



Evaluation of the effects of chitosan-loaded Curcumin-Vancomycin nanoparticles on biofilm formation in *Staphylococcus aureus*

Mohammad Amin Mohammadi, Zahra Keshtmand^{*}

Department of Biology, CT.C., Islamic Azad University, Tehran, Iran

ABSTRACT

Article info:

Received: 23 Jun 2026
Accepted: 17 Aug 2026

Keywords:

Staphylococcus aureus
Biofilms
Chitosan
Curcumin
Vancomycin

Staphylococcus aureus is a major opportunistic pathogen responsible for persistent and device-related infections, largely due to its strong biofilm-forming capacity and increasing antimicrobial resistance. This study aimed to evaluate the antibiofilm effects of chitosan-loaded curcumin-vancomycin nanoparticles against *S. aureus* clinical isolates. Nanoparticles were synthesized via ionic gelation and characterized using dynamic light scattering and electrophoretic light scattering techniques. Biofilm formation was quantified using the crystal violet microtiter plate assay, and gene expression analysis of the *icaA* gene was performed in biofilm-producing *S. aureus* exposed to sub-minimum inhibitory concentration (MIC) concentrations of nanoparticles using real-time PCR and the $2^{-\Delta\Delta C_t}$ method. Results showed a significant reduction in *icaA* expression following treatment with curcumin, vancomycin, and particularly curcumin-vancomycin-loaded chitosan nanoparticles compared to untreated controls ($P < 0.05$). The greatest suppression of *icaA* expression was observed in the nanoparticle-treated group, indicating enhanced antibiofilm activity. The mean hydrodynamic diameter was 333.8 ± 9.2 nm with a polydispersity index (PDI) of 0.236, indicating a relatively uniform size distribution. The zeta potential was +28.9 mV, confirming good colloidal stability. All tested isolates were strong biofilm producers. MIC of nanoparticles ranged from 64 to 256 $\mu\text{g/mL}$, demonstrating strain-dependent antimicrobial activity. The findings demonstrate that curcumin-vancomycin-loaded chitosan nanoparticles effectively inhibit biofilm formation and downregulate key biofilm-associated genes in *S. aureus*. This synergistic nano-formulation offers a promising strategy for overcoming biofilm-associated infections and improving the efficacy of conventional antibiotics.

*Corresponding Author(s):

Zahra Keshtmand, PhD

Address: Department of Biology, Islamic Azad University, Tehran, Iran.

Tel: +98 2144600196

E-mail: Zahra.keshtmand@iaau.ac.ir



Copyright © 2026: Author(s)

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license (<https://creativecommons.org/licenses/by-nc/4.0/>).
Noncommercial uses of the work are permitted, provided the original work is properly cited.

1. Introduction

Staphylococcus aureus is one of the most important opportunistic bacterial pathogens responsible for a wide range of community- and hospital-acquired infections, including skin and soft tissue infections, wound infections, osteomyelitis, endocarditis, and device-associated infections [1,2]. The remarkable ability of *S. aureus* to form biofilms significantly contributes to its pathogenicity and persistence in clinical settings [1]. Biofilms are structured microbial communities embedded within a self-produced extracellular polymeric substance (EPS) matrix that protects bacterial cells from environmental stresses, host immune responses, and antimicrobial agents [3]. Consequently, biofilm-associated bacteria can exhibit up to 1000-fold greater resistance to antibiotics than their planktonic counterparts, leading to chronic and recurrent infections [4]. Biofilm formation in *S. aureus* occurs through a complex, multi-step process involving initial adhesion, accumulation, maturation, and dispersal [3]. This process is regulated by several genetic and environmental factors, including the intercellular adhesion (*ica*) operon, accessory gene regulator (*agr*) system, and various microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [5]. The ability of *S. aureus* to establish robust biofilms has become a major therapeutic challenge, particularly with the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) strains and the emergence of multidrug resistance [2,5]. Conventional antibiotic therapy often fails to eradicate biofilm-associated infections because antimicrobial agents have limited penetration through the EPS matrix, and bacterial cells within biofilms frequently adopt metabolically dormant states that reduce antibiotic susceptibility [1,4].

Vancomycin remains one of the most widely used antibiotics for the treatment of serious *S. aureus* infections, especially those caused by MRSA [6]. However, the efficacy of vancomycin against biofilm-associated infections is often compromised due to poor penetration into mature biofilms and the development of adaptive resistance mechanisms [6,7]. Therefore, innovative therapeutic approaches are needed to improve antibiotic delivery and enhance antibiofilm activity. Nanotechnology-based drug delivery systems have emerged as promising strategies to overcome these limitations by improving drug stability, targeted delivery, and controlled release profiles [8]. Such systems can facilitate deeper penetration into biofilm matrices and increase the local concentration of antimicrobial agents at infection sites [8,9].

Curcumin, a natural polyphenolic compound derived from *Curcuma longa* (turmeric), has attracted considerable attention due to its broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and antibiofilm properties [10]. Previous studies have demonstrated that curcumin can inhibit *S. aureus* biofilm formation by interfering

with bacterial adhesion, quorum sensing pathways, and biofilm-associated gene expression [11,12]. Nevertheless, the clinical application of curcumin is limited by its poor aqueous solubility, rapid metabolism, and low bioavailability [10]. Encapsulation of curcumin within nanoparticle-based delivery systems has been proposed as an effective strategy to overcome these drawbacks and enhance its therapeutic efficacy [13]. Chitosan, a naturally occurring cationic polysaccharide obtained from chitin, possesses excellent biocompatibility, biodegradability, and intrinsic antimicrobial properties [14]. Due to its positive surface charge, chitosan can interact electrostatically with negatively charged bacterial cell membranes and biofilm matrices, facilitating improved penetration and disruption of established biofilms [15]. Furthermore, chitosan nanoparticles have been widely investigated as drug carriers because they provide controlled drug release, enhanced stability, and increased retention at infection sites [14,16]. Several studies have reported that chitosan-based nanoparticles can significantly inhibit biofilm formation and improve the antibacterial efficacy of encapsulated compounds against *S. aureus* [16,17].

Recent evidence suggests that combining conventional antibiotics with natural antibiofilm agents within nanocarrier systems may produce synergistic antimicrobial effects [18]. The co-delivery of vancomycin and curcumin using chitosan nanoparticles may simultaneously target bacterial viability and biofilm integrity, thereby enhancing treatment efficacy against biofilm-forming *S. aureus* [18,19]. However, despite the growing interest in nanoparticle-mediated antimicrobial therapies, limited information is available regarding the antibiofilm activity of chitosan-loaded curcumin-vancomycin nanoparticles against *S. aureus* biofilms [19]. Therefore, the present study aimed to evaluate the effects of chitosan-loaded curcumin-vancomycin nanoparticles on biofilm formation in *S. aureus*, with the goal of exploring an effective strategy for combating biofilm-associated staphylococcal infections.

2. Materials and Methods

2.1 Chemicals and reagents

Chitosan (medium molecular weight; degree of deacetylation $\geq 85\%$), sodium tripolyphosphate (TPP), curcumin ($\geq 95\%$ purity), and vancomycin hydrochloride were purchased from Sigma-Aldrich (Merck KGaA, St. Louis, MO, USA). Tryptic Soy Broth (TSB), Tryptic Soy Agar (TSA), crystal violet, glacial acetic acid, dimethyl sulfoxide (DMSO), ethanol, and phosphate-buffered saline (PBS) were obtained from Merck (Darmstadt, Germany). The RNA extraction kit, cDNA synthesis kit, and SYBR Green Master Mix were purchased from Sinaclon BioScience Co. (Tehran, Iran). All reagents and chemicals used in this study were of

analytical grade and were used according to the manufacturers' instructions.

2.2 Bacterial isolates

Clinical isolates of *S. aureus* were obtained from clinical specimens collected from patients with diabetic foot osteomyelitis at Ayatollah Rouhani Hospital, Babol, Iran. The isolates were recovered from the hospital microbiology laboratory and subsequently transferred to the microbiology laboratory for further analysis. Only non-duplicate clinical isolates were included in the study. Identification of *S. aureus* was initially performed using conventional microbiological and biochemical methods and was subsequently confirmed by PCR amplification of the species-specific *nuc* gene [2]. Confirmed isolates were stored at -80°C in Tryptic Soy Broth (TSB) supplemented with 20% glycerol until further experiments.

2.3 Synthesis of chitosan-loaded curcumin-vancomycin nanoparticles

Chitosan-loaded curcumin-vancomycin nanoparticles were synthesized using the ionic gelation method. Briefly, chitosan was dissolved in 1% (v/v) acetic acid solution to obtain a final concentration of 2 mg/mL and stirred overnight at room temperature. Curcumin was dissolved in a minimal volume of DMSO and subsequently added to the chitosan solution under continuous magnetic stirring. Vancomycin hydrochloride was then dissolved in distilled water and incorporated into the chitosan-curcumin mixture. Subsequently, sodium tripolyphosphate (TPP; 1 mg/mL) was added dropwise to the mixture under constant stirring at room temperature. Nanoparticle formation occurred spontaneously through electrostatic interactions between the positively charged amino groups of chitosan and negatively charged phosphate groups of TPP. The suspension was stirred for 2 h to ensure complete nanoparticle formation. The obtained nanoparticles were collected by centrifugation at $15,000 \times g$ for 30 min, washed twice with distilled water to remove unbound compounds, and lyophilized for further analyses [20].

2.4 Particle size and zeta potential analysis

The hydrodynamic diameter, polydispersity index (PDI), and zeta potential of the synthesized nanoparticles was determined using Dynamic Light Scattering (DLS) and electrophoretic light scattering techniques (Zetasizer Nano ZS, Malvern Instruments, UK). Prior to measurement, nanoparticle suspensions were diluted in deionized water and sonicated for 5 min to avoid aggregation. Measurements were performed at 25°C in triplicate. The mean particle size, PDI, and zeta potential values were recorded and reported as mean $\pm$ standard deviation.

2.5 Determination of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of free vancomycin, free curcumin, and chitosan-loaded curcumin-vancomycin nanoparticles against the clinical *S. aureus* isolates were determined using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2025) [21]. Two-fold serial dilutions of each tested agent were prepared in Mueller-Hinton broth (MHB). For the chitosan-loaded curcumin-vancomycin nanoparticles, the final concentrations tested ranged from 16 to 512 $\mu\text{g/mL}$, corresponding to final concentrations of 16, 32, 64, 128, 256, and 512 $\mu\text{g/mL}$ in the microdilution series. Thus, the final concentration in the first well was 512 $\mu\text{g/mL}$. Bacterial suspensions were adjusted to the turbidity of a 0.5 McFarland standard and inoculated into the wells. The microplates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration showing no visible bacterial growth. Appropriate growth and sterility controls were included in each assay, and all measurements were performed in triplicate.

2.6 Phenotypic evaluation of biofilm formation

Biofilm-forming ability of *S. aureus* isolates was assessed using the microtiter plate crystal violet assay. Briefly, overnight bacterial cultures were diluted 1:100 in TSB supplemented with 1% glucose. Aliquots (200 μL) were transferred into sterile 96-well polystyrene microplates and incubated at 37°C for 24 h. Following incubation, planktonic cells were removed and wells were gently washed three times with PBS. The adherent biofilm was fixed with methanol for 15 min and stained with 0.1% crystal violet for 20 min. Excess stain was removed by washing with distilled water. The bound dye was solubilized using 33% acetic acid, and absorbance was measured at 570 nm using a microplate reader. Biofilm production was classified as weak, moderate, or strong according to the criteria described by Stepanović et al. [22].

2.7 Quantitative real-time PCR analysis

To investigate the antibiofilm effect of nanoparticles independent of growth inhibition, biofilm-producing isolates were exposed to sub-MIC concentrations of free curcumin, free vancomycin, and chitosan-loaded curcumin-vancomycin nanoparticles. Bacterial suspensions were cultured in TSB containing the selected sub-MIC concentration and incubated for 24 h at 37°C . Untreated cultures served as controls. Following incubation, biofilm formation was quantified using the crystal violet microtiter plate assay as described above. Total RNA was extracted from untreated and treated biofilm-forming *S. aureus* isolates using the RNX-Plus™ Kit (Sinaclon Bioscience,

Tehran, Iran) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically, and RNA integrity was verified by agarose gel electrophoresis. To avoid interference from genomic DNA, RNA samples were treated with DNase I prior to reverse transcription. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a commercial cDNA synthesis kit. The relative expression of the *icaA* gene was quantified by real-time PCR using the following primers: Forward: 5'-GAGGTAAAGCCAACGCACTC-3' and Reverse: 5'-CCTGTAACCGCACCAAGTTT-3' (151 bp), previously described by Atshan et al. [23]. The housekeeping gene *gyrB* was used as the internal reference gene. Amplification reactions were performed in a final volume of 20 µL containing SYBR Green Master Mix, gene-specific primers, nuclease-free water, and cDNA template. Thermal cycling conditions consisted of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Expression levels of *icaA* in nanoparticle-treated isolates were compared with those of untreated controls. Melting curve analysis was performed to confirm the specificity of the amplified products. Total RNA was extracted from untreated and nanoparticle-treated biofilm-forming *S. aureus* isolates using the RNX-Plus™ Kit (Sinaclon Bioscience, Tehran, Iran) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically using a NanoDrop instrument (Thermo Scientific, USA), and RNA integrity was verified by agarose gel electrophoresis. To avoid interference from genomic DNA, RNA samples were treated with DNase I prior to reverse transcription. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the cDNA Synthesis Kit (Sinaclon Bioscience, Tehran, Iran) following the

manufacturer's protocol. The synthesized cDNA was stored at -20°C and subsequently used as the template for quantitative real-time PCR analysis. Amplification reactions were performed in a final volume of 20 µL containing SYBR Green Master Mix, gene-specific primers, nuclease-free water, and cDNA template. The housekeeping gene *gyrB* was used as the internal reference gene. Thermal cycling conditions consisted of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 60 s. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Expression levels of *icaA* in nanoparticle-treated isolates were compared with those of untreated controls.

2.8 Statistical analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analyses were carried out using GraphPad Prism version 9.0. Differences between treated and untreated groups were analyzed using Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value <0.05 was considered statistically significant.

3. Results

All 20 clinical isolates of *S. aureus* were strong biofilm producers. The particle size distribution and PDI of the synthesized nanoparticles were determined using DLS. Samples were analyzed in disposable polystyrene cuvettes at 25°C with a refractive index of 1.335. The mean hydrodynamic diameter of the nanoparticles was 333.8 ± 9.2 nm, with a PDI of 0.236, indicating a relatively narrow size distribution. The synthesized nanoparticles exhibited a positive zeta potential of +28.9 mV, indicating good colloidal stability. The electrical conductivity of the suspension was 2.69 mS/cm (Figure 1).

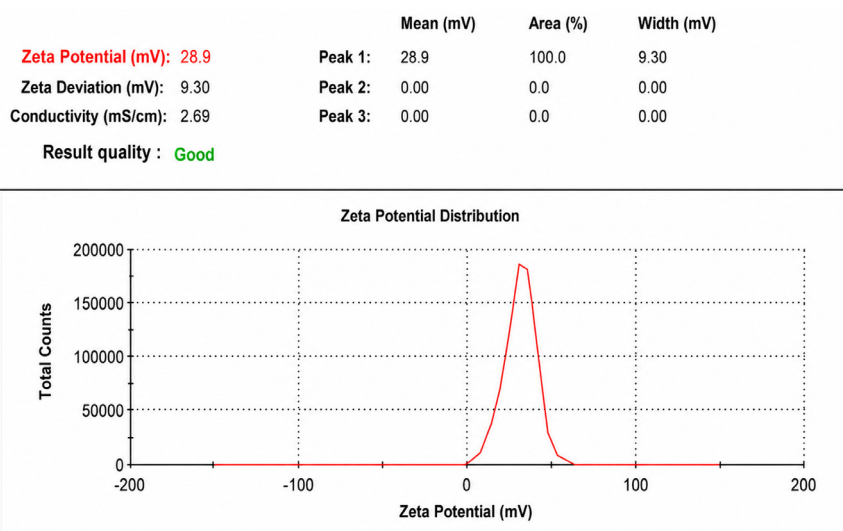


Figure 1. Calculation of the zeta potential of chitosan nanoparticles using a Zetasizer Nano ZS.

The MIC values of free vancomycin ranged from 1 to 8 µg/mL (MIC₅₀ = 2 µg/mL; MIC₉₀ = 4 µg/mL), while free curcumin showed higher MICs ranging from 128 to 512 µg/mL (MIC₅₀ = 256 µg/mL; MIC₉₀ = 512 µg/mL). The co-loaded curcumin-vancomycin chitosan nanoparticles exhibited MIC values between 64 and 256 µg/mL (MIC₅₀ = 128 µg/mL; MIC₉₀ = 256 µg/mL).

Gene expression analysis of the biofilm-associated gene *icaA* was performed in strong biofilm-producing isolates exposed to sub-MIC concentrations of free curcumin, free vancomycin, and curcumin-vancomycin-loaded chitosan nanoparticles using real-time PCR. The *gyrB* gene was used as the internal control, and relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Treatment with curcumin, vancomycin, and especially the curcumin-vancomycin nanoparticles significantly reduced *icaA* expression compared with the untreated control ($P < 0.05$). The greatest reduction was observed in the nanoparticle-treated group, in which *icaA* expression decreased approximately 0.8-fold relative to the control (Figure 2).

4. Discussion

In the present study, all 20 clinical isolates of *S. aureus* exhibited strong biofilm-forming capacity. This finding is consistent with the well-established role of biofilm as a major virulence determinant that confers high-level tolerance to antibiotics and host immune defenses, thereby contributing to persistent and recurrent infections [24,25]. The ability of *S. aureus* to form robust biofilms continues to represent a significant clinical challenge, particularly in device-related and chronic infections, and underscores the need for therapeutic strategies that specifically target biofilm matrix development and associated gene expression [26]. Physicochemical characterization revealed that the curcumin-vancomycin co-loaded chitosan nanoparticles possessed a mean hydrodynamic diameter of 333.8 ± 9.2 nm, a polydispersity index of 0.236, and a positive zeta potential of +28.9 mV. Nanoparticles within this size range are generally considered suitable for interaction

with bacterial surfaces and penetration into biofilm matrices, while a low PDI indicates a relatively homogeneous formulation [27]. The positive surface charge is particularly advantageous, as it promotes electrostatic attraction to the negatively charged bacterial cell wall and extracellular polymeric substances, thereby facilitating closer contact and improved delivery of the encapsulated agents [28,29].

The antimicrobial activity of the co-loaded nanoparticles was superior to that of free curcumin and, for several isolates, comparable or advantageous relative to free vancomycin when drug content is taken into account. These results support previous observations that nanoencapsulation can enhance the effective concentration of poorly soluble natural compounds and improve antibiotic delivery into biofilm communities [30,31]. The strain-dependent variation in MIC values is likely attributable to differences in cell-surface properties, biofilm density, and the extent of nanoparticle-bacterium interaction among clinical isolates. At the molecular level, sub-inhibitory concentrations of free curcumin, free vancomycin, and especially the co-loaded nanoparticles significantly downregulated *icaA* expression. The greatest reduction (approximately 0.8-fold relative to the untreated control) was observed with the nanoparticle formulation. The *icaA* gene encodes *N*-acetylglucosaminyltransferase, a key enzyme required for the synthesis of polysaccharide intercellular adhesin (PIA), the major component of the staphylococcal biofilm matrix [32]. Suppression of *icaA* therefore directly impairs biofilm structural integrity. The enhanced effect of the co-loaded nanoparticles compared with the free agents suggests improved cellular uptake and sustained release of both curcumin and vancomycin within the biofilm microenvironment, resulting in a more pronounced interference with PIA biosynthesis [33,34]. Several limitations of the present work should be acknowledged. The experiments were performed exclusively *in vitro*; consequently, pharmacokinetic behavior, potential cytotoxicity, and *in-vivo* efficacy remain to be established.

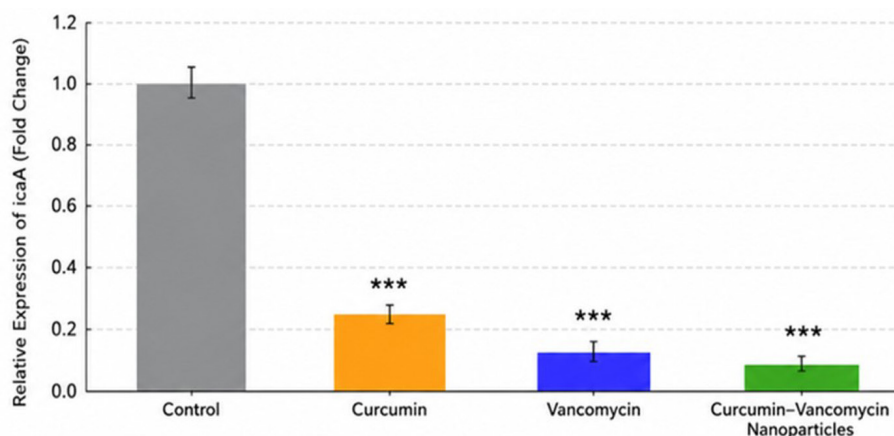


Figure 2. Relative expression of *icaA* gene in biofilm-producing *S. aureus* strains after treatment with nanoparticles compared to untreated.

Only a single biofilm-related gene was examined; broader transcriptomic or proteomic analyses would provide a more comprehensive understanding of the molecular mechanisms involved. In addition, the clinical isolates were obtained from a single center, which may limit the generalizability of the findings. Future studies incorporating animal models of biofilm infection, multi-center isolate collections, and multi-omics approaches are warranted to further validate and expand these observations. Collectively, the physicochemical stability of the formulation, the improved antimicrobial profile relative to free curcumin, and the significant downregulation of *icaA* support the potential of chitosan-based co-delivery of curcumin and vancomycin as a promising antibiofilm strategy against clinical *S. aureus* isolates.

The present study demonstrated that chitosan-loaded curcumin-vancomycin nanoparticles exhibited antibiofilm activity against clinical *S. aureus* isolates and were associated with reduced expression of the biofilm-associated *icaA* gene. Although the greatest reduction in *icaA* expression was observed following treatment with the co-loaded nanoparticles, free curcumin and vancomycin also produced significant reductions compared with the untreated control. Therefore, the present findings do not establish a synergistic effect of the combined nanoformulation or demonstrate clear superiority over the individual agents. Further studies using formal synergy assays, equivalent drug concentrations, additional biofilm-related targets, and *in vivo* models are warranted to determine whether nanoencapsulation provides a meaningful advantage over free curcumin or vancomycin.

Declaration of artificial intelligence (AI) in the writing process

The authors declare whether AI or AI-assisted technologies were used during the preparation of this manuscript. If used, AI tools were employed solely to improve language quality, grammar, readability, and organizational structure. The authors carefully reviewed and edited all AI-generated content and take full responsibility for the accuracy, integrity, and originality of the final manuscript. No AI tool was used to generate, analyze, or interpret scientific data or images, or to draw scientific conclusions. The use of AI-assisted technologies complies with current publication ethics recommendations and journal policies.

Authors' contributions

MAM: Conceptualization, methodology, investigation, data collection, laboratory experiments, data analysis, and writing of the original draft. ZK: Supervision, conceptualization, methodology, interpretation of the results, critical revision of the manuscript, and project administration. All authors have read and approved the final version of the manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical declarations

The study protocol was reviewed and approved by the Institutional Ethics Committee of the Islamic Azad University, Central Tehran Branch (Approval No. IR.IAU.CTB.REC.1404.147). The study was conducted in accordance with the ethical principles and regulations governing research involving human-derived clinical specimens. All procedures involving clinical isolates were performed in accordance with the approved study protocol and applicable institutional ethical requirements.

Financial support

Self-funded.

References

- Schilcher K, Horswill AR. Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies. *Microbiol Mol Biol Rev.* 2020;84(3):e00026-19. DOI: [10.1128/MMBR.00026-19](https://doi.org/10.1128/MMBR.00026-19) PMID: [32792334](https://pubmed.ncbi.nlm.nih.gov/32792334/)
- Agbo MC, Ezeonu IM, Onodagu BO, Ezech CC, Ozioko CA, Emencheta SC. Antimicrobial resistance markers distribution in *Staphylococcus aureus* from Nsukka, Nigeria. *BMC Infect Dis.* 2024;24(1):320. DOI: [10.1186/s12879-024-09126-1](https://doi.org/10.1186/s12879-024-09126-1) PMID: [38491352](https://pubmed.ncbi.nlm.nih.gov/38491352/)
- Idrees M, Sawant S, Karodia N, Rahman A. *Staphylococcus aureus* Biofilm: Morphology, Genetics, Pathogenesis and Treatment Strategies. *Int J Environ Res Public Health.* 2021;18(14):7602. DOI: [10.3390/ijerph18147602](https://doi.org/10.3390/ijerph18147602) PMID: [34300053](https://pubmed.ncbi.nlm.nih.gov/34300053/)
- Seethalakshmi PS, Rajeev R, Kiran GS, Selvin J. Promising treatment strategies to combat *Staphylococcus aureus* biofilm infections: an updated review. *Biofouling.* 2020;36(10):1159-1181. DOI: [10.1080/08927014.2020.1857743](https://doi.org/10.1080/08927014.2020.1857743) PMID: [33353409](https://pubmed.ncbi.nlm.nih.gov/33353409/)
- Tuon FF, Suss PH, Telles JP, Dantas LR, Borges NH, Ribeiro VST. Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics (Basel).* 2023;12(1):87. DOI: [10.3390/antibiotics12010087](https://doi.org/10.3390/antibiotics12010087) PMID: [36671287](https://pubmed.ncbi.nlm.nih.gov/36671287/)
- Nasser A, Dallal MMS, Jahanbakhshi S, Azimi T, Nikouei L. *Staphylococcus aureus*: Biofilm Formation and Strategies Against it. *Curr Pharm Biotechnol.* 2022;23(5):664-678. DOI: [10.2174/1389201022666210708171123](https://doi.org/10.2174/1389201022666210708171123) PMID: [34238148](https://pubmed.ncbi.nlm.nih.gov/34238148/)
- Guo N, Bai X, Shen Y, Zhang T. Target-based screening for natural products against *Staphylococcus aureus* biofilms. *Crit Rev Food Sci Nutr.* 2023;63(14):2216-2230. DOI: [10.1080/10408398.2021.1972280](https://doi.org/10.1080/10408398.2021.1972280) PMID: [34491124](https://pubmed.ncbi.nlm.nih.gov/34491124/)
- Zheng D, Huang C, Huang H, Zhao Y, Khan MRU, Zhao H, et al. Antibacterial Mechanism of Curcumin: A Review. *Chem Biodivers.* 2020;17(8):e2000171. DOI: [10.1002/cbdv.202000171](https://doi.org/10.1002/cbdv.202000171) PMID: [32533635](https://pubmed.ncbi.nlm.nih.gov/32533635/)
- Gao K, Zhang B, Zhao F. Antibiofilm Effect of Curcumin Against *Staphylococcus aureus* Surface Wound Biofilm-Associated Infection: In Vitro and In Silico. *Appl Biochem Biotechnol.* 2023;195(9):5329-5337. DOI: [10.1007/s12010-022-03844-5](https://doi.org/10.1007/s12010-022-03844-5) PMID: [35166997](https://pubmed.ncbi.nlm.nih.gov/35166997/)
- Batista de Andrade Neto J, Pessoa de Farias Cabral V, Brito Nogueira LF, Rocha da Silva C, Gurgel do Amaral Valente Sá L,

- Ramos da Silva A, et al. Anti-MRSA activity of curcumin in planktonic cells and biofilms and determination of possible action mechanisms. *Microb Pathog.* 2021;155:104892. DOI: [10.1016/j.micpath.2021.104892](https://doi.org/10.1016/j.micpath.2021.104892) PMID: [33894289](https://pubmed.ncbi.nlm.nih.gov/33894289/)
11. Ma S, Moser D, Han F, Leonhard M, Schneider-Stickler B, Tan Y. Preparation and antibiofilm studies of curcumin loaded chitosan nanoparticles against polymicrobial biofilms of *Candida albicans* and *Staphylococcus aureus*. *Carbohydr Polym.* 2020;241:116254. DOI: [10.1016/j.carbpol.2020.116254](https://doi.org/10.1016/j.carbpol.2020.116254) PMID: [32507182](https://pubmed.ncbi.nlm.nih.gov/32507182/)
12. Pamukcu A, Erdogan N, Şen Karaman D. Polyethylenimine-grafted mesoporous silica nanocarriers markedly enhance the bactericidal effect of curcumin against *Staphylococcus aureus* biofilm. *J Biomed Mater Res B Appl Biomater.* 2022;110(11):2506-2520. DOI: [10.1002/jbm.b.35108](https://doi.org/10.1002/jbm.b.35108) PMID: [35735075](https://pubmed.ncbi.nlm.nih.gov/35735075/)
13. Xu J, Lin Q, Sheng M, Ding T, Li B, Gao Y, et al. Antibiofilm Effect of Cinnamaldehyde-Chitosan Nanoparticles against the Biofilm of *Staphylococcus aureus*. *Antibiotics (Basel).* 2022;11(10):1403. DOI: [10.3390/antibiotics11101403](https://doi.org/10.3390/antibiotics11101403) PMID: [36290061](https://pubmed.ncbi.nlm.nih.gov/36290061/)
14. Zhang Z, Chen J, Zou L, Tang J, Zheng J, Luo M, et al. Preparation, Characterization, and *Staphylococcus aureus* Biofilm Elimination Effect of Baicalein-Loaded β -Cyclodextrin-Grafted Chitosan Nanoparticles. *Int J Nanomedicine.* 2022;17:5287-5302. DOI: [10.2147/IJN.S383182](https://doi.org/10.2147/IJN.S383182) PMID: [36411767](https://pubmed.ncbi.nlm.nih.gov/36411767/)
15. Rashki S, Safardoust-Hojaghan H, Mirzaei H, Abdulsahib WK, Mahdi MA, Salavati-Niasari M, et al. Delivery LL37 by chitosan nanoparticles for enhanced antibacterial and antibiofilm efficacy. *Carbohydr Polym.* 2022;291:119634. DOI: [10.1016/j.carbpol.2022.119634](https://doi.org/10.1016/j.carbpol.2022.119634) PMID: [35698353](https://pubmed.ncbi.nlm.nih.gov/35698353/)
16. Etemadi S, Barhaghi MHS, Leylabadlo HE, Memar MY, Mohammadi AB, Ghotaslou R. The synergistic effect of turmeric aqueous extract and chitosan against multidrug-resistant bacteria. *New Microbes New Infect.* 2021;41:100861. DOI: [10.1016/j.nmni.2021.100861](https://doi.org/10.1016/j.nmni.2021.100861) PMID: [33898041](https://pubmed.ncbi.nlm.nih.gov/33898041/)
17. Cai M, Wang Y, Wang R, Li M, Zhang W, Yu J, et al. Antibacterial and antibiofilm activities of chitosan nanoparticles loaded with *Ocimum basilicum* L. essential oil. *Int J Biol Macromol.* 2022;202:122-129. DOI: [10.1016/j.ijbiomac.2022.01.066](https://doi.org/10.1016/j.ijbiomac.2022.01.066) PMID: [35041880](https://pubmed.ncbi.nlm.nih.gov/35041880/)
18. Mastoor S, Nazim F, Rizwan-Ul-Hasan S, Ahmed K, Khan S, Ali SN, et al. Analysis of the Antimicrobial and Anti-Biofilm Activity of Natural Compounds and Their Analogues against *Staphylococcus aureus* Isolates. *Molecules.* 2022;27(20):6874. DOI: [10.3390/molecules27206874](https://doi.org/10.3390/molecules27206874) PMID: [36296467](https://pubmed.ncbi.nlm.nih.gov/36296467/)
19. Khaleghian M, Sahrayi H, Hafezi Y, Mirshafeyan M, Moghaddam ZS, Farasati Far B, et al. In silico design and mechanistic study of niosome-encapsulated curcumin against multidrug-resistant *Staphylococcus aureus* biofilms. *Front Microbiol.* 2023;14:1277533. DOI: [10.3389/fmicb.2023.1277533](https://doi.org/10.3389/fmicb.2023.1277533) PMID: [38098658](https://pubmed.ncbi.nlm.nih.gov/38098658/)
20. Cerchiara T, Abruzzo A, di Cagno M, Bigucci F, Bauer-Brandl A, Parolin C, et al. Chitosan based micro- and nanoparticles for colon-targeted delivery of vancomycin prepared by alternative processing methods. *Eur J Pharm Biopharm.* 2015;92:112-9. DOI: [10.1016/j.ejpb.2015.03.004](https://doi.org/10.1016/j.ejpb.2015.03.004) PMID: [25769679](https://pubmed.ncbi.nlm.nih.gov/25769679/)
21. Wayne P. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 35th informational supplement. CLSI document M100-S35. 2025. URL: <https://clsi.org/shop/standards/m100>
22. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS.* 2007;115(8):891-9. DOI: [10.1111/j.1600-0463.2007.apm_630.x](https://doi.org/10.1111/j.1600-0463.2007.apm_630.x) PMID: [17696944](https://pubmed.ncbi.nlm.nih.gov/17696944/)
23. Atshan SS, Shamsudin MN, Karunanidhi A, van Belkum A, Lung LT, Sekawi Z, et al. Quantitative PCR analysis of genes expressed during biofilm development of methicillin resistant *Staphylococcus aureus* (MRSA). *Infect Genet Evol.* 2013;18:106-12. DOI: [10.1016/j.meegid.2013.05.002](https://doi.org/10.1016/j.meegid.2013.05.002) PMID: [23669446](https://pubmed.ncbi.nlm.nih.gov/23669446/)
24. Kashi M, Noei M, Chegini Z, Shariati A. Natural compounds in the fight against *Staphylococcus aureus* biofilms: a review of antibiofilm strategies. *Front Pharmacol.* 2024;15:1491363. DOI: [10.3389/fphar.2024.1491363](https://doi.org/10.3389/fphar.2024.1491363) PMID: [39635434](https://pubmed.ncbi.nlm.nih.gov/39635434/)
25. Costa EM, Silva S, Vicente S, Neto C, Castro PM, Veiga M, et al. Chitosan nanoparticles as alternative anti-staphylococci agents: Bactericidal, antibiofilm and antiadhesive effects. *Mater Sci Eng C Mater Biol Appl.* 2017;79:221-226. DOI: [10.1016/j.msec.2017.05.047](https://doi.org/10.1016/j.msec.2017.05.047) PMID: [28629011](https://pubmed.ncbi.nlm.nih.gov/28629011/)
26. Tan Y, Ma S, Leonhard M, Moser D, Haselmann GM, Wang J, et al. Enhancing antibiofilm activity with functional chitosan nanoparticles targeting biofilm cells and biofilm matrix. *Carbohydr Polym.* 2018;200:35-42. DOI: [10.1016/j.carbpol.2018.07.072](https://doi.org/10.1016/j.carbpol.2018.07.072) PMID: [30177175](https://pubmed.ncbi.nlm.nih.gov/30177175/)
27. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics.* 2018;10(2):57. DOI: [10.3390/pharmaceutics10020057](https://doi.org/10.3390/pharmaceutics10020057) PMID: [29783687](https://pubmed.ncbi.nlm.nih.gov/29783687/)
28. Honary S, Zahir F. Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2). *Trop J Pharm Res.* 2013;12(2):265-273. DOI: [10.4314/tjpr.v12i2.20](https://doi.org/10.4314/tjpr.v12i2.20)
29. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: a state of the art review. *Int J Food Microbiol.* 2010;144(1):51-63. DOI: [10.1016/j.ijfoodmicro.2010.09.012](https://doi.org/10.1016/j.ijfoodmicro.2010.09.012) PMID: [20951455](https://pubmed.ncbi.nlm.nih.gov/20951455/)
30. Gayani B, Dilhari A, Wijesinghe GK, Kumarage S, Abayaweera G, Samarakoon SR, et al. Effect of natural curcuminoids-intercalated layered double hydroxide nanohybrid against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*: A bactericidal, antibiofilm, and mechanistic study. *Microbiologypopen.* 2019;8(5):e00723. DOI: [10.1002/mbo3.723](https://doi.org/10.1002/mbo3.723) PMID: [30221843](https://pubmed.ncbi.nlm.nih.gov/30221843/)
31. Luan L, Chi Z, Liu C. Chinese White Wax Solid Lipid Nanoparticles as a Novel Nanocarrier of Curcumin for Inhibiting the Formation of *Staphylococcus aureus* Biofilms. *Nanomaterials (Basel).* 2019;9(5):763. DOI: [10.3390/nano9050763](https://doi.org/10.3390/nano9050763) PMID: [31109013](https://pubmed.ncbi.nlm.nih.gov/31109013/)
32. O'Gara JP. ica and beyond: biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. *FEMS Microbiol Lett.* 2007;270(2):179-88. DOI: [10.1111/j.1574-6968.2007.00688.x](https://doi.org/10.1111/j.1574-6968.2007.00688.x) PMID: [17419768](https://pubmed.ncbi.nlm.nih.gov/17419768/)
33. Targhi AA, Moammeri A, Jamshidifar E, Abbaspour K, Sadeghi S, Lamakani L, et al. Synergistic effect of curcumin-Cu and curcumin-Ag nanoparticle loaded niosome: Enhanced antibacterial and anti-biofilm activities. *Bioorg Chem.* 2021;115:105116. DOI: [10.1016/j.bioorg.2021.105116](https://doi.org/10.1016/j.bioorg.2021.105116) PMID: [34333420](https://pubmed.ncbi.nlm.nih.gov/34333420/)
34. Ramasamy M, Lee JH, Lee J. Development of gold nanoparticles coated with silica containing the antibiofilm drug cinnamaldehyde and their effects on pathogenic bacteria. *Int J Nanomedicine.* 2017;12:2813-2828. DOI: [10.2147/IJN.S132784](https://doi.org/10.2147/IJN.S132784) PMID: [28435260](https://pubmed.ncbi.nlm.nih.gov/28435260/)