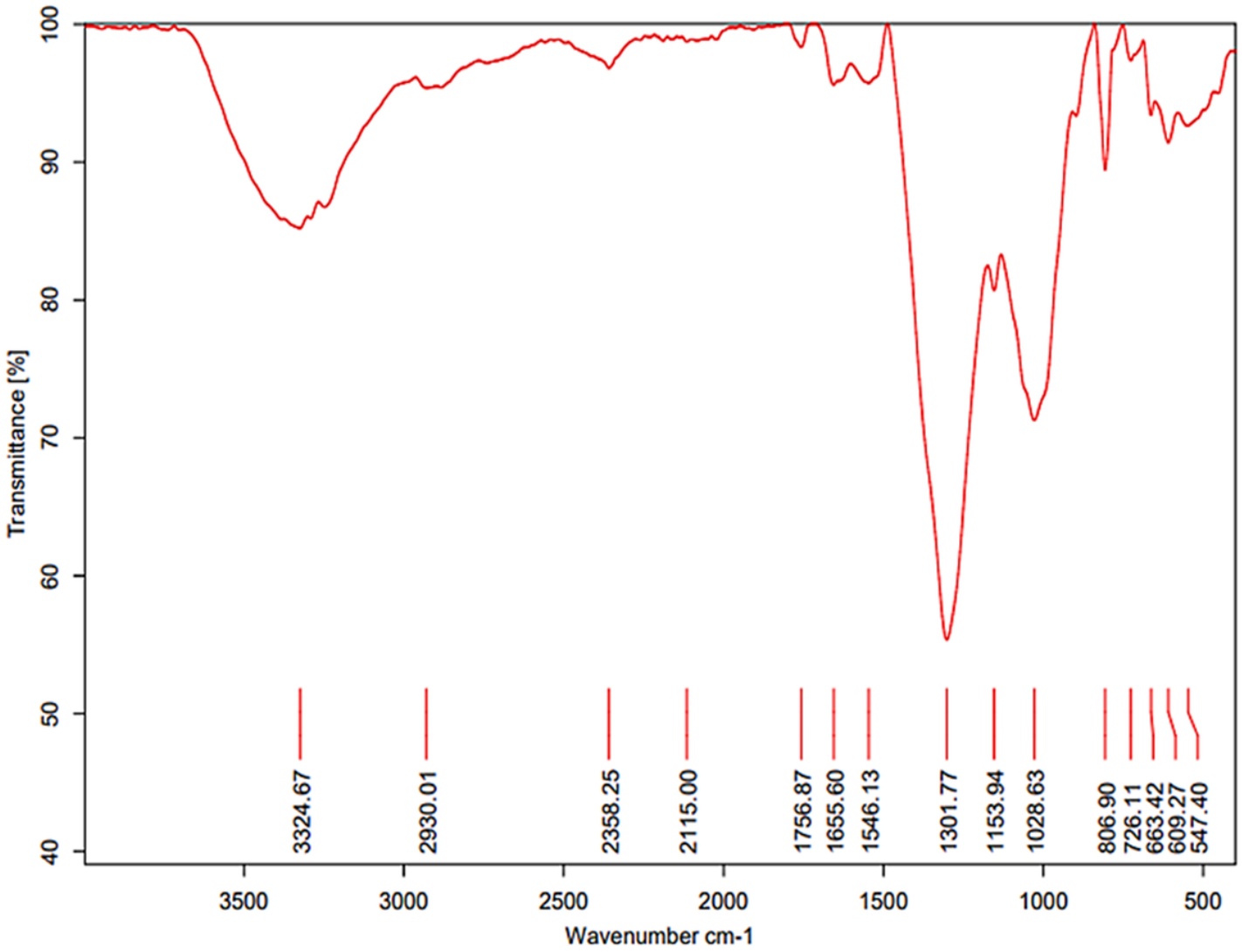

Figure 2

FTIR spectra of the biosynthesized Ch-AgNPs.

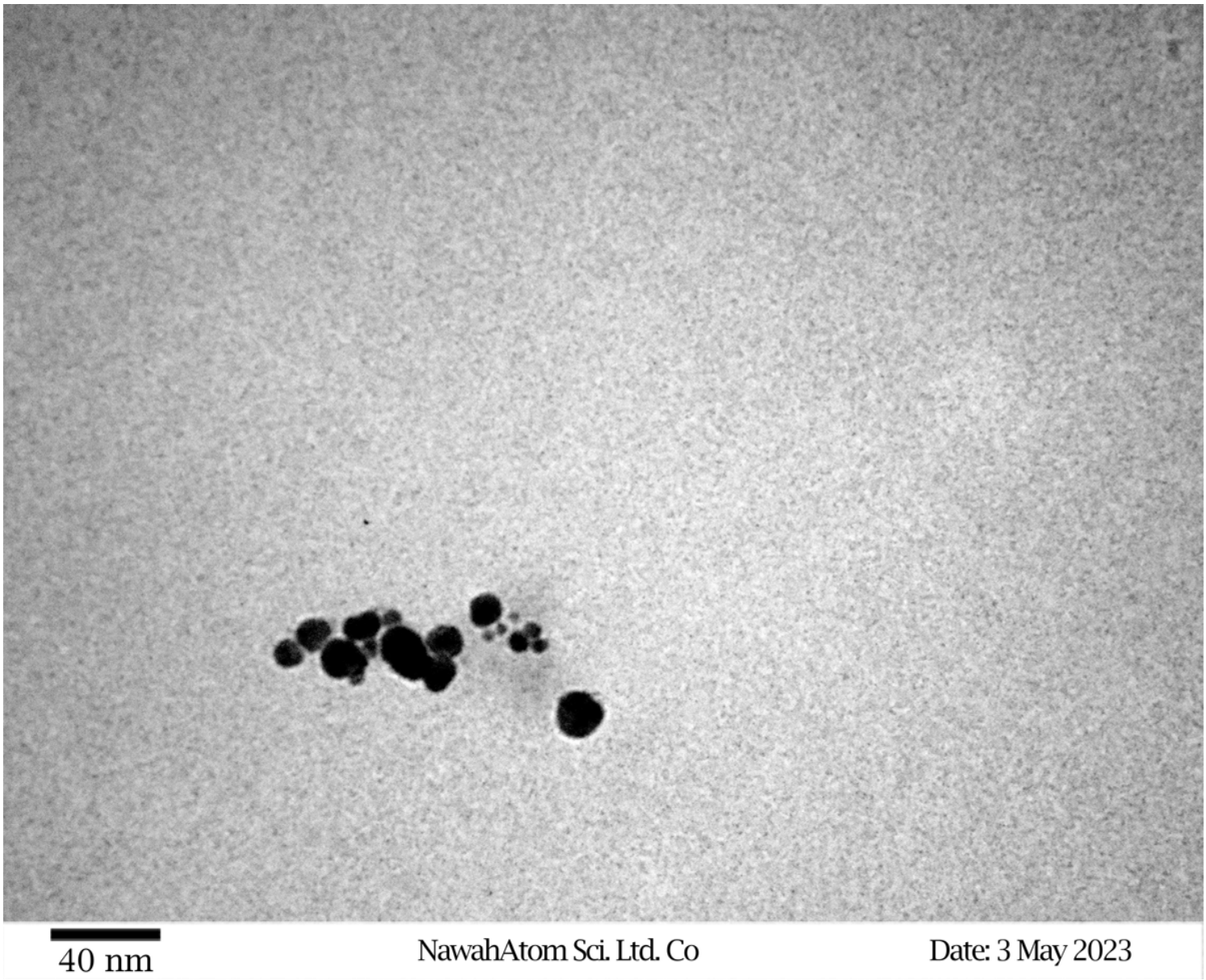

Figure 3

TEM imaging of the biosynthesized Ch-AgNPs.

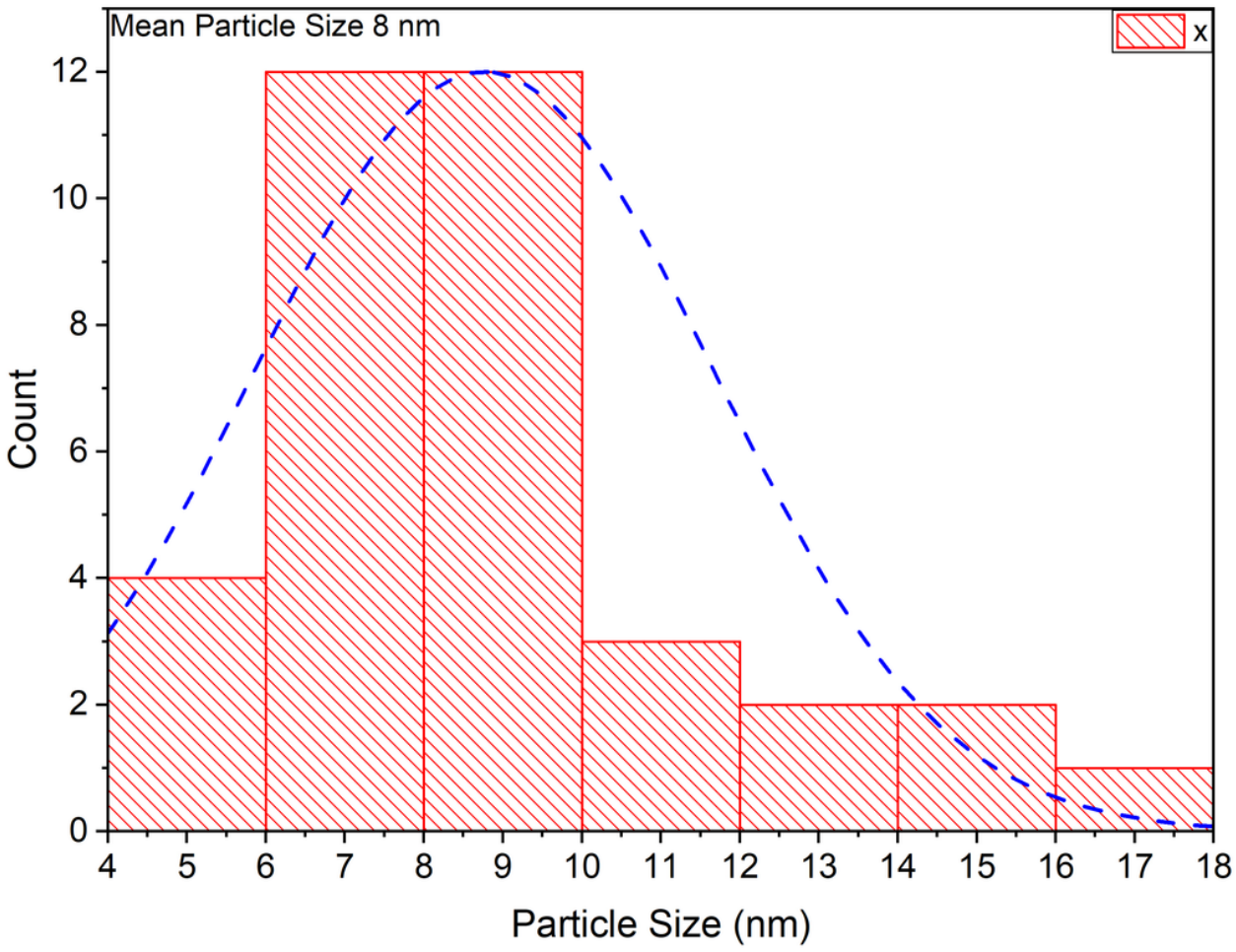

Figure 4

The average size of the biosynthesized nanoparticles.

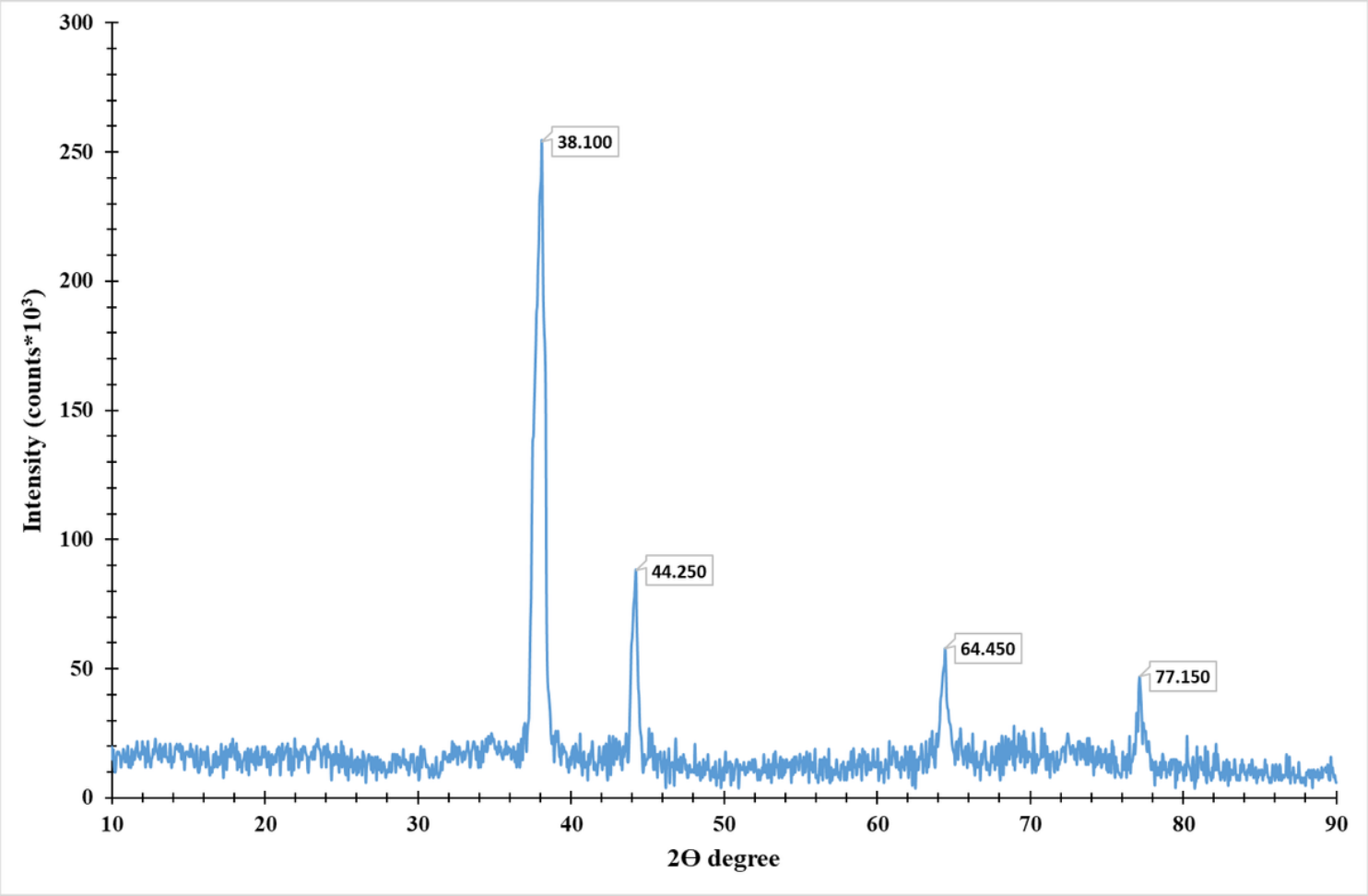

Figure 5

XRD test for Ch-AgNPs.

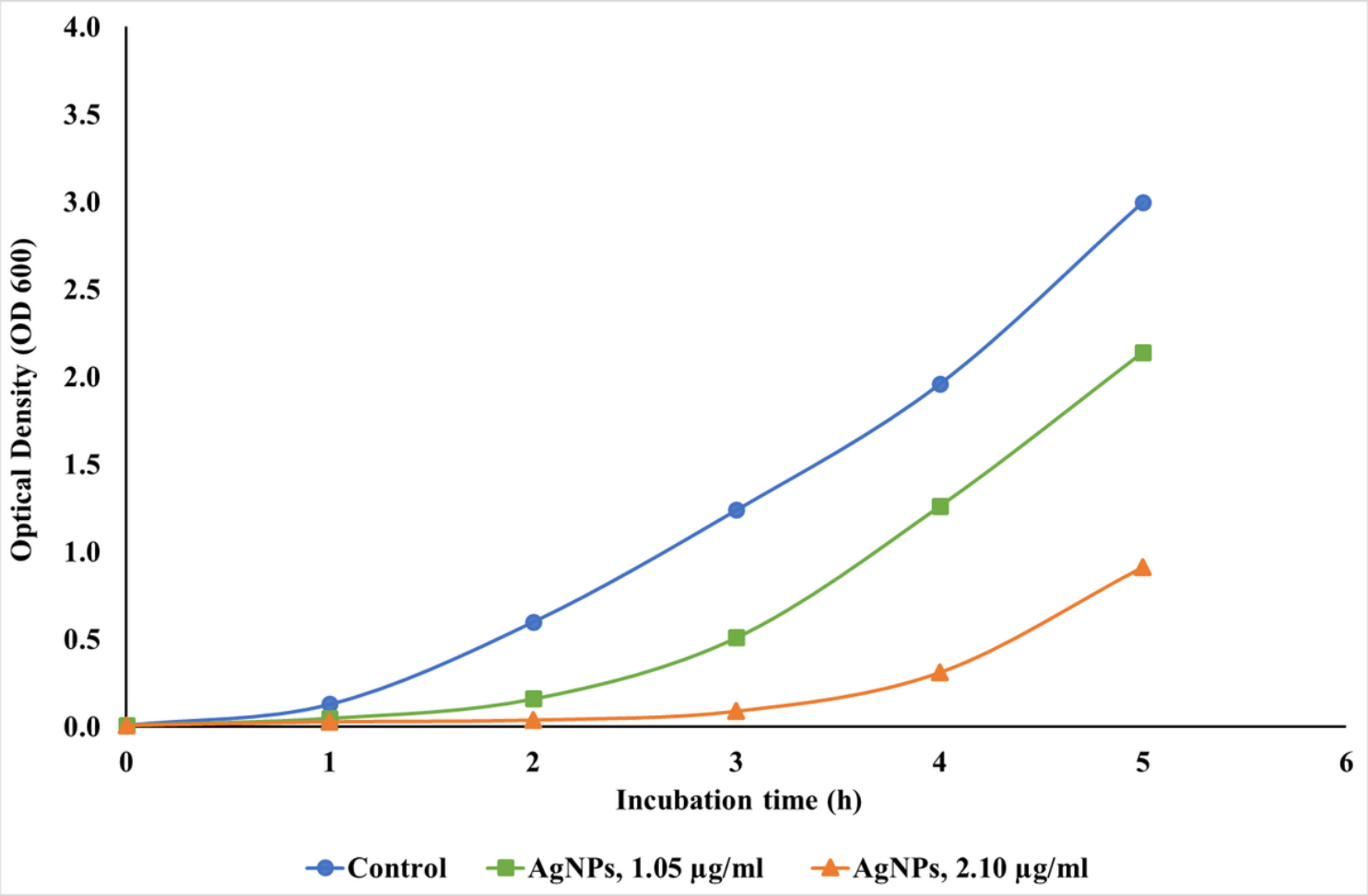

Figure 6

Growth curve of *Staphylococcus aureus*.

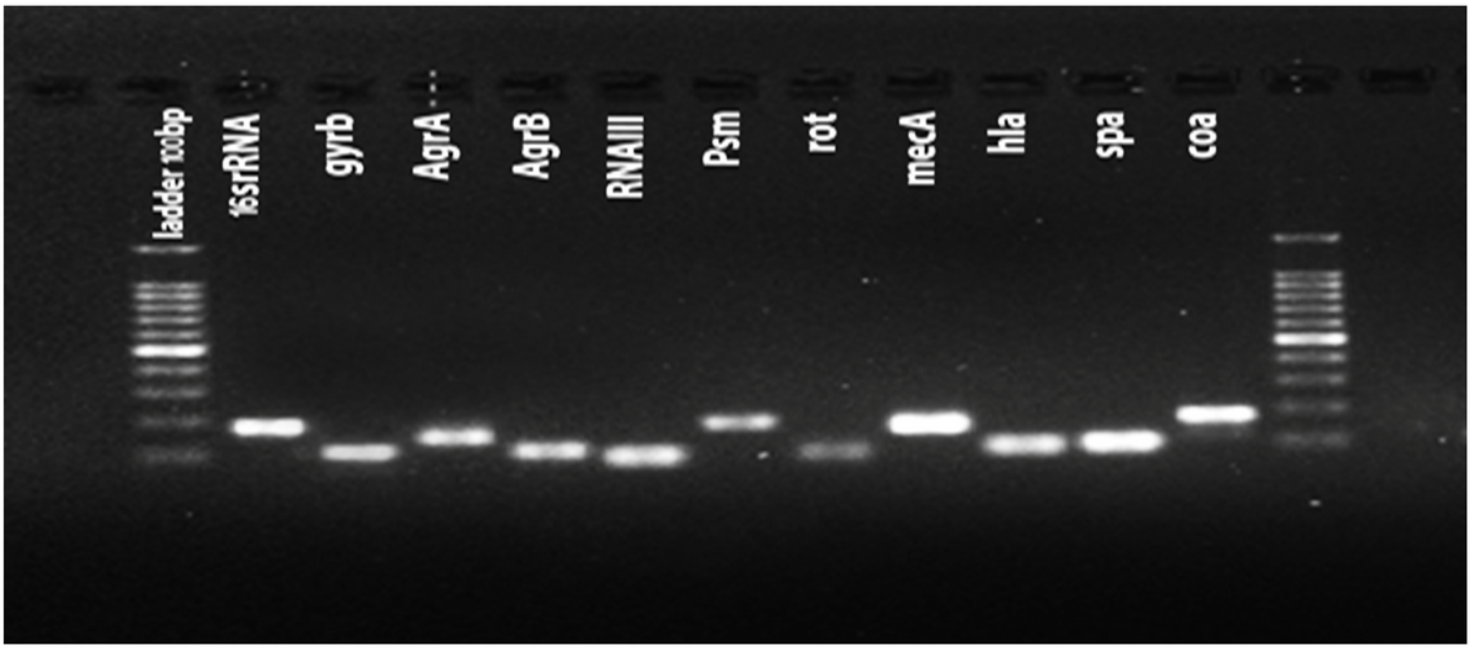

Figure 7

Gel electrophoresis of PCR product for 11 different genes of *S. aureus* with 1.5% agarose, 1X TAE, and at 70 volts for 55 min.

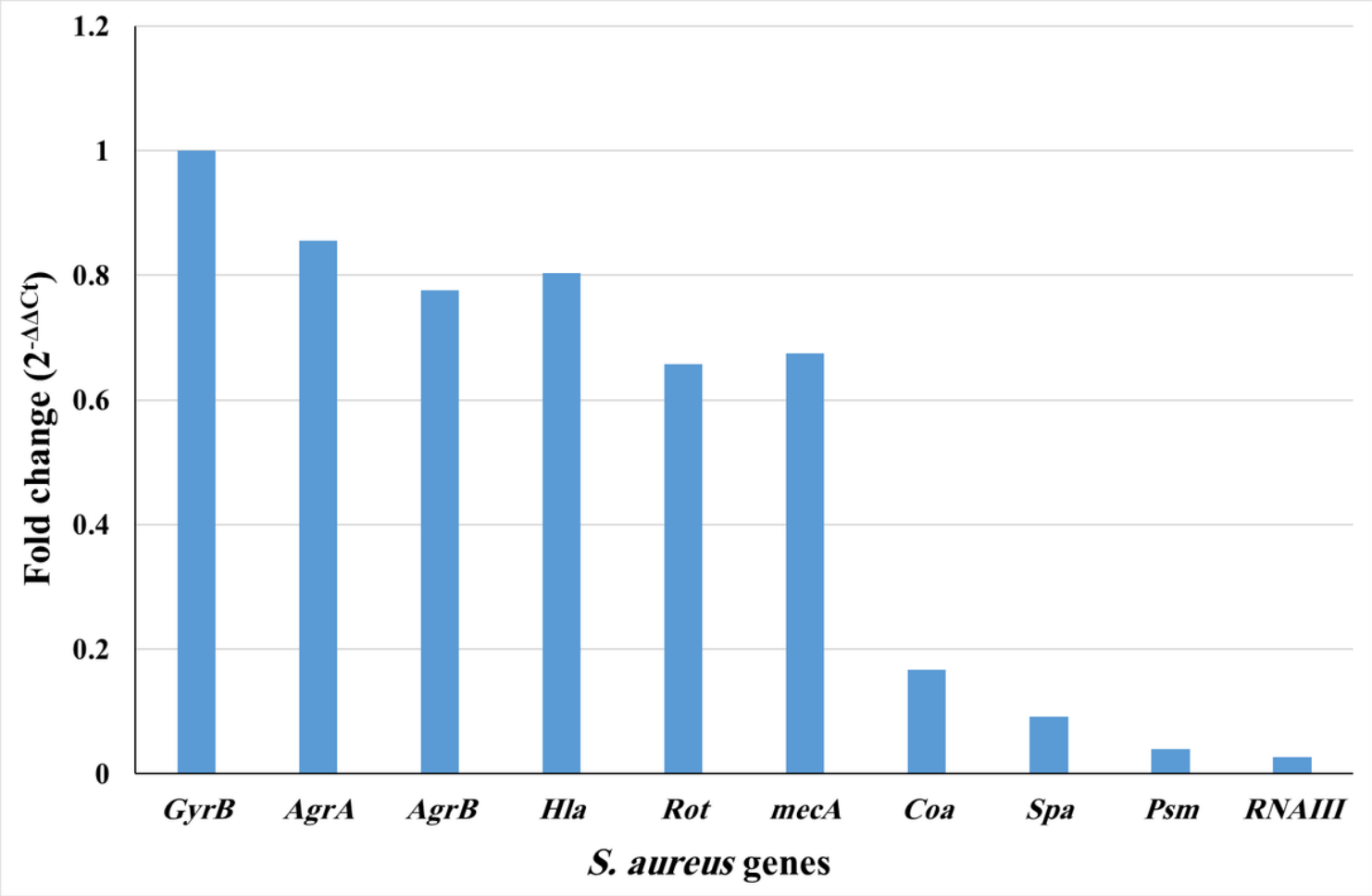

Figure 8

Relative change in expression of genes associated with QS and virulence factors in *S. aureus*