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Original Article

Nanocurcumin: An Innovative Strategy to Combat Virulence and Methicillin Resistance in *Staphylococcus aureus* Isolated from Burn Wounds

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What's Known

- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen frequently harboring the *mecA* gene, which confers resistance to β-lactam antibiotics. Its capacity to form biofilms and produce a wide range of toxins further complicates treatment strategies. Therefore, alternative approaches targeting bacterial virulence factors are urgently needed.

What's New

- Nanocurcumin significantly downregulated the expression of critical virulence and resistance genes in *S. aureus*, including *icaA*, *hla*, *pvl*, *femA*, and *mecA*. In contrast, it exhibited no notable effect on *fnbA* and *ftsZ*, indicating a selective genetic modulation of bacterial pathogenicity.

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that causes various infections and exhibits antibiotic resistance and virulence factors, requiring alternative therapies. This study aimed to evaluate the effects of nanocurcumin on gene expression of *S. aureus* isolates from burn wounds in Iraqi patients, focusing on inhibiting resistance and virulence genes.

Methods: From March 2023 to May 2024, burn wound samples from Iraqi patients yielded 110 *S. aureus* isolates. Identification was conducted by Gram staining, biochemical assays, and culture techniques. Fifty isolates were randomly selected for antibiotic susceptibility testing using the VITEK 2 Compact System. Ten isolates showing the highest resistance to multiple antibiotics were selected for molecular characterization via Multiplex polymerase chain reaction (Multiplex PCR) to detect *fnbA, icaA, icaB, ftsZ, hla, pvl, femA,* and *mecA* genes. The ten isolates were then divided into two groups: a treatment group exposed to nanocurcumin and an untreated control group. The MIC (minimum inhibitory concentration) of nanocurcumin was determined using the broth microdilution method in a 96-well plate. The 16S *rRNA* gene served as an internal control for evaluating the molecular effects. A two-tailed *t* test was used to assess the significance of gene expression differences.

Results: All 110 isolates were confirmed as *S. aureus*. The 50 selected isolates were resistant to cefoxitin, amoxicillin, benzylpenicillin, ampicillin/sulbactam, piperacillin/tazobactam, cloxacillin, oxacillin, and azithromycin. *MecA* gene was detected in all isolates. Among the ten tested, *femA, icaA, hla*, and *ftsZ* were present in 70%; *pvl* in 50%; *icaB* in 20%; and *fnbA* in 10%. Quantitative reverse transcription polymerase chain reaction (qRT-PCR), showed significant downregulation of *icaA, hla, pvl, femA*, and *mecA* in treated isolates. No significant changes were seen in *fnbA* and *ftsZ*.

Conclusion: Nanocurcumin inhibits *S. aureus* virulence and resistance genes, reducing biofilm formation and toxin production, but lacks effect on *fnbA* and *ftsZ*, requiring further research.

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Introduction

Burn wounds provide an optimal environment for bacterial proliferation and serve as more enduring and abundant sources of infection than surgical wounds, mostly due to the greater surface area affected and the prolonged duration of hospitalization.[1] *Staphylococcus aureus* infections range from superficial skin and soft tissue infections (SSTIs) to severe invasive diseases such as bacteremia, endocarditis, and toxic shock syndrome.[2] The pathogenicity of the organism is a multifaceted process. This process involves the synchronization of several variables, facilitating tissue colonization, inducing tissue damage, and finally resulting in systemic diseases.[3] Among these infections, Methicillin-resistant *Staphylococcus aureus* (MRSA) presents a considerable global challenge because of its antimicrobial resistance properties, especially within healthcare environments and in developing countries such as Iraq.[4]

A polyphenolic molecule generated from turmeric, curcumin, has antioxidant, anti-inflammatory, and antibacterial characteristics. Hence, it is a potential antibiotic option for fighting MRSA.[3] Nanocurcumin, a nanoparticle-enhanced form of curcumin, addresses its low solubility and bioavailability, enabling better bacterial penetration, biofilm disruption, and quorum-sensing regulation to reduce MRSA virulence.[5] Studies demonstrate its effectiveness against drug-resistant MRSA and its potential for broader applications, including viral pathogens such as Human Immunodeficiency Virus type 1 (HIV-1) and Coronavirus Disease 2019 (COVID-19).[6, 7] Although numerous studies have examined antibiotic resistance and virulence factors in *S. aureus*, there remains a significant gap in evaluating nanocurcumin as a potential alternative treatment, particularly in clinical isolates derived from burn wound infections in Iraqi patients. Furthermore, the molecular mechanisms through which nanocurcumin influences the expression of resistance and virulence genes have not been comprehensively investigated. Addressing this gap is especially relevant in light of the increasing prevalence of multidrug-resistant *S. aureus* and the urgent need for novel therapeutic strategies. This study aims to investigate the effect of nanocurcumin on the expression of key resistance and virulence genes (*icaA*, *hla*, *pvl*, *femA*, *fnbA*, *ftsZ*, and *mecA*) in clinical *S. aureus* isolates, providing insights into its potential as an alternative antimicrobial agent.

Materials and Methods

Ethical Considerations

As stated in the ethical agreement, all subjects voluntarily consented to provide the researcher with bacterial samples. Following the guidelines laid forth by the Declaration of Helsinki, all subjects provided their written informed consent. This study was approved by the Iraqi Health Committee according to the approval letter (03/2023/276) issued on February 21, 2023.

Sample Collection and Identification

A Baghdad Teaching-Hospital, in Baghdad, Iraq, identified 110 *S. aureus* isolates in burn wound infections among patients from March 2023 to May 2024, using routine laboratory tests, Gram-staining, biochemical tests, and culture methods to identify the *S. aureus* bacteria. The isolates underwent cultivation on blood and mannitol salt agar plates, followed by incubation at 37 °C for a duration of 24 hours.

Identification Using the VITEK 2 System

The bacterial isolates were identified using the VITEK 2 system with ID-GP identification cards (bioMérieux, France). Each isolate was cultured on mannitol salt agar (Oxoid, UK) and incubated overnight at 37 ºC. A bacterial suspension was prepared by picking several colonies and suspending them in 3 mL of physiological saline, adjusting the turbidity to 0.50–0.63. The ID-GP card was labeled with the isolate number, manually transferred to the transfer unit, and placed inside the vacuum chamber, where the suspension was automatically injected. The card was incubated at 35.5 ºC for up to 8 hours. During incubation, the system analyzed biochemical reactions and stored data. Finally, the software performed analysis and printed diagnostic reports according to the manufacturer›s instructions (bioMérieux, France).

Antimicrobial Susceptibility

The guidelines laid down by the Clinical and Laboratory Standards Institute, 2024 (CLSI), were followed.[8] After letting the samples sit overnight at 37 °C, 50 samples were tested for antibiotic response, using cefoxitin, benzylpenicillin, amoxicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cloxacillin, oxacillin, gentamicin, ciprofloxacin, moxifloxacin, norfloxacin, inducible clindamycin resistance, azithromycin, clarithromycin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, doxycycline, minocycline, tetracycline, tigecycline, fusidic acid, rifampicin, and trimethoprim/

sulfamethoxazole. to assess the profiles related to antibiotic susceptibility and classify these samples as MRSA.

DNA Extraction

Genomic DNA was isolated from bacterial growth according to the protocol of the ABIOpure kit (USA). In a microcentrifuge tube, we extracted DNA from bacterial harvesting cells for 1 min at 13000 rpm. The cells were suspended and mixed with G-buffer solution and binding buffer, and then the tubes were incubated at 65 °C for 15 min, flipping them every 5 min to aid in cell lysis. The cell lysate was loaded into a column, and the mixture was centrifuged at 13,000 rpm for 1 min. For sample volumes exceeding 800 µL, the sample was loaded and spun again. 500 µL of sample was applied to the column, washed with buffers A and B, and centrifuged at 13000 rpm for 1 min. Next, a certain amount of elution buffer was added to a clean microcentrifuge tube with the G-spinTM column, and the tube was left at room temperature for 1 min.

Primer Selection

The primers were provided in a lyophilized form (Macrogen, Korea) and then prepared in sterile nuclease-free water. To create a functional solution of these primers, 10 µL of primer stock solution was mixed with 90 µL of nuclease-free water to reach a final concentration of 100 µM and then kept at -20 °C until used. Reference of primers designed in this study, as mentioned in table 1. The oligonucleotide primers employed in this investigation were designed to evaluate the existence of virulence and methicillin genes. From the highest antibiotic resistance among the entire 50 *S. aureus* clinical strains, 10 samples were selected for the multiplex PCR assay, divided into two groups, as reported in figure 1. The simultaneous amplification of the *mecA* and *femA* genes has demonstrated great efficacy in quickly identifying methicillin resistance in 10 MRSA samples.

Table 1: Sequences of oligonucleotide primers and the PCR assay protocol employed in this work

Primer Name	Sequence 5`-3`	Primers Reference	Steps	°C	m:s	Cycle
IcaB-R	TTTTCTTCCCCAACATGACCT	This study	RT. Enzyme Activation	37	15:00	1
IcaB-F	TATTGCCTGTAAGCACACTGG					
IcaA-R	AGTAATACTTCGTGTCCCCCT					
IcaA-F	GAAAACAGAGGTAAAGCCAACG					
Hla-R	AGTTGATTGCCATATACCGGG					
Hla-F	AGCGAAGAAGGTGCTAACAAA					
MecA-R	GCTGATTCAGGTTACGGACAA					
MecA-F	CCCAATTTGTCTGCCAGTTTC		Initial Denaturation	95	5:00	
FnbA-R	ATTTGCCATCGGAAAAAGCTG					
FnbA-F	GTCACTGTTGTTGGGTCGATA					
PvI-R	CGGCGATGAATTATTGTGTGG					
PvI-F	GCGTTCCATCATAAAGCCATG					
FemA -R	ACGAATTTGTAGCACAGGATCA		Denaturation	95	0:20	40
FemA-F	TCATTTCACGCAAACTGTTGG					
FtsZ-R	CTTGTTCCGAATCCAGTGCTA		Annealing	51	0:20	
FtsZ-F	AAATAGAGCGGTAGAAGCTGC		Extension	72	0:20	

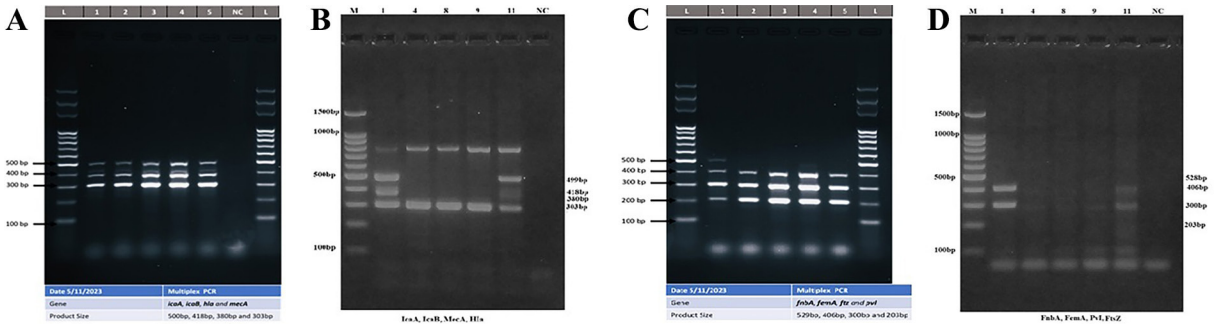

Figure 1: Multiplex PCR of virulence and resistance genes among *S. aureus* strains. (A) Results of the amplification of (303bp *MecA*, 380bp *hlA*, icaB 418pb and 500bp *icA*). PCR products were: 5 bands (*mecA*), 5 bands (*hlA*) and 5 bands (*icaA*). (B) Results of the amplification of (303bp *MecA*, 380bp *hlA*, icaB 418pb and 500bp *icA*). PCR products were: 5 bands (*mecA*), 2 bands (*hlA*) and 2 bands (*icaA*). (C) Results of the amplification of (*pvl* 203bp, 300bp *ftsZ*, 406bp *femA* and 529 bp *fnbA*). PCR products were: 5 bands (*pvl*), 5bands (*ftsZ*), 5 bands (*femA*) and 2 bands (*icaB*). (D) Results of the amplification of (*pvl* 203bp, 300bp *ftsZ*, 406bp *femA* and 529 bp*fnbA*). PCR products were: (2) bands (*ftsZ*) and (2) bands (*femA*). Bacterial species' genes were separated using 2% agarose gel electrophoresis and stained with ethidium bromide. The DNA marker used was a 100 bp ladder.

Multiplex-Polymerase Chain Reaction (PCR) and Amplification Conditions

Out of the 10 MRSA clinical strains that were the most resistant ones to antibiotics, samples were chosen for Multiplex PCR to check for the presence of the *Fnb, icaA, icaB, ftz, hla, pvl, femA,* and *mecA* genes. The 16S *rRNA* gene was used as a reference gene. The complete multiplex-PCR program was optimized by using the Tm Calculator website.[9] The PCR reaction was conducted in a final volume of 24 µL, consisting of 3 µL each of template DNA (21.4–28.2 ng/µL), 1 µL of forward and reverse primers (10 µM), and 7.5 µL of nuclease-free water, and the remaining volume was made up with the Promega Master Mix (USA). The Eppendorf tubes were briefly centrifuged before placing them into the PCR equipment. The cycling parameters were as follows: an initial denaturation cycle of 5 min at 94 °C, followed by 38 repeated cycles of denaturation at 94 °C for 30 sec, annealing at 57 °C for 45 sec, and extension at 72 °C for 45 sec. Finally, there was a single cycle of extension at 72 °C for 7 min. Table 1 presents the primer sequences, the PCR reaction protocol, and the size of the amplification products. A 1.5% agarose gel was prepared with 1X TAE buffer (0.08 M Tris, 0.08 M acetic acid, and 0.02 M EDTA) and RedSafe™ Nucleic Acid Staining Solution (Intron Biotechnology, Korea). Eight µL of DNA were loaded, and electrophoresis was run at 70 V for 75 min. DNA bands were visualized with UV light (336 nm) and photographed. Multiplex PCR amplified *Fnb, icaA, icaB, ftz, hla, pvl, femA,* and *mecA* genes using a 1000 bp marker.

Nanocurcumin Preparations

The nanocurcumin source used in this study offered details about curcumin production, encapsulation strategies, composition characterization, and stability evaluation.[10]

Antibacterial Activities of Nanocurcumin Assay

The strains under study were serially diluted from standardized suspensions of fresh bacterial cultures. A 0.5 MacFarland standard tube's worth of colony-forming units per milliliter (cfu/mL) was used to adjust the turbidity of the bacterial suspensions, which were then vigorously mixed in the test tubes. The agar well diffusion technique was used to evaluate the antibacterial activity of nanocurcumin against five reference microorganisms. To determine the effect of nanocurcumin on *S. aureus*, 100 µL (10^6 cfu) of the bacterial suspension was equally distributed onto Muller-Hinton agar plates (Oxoid, England) and inoculated with a concentration of 1 mg/mL, produced from a 100 mg/mL stock solution. Before being incubated at 37 °C for 24 hours, the plates were kept at room temperature for 1 hour. Diameter measurements of the clear inhibitory zones were used to assess the antibacterial activity. A positive reference standard of 4 µg of amoxicillin was used to test the strain's sensitivity. In the first experiment, the medium containing just nanocurcumin had no inhibitory effects; this substance served as a negative control.

Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution technique was used to determine the MIC of nanocurcumin against *S. aureus* strains, following the directions of the CLSI.[8] In 96-well microplates, the MIC of nanoparticles was determined by repeated dilution. In order to achieve this goal, 10 wells were used for serial dilutions at doses of 1000, 500, 250, 125, 62, 31, 16, 8, 4, 2, and 1 µg/mL. Two wells served as positive controls, comprising culture medium and microbial suspension, while two wells served as negative controls, comprising culture media alone. Before adding 20 µL of resazurin to each well after sufficient growth, 10 µL of bacterial suspension, which corresponds to a 0.5 McFarland turbidity standard, was added to wells containing different concentrations of nanoparticles. The plates were then incubated overnight at 37 °C. Keeping an eye on the color shift in the wells allows one to determine the MIC. The growth control columns went from blue to pink or light pink, while all the sterility control wells stayed blue.[11]

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Only five samples were chosen for (qRT-PCR) after nanocurcumin treatment. The study assessed nanocurcumin's potential inhibitory effects on the following genes in *S. aureus* strains: *FnbA, icaA, icaB, ftsZ, hla, pvl, femA,* and *mecA*, using qRT-PCR and a housekeeping gene for normalization. The qRT-PCR kits were developed and manufactured by Promega (USA). The process included two stages, and the Master Mix (Thermo Fisher Scientific, USA) included SYBR Green dye for marking purposes. First, the sample's RNA was extracted using the TRIzol™ method (Thermo Fisher Scientific, USA). The concentration of extracted RNA and the quality of the sample were evaluated using a Quantum Fluorometer (Promega, USA). A mixture of 1 µL of RNA and 200 µL of diluted QuantiFluor dye was made. The concentration of RNA was determined after incubating at room temperature in darkness for 5 min.

Table 1 provides a reference to the qRT-PCR software. High-capacity complementary DNA (cDNA) reverse transcription kits (Applied Biosystems, US) were used for cDNA synthesis.

Statistical Analysis

Gene expression analysis was performed using GraphPad Software, USA (part of Dotmatics Global Company), applying the 2^−ΔΔCt Livak method. A fold change of less than 1 was considered statistically significant. Additionally, Microsoft Excel 2010 (Microsoft Corporation, USA) was used to calculate P values using a paired *t* test, with statistical significance set at P<0.001.

Results

Identification of MRSA

A total of (110) isolates were confirmed as *S. aureus* based on colony morphology on blood, mannitol salt agar (figure 2), and Muller-Hinton agar, positive Gram staining, and biochemical test results. Identification was further confirmed phenotypically using the VITEK 2. All isolates were catalase- and coagulase-positive and genotyped by qRT-PCR. All isolates showed growth in the presence of cefoxitin (4 µg/mL), indicating methicillin resistance.

Antibiotic Susceptibility Testing

Antibiotic susceptibility results via VITEK 2, as represented in table 2, show the susceptibility testing against *S. aureus* for all isolates. The antibiotic susceptibility data show 100% resistance to cefoxitin, amoxicillin, benzylpenicillin, ampicillin/sulbactam, piperacillin/tazobactam, cloxacillin, oxacillin, and azithromycin. Conversely, all isolates were sensitive to doxycycline, minocycline, tigecycline, trimethoprim/sulfamethoxazole, and rifampicin.

Figure 2: Identification and selective cultivation of *Staphylococcus aureus* on selective media. *S. aureus* colonies were grown on mannitol salt agar, where they appeared as yellow colonies due to mannitol fermentation, indicating selective growth. Growth was also observed in blood culture, supporting the identification of *S. aureus* based on its hemolytic activity and characteristic morphology.

Multiplex PCR Assay

The *mecA* gene, identified in 100% of the isolates, is the definitive marker for methicillin resistance, as it encodes penicillin-binding protein 2a (PBP2a). While the *femA* gene, crucial for maintaining methicillin tolerance by preserving peptidoglycan integrity, was found in 70% of the isolates. The *mecA* gene was detected in all 10 isolates (100%), thereby confirming them as MRSA. The *ica* operon, essential for biofilm formation, was also analyzed. Genes such as *icaA* and *icaB* were detected at 70% and 20%, respectively. Virulence factors, including toxins and adhesins, were also studied. The *hla* gene, responsible for producing alpha-hemolysin, was found in 70% of isolates, highlighting its critical role in host cell damage. Similarly, the *pvl* gene, linked to severe skin and soft tissue infections, was present in 50% of the isolates. The *fnbA* gene was detected in 10% of isolates. The *ftsZ* gene was identified in 70% of our isolates, highlighting its significant role in bacterial survival and cytokinesis.

Table 2: Antibiotic resistance frequency among clinical strains isolated from burn wound infections

Antimicrobial	Sensitive N (%)	Resistance N (%)	Antimicrobial	Sensitive N (%)	Resistance N (%)
Cefoxitin	0 (0)	50 (100)	Clarithromycin	14 (28)	36 (72)
Benzylpenicillin	0 (0)	50 (100)	Erythromycin	20 (40)	30 (60)
Amoxicillin	0 (0)	50 (100)	Clindamycin	9 (18)	41 (82)
Amoxicillin/Clavulanic Acid	5 (10)	45 (90)	Linezolid	40 (80)	10 (20)
Ampicillin/Sulbactam	0 (0)	50 (100)	Teicoplanin	40 (80)	10 (20)
Piperacillin/Tazobactam	0 (0)	50 (100)	Vancomycin	45 (90)	5 (10)
Cloxacillin	0 (0)	50 (100)	Doxycycline	50 (100)	0 (0)
Oxacillin	0 (0)	50 (100)	Minocycline	50 (100)	0 (0)
Gentamicin	19 (38)	31 (62)	Tetracycline	38 (76)	12 (24)
Ciprofloxacin	34 (68)	16 (32)	Tigecycline	50 (100)	0 (0)
Moxifloxacin	40 (80)	10 (20)	Fusidic acid	10 (20)	40 (80)
Norfloxacin	40 (80)	10 (20)	Rifampicin	50 (100)	0 (0)
Inducible Clindamycin Resistance	0 (0)	50 (100)	Trimethoprim/ sulfamethoxazole	50 (100)	0 (0)
Azithromycin	0 (0)	50 (100)			

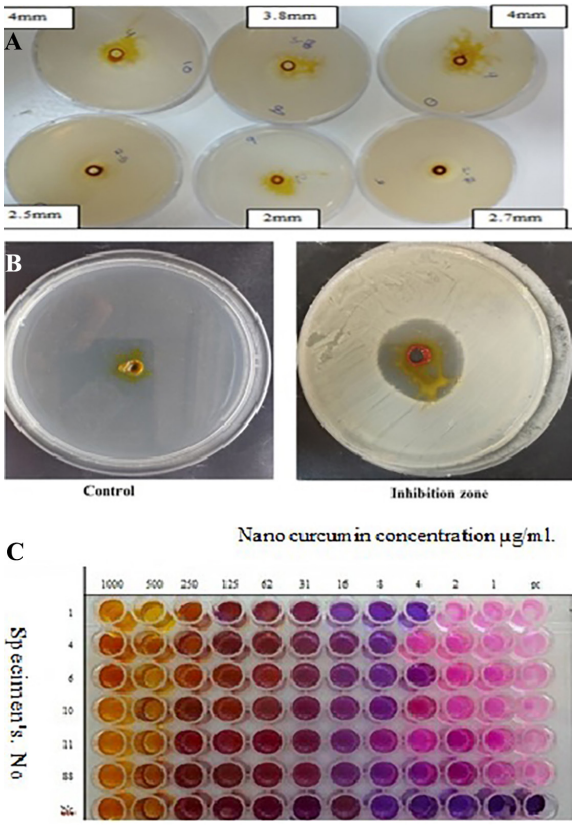

Figure 3: Evaluation of Nanocurcumin Antibacterial Activity Against Methicillin-resistant *Staphylococcus aureus* (MRSA). (A) The *S. aureus* Inhibition zones are present at a concentration of 1 mg/mL nanocurcumin in each experimental configuration. Antibacterial activity of nanocurcumin and the corresponding inhibition zones in compared to the control sample against *S. aureus*. This indicates that nanocurcumin is a more potent antibacterial agent owing to its decreased particle size. (B) Following a 24-hour period, 20 µL of Resazurin was added to each well and incubated for an additional 2-4 hours at 37°C. (C) Color alterations were seen and recorded. The concentration at which the color shift occurred was termed the Minimum Inhibitory Concentration (MIC).

Antibacterial Activities of Nanocurcumin Assay and Minimum Inhibitory Concentration (MIC)

The inhibition zones of the nanocurcumin antibacterial activity against six MRSA isolates were as follows (figure 3A): 2±2.5, 2.7±3.8, and 4 mm for nanocurcumin, respectively. Figure 3B displays the MIC values for the six distinct samples influenced by nanocurcumin. Each sample exhibited a distinct concentration of sub-inhibition, with the minimum concentration of nanocurcumin that entirely suppresses bacterial growth being determined. The data were organized in increasing order: 4, 4, 8, 8, 8, and 16 µg/mL. The minimum dose of nanocurcumin that successfully suppressed bacterial growth in the examined samples was established as 4 µg/mL.

Gene Expression Assay

The results indicated that the expression of virulence and methicillin resistance genes related to resistance, stress response, pathogenicity, adhesion, and biofilm formation was analyzed for five clinical strains of MRSA. To investigate the molecular effects of nanocurcumin on MRSA strains, virulence genes associated with pathogenicity were chosen for transcriptional expression quantification with qRT-PCR, subsequently compared to untreated bacteria. The average relative quantities of target genes were standardized to the average relative quantity of the 16S *rRNA* gene reference gene within the same sample. The addition of nanocurcumin strongly inhibits the expression of virulence and methicillin genes, as anticipated. The *femA* gene expression (adhesion and biofilm formation) was also significantly downregulated by (0.668-0.090-0.865-0.123 and 0.225) fold, *hla* gene (0.768-0.652 and 0.502) fold, *icaA* (0.435-0.074-0.893 and 0.448) fold, and other virulence factors that were downregulated in the MRSA *mecA* (0.129-0.458-0.862 and 0.208). All virulence genes were suppressed in *S. aureus*. The resulting amplification curves of qRT-PCR are shown in figure 4. Each curve represents the amplification of *femA, icaA, hlA*, *mecA, fnbA, pvl,* and *ftsZ*, and the 16S *rRNA* gene (housekeeping gene), respectively.

Our study demonstrates that nanocurcumin significantly reduces the expression of key virulence and resistance genes in *S. aureus*, including *icaA*, *hla*, *pvl*, *femA*, and *mecA*, compared to control samples. The results emphasize the wide-range effects of nanocurcumin on the structural and toxic elements of MRSA, offering a multifaceted strategy for managing infections. Overall, the analysis of gene expression folding patterns reveals that nanocurcumin exerts a significant influence on MRSA by specifically targeting crucial genes associated with biofilm formation, cell wall integrity, viral potency, and antibiotic resistance (figure 5).

Discussion

Overall, the results of this study showed 90%, 82%, 80%, 72%, 60%, 32%, and 24% resistance for amoxicillin/clavulanic acid, clindamycin, erythromycin, fusidic acid, clarithromycin, gentamicin, ciprofloxacin, and tetracycline, respectively. It also revealed that 20% of the isolates were resistant to moxifloxacin, norfloxacin, linezolid, and teicoplanin, while the resistance to vancomycin was 10%. Based on the results of the antibiotic sensitivity test in our study, particularly for cefoxitin, amoxicillin, and oxacillin, 100% of strains were MRSA compared with other studies, which showed (4%), (21.2%), (61%), and (68.5%) to

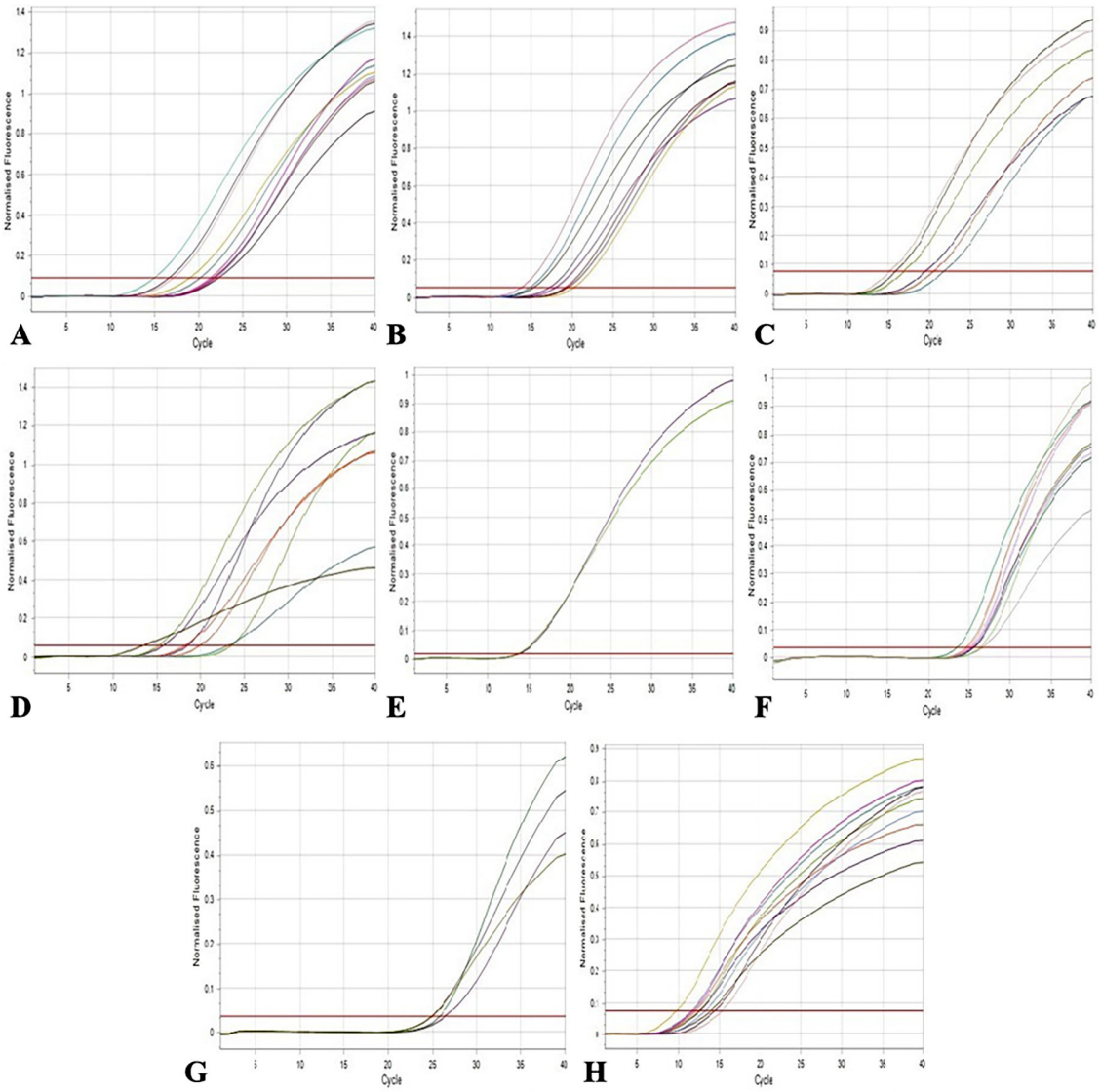

Figure 4: Quantitative reverse transcription PCR (qRT-PCR) analysis was conducted to assess the relative expression levels of virulence and resistance-associated genes in *Staphylococcus aureus* isolates following treatment with nanocurcumin compared to untreated controls. (A) The *femA* gene exhibited decreased transcriptional activity in nanocurcumin-treated isolates. (B) Expression of the *icaA* gene was reduced after treatment. (C) The *hlA* gene showed downregulated expression in response to nanocurcumin. (D) The *mecA* gene demonstrated a significant decrease in expression in treated samples relative to controls. (E) The *fnbA* gene also displayed lowered transcriptional levels following treatment. (F) The *ftsZ* gene showed a reduction in expression after nanocurcumin exposure. (G) Expression of the *pvl* gene was suppressed in treated isolates. (H) The 16S *rRNA* gene, used as an internal control, maintained stable expression across both treated and untreated groups, validating the consistency of the qRT-PCR data.

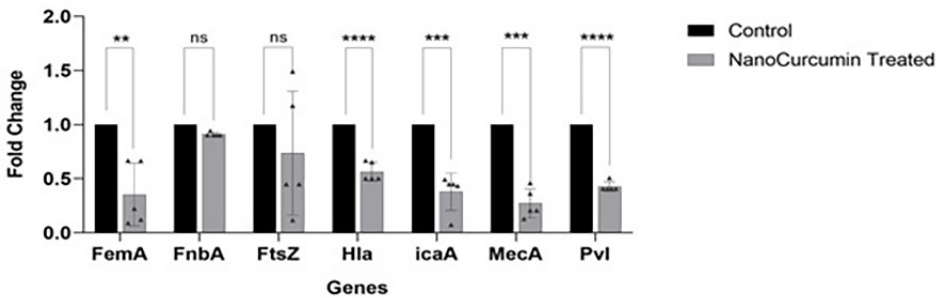

Figure 5: The expression levels of key antibiotic resistance and virulence genes in five extensively drug-resistant (XDR) clinical strains of Methicillin-resistant *Staphylococcus aureus* (MRSA) were analyzed before and after treatment with nanocurcumin using quantitative real-time PCR. The results demonstrated a statistically significant downregulation of *femA*, *hla*, *icaA*, *mecA*, and *pvl* following nanocurcumin treatment (***P<0.001), while no significant alterations were observed in the expression levels of *fnbA* and *ftsZ*. Statistical significance indicators: (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001)

be methicillin-resistant among MRSA isolates.[12-15] On the other hand, Salman and his team reported 100% resistance to cefotaxime of strains isolated from urine specimens.[16] Concerning the results of Ansari and colleagues,[12] that noted (36.7%) resistance to ciprofloxacin, whereas other study mentioned 8.5% multidrug resistance (MDR),[17] in contrast, the study by Jafari-Sales and Jafari showed[18] a 100% penicillin resistance and 91.66% co-amoxiclav resistance. The bacterial resistance percentages of penicillin G, cloxacillin, tetracycline, and amoxicillin were found to be 87.9%, 75.9%, 65.2%, and 55.6%, respectively.[19] The variation in MRSA isolation rates across studies can be attributed to disparities in geographical locations and timeframes, variations in hygienic practices followed in different hospitals, the quality of healthcare services provided by the hospital, the effectiveness of infection control programs, and the judicious use of antibiotics, which can differ among hospitals.[20] Based on the available studies, it is clear that MRSA has unusually high rates, and there is a need to deal with this issue. The inappropriate and frequent prescription of antibiotics in healthcare facilities, particularly in Iraq, is becoming a problematic issue. Furthermore, it is recommended to use doxycycline, minocycline, tetracycline, rifampicin, trimethoprim/sulfamethoxazole, and tigecycline against MRSA as they have been proven effective in killing MRSA according to the results of our study. The community, particularly in health development centers, has gained a reputation for neglecting precautionary measures; issues with proper hand washing and isolation of contaminated children contribute to the spread of MRSA bacteria. Iraq's healthcare resources, reduced availability, and severely damaged infrastructure have resulted in severe suffering for both the nation and its people. The tendency for outside hospital circumstances to involve human MRSA bacterial colony contributors also exacerbates the situation. Solving these issues is crucial to effectively stopping the spread of MRSA infection.

The identification of the *mecA* gene serves as the definitive method for detecting bacteria that exhibit resistance to methicillin. The *mecA* gene, identified in 100% of the isolates, is the definitive marker for methicillin resistance, as it encodes penicillin-binding protein 2a (PBP2a), reducing β-lactam antibiotic efficacy.[21] The *femA* gene, crucial for maintaining methicillin tolerance by preserving peptidoglycan integrity, was found in 70% of the isolates, consistent with previous findings.[22] The *mecA* percentage of this study confirmed for 10 isolates was 100% (MRSA). An analysis of research on the *mecA* gene of MRSA reveals that certain sources indicate success rates below 50%,[18, 23] while other sources offer success rates beyond 50%.[24] On the other hand, the majority of studies examining the *femA* gene provide ratios that surpass 50%, as evidenced in sources.[23] The ica operon, essential for biofilm formation, was also analyzed. Genes such as *icaA* and *icaB* were detected at 70% and 20%, respectively, aligning with their established role in enhancing biofilm production and persistence in infections.[25] The presence of *ica* genes correlates with biofilm-forming ability, with isolates possessing more *ica* genes demonstrating increased biofilm production.[25] Additionally, antibiotic treatments such as gentamicin can upregulate *ica* gene expression, further complicating treatment outcomes.[26] Virulence factors, including toxins and adhesins, were also studied. The *hla* gene, responsible for producing alpha-hemolysin, was found in 70% of isolates, highlighting its critical role in host cell damage.[27] Similarly, the *pvl* gene, linked to severe skin and soft tissue infections, was present in 50% of the isolates, consistent with earlier studies,[28] while a below 50% record was documented by Rasmi and his team in 2022.[17] Adhesins such as fibronectin-binding proteins (*FnBPA* and *FnBPB*) play a vital role in host colonization and biofilm formation.[29] The *fnbA* gene was detected in 10% of isolates, reflecting variable prevalence across studies[30] that reported prevalence ratios below 50%. Rasmi and his team (2022) and Idrees and his team (2023) recorded greater ratios.[17, 24] The *ftsZ* gene in *S. aureus* plays a crucial role in cytokinesis by encoding a protein that forms a contractile ring at the location where the septum will develop.[31] The *ftsZ* gene was identified in 70% of our isolates, highlighting its significant role in bacterial survival and cytokinesis. Similarly, the presence of the *ftsZ* gene in 43% of MRSA isolates was reported, further emphasizing its essential function in the survival of these bacteria.[32] Collectively, these findings underscore the complexity of *S. aureus* pathogenesis and its reliance on multiple virulence determinants. Overall**,** the *mecA* gene, which confers methicillin resistance, is a key marker for identifying MRSA strains commonly isolated from both wound and burn infections. Investigating additional virulence determinants—such as *ftsZ, femA, hla, pvl, icaB,* and *fnbA*—provides deeper insight into how these organisms establish infection and persist despite treatment. Notably, our staphylococcal isolates showed an exceptionally high prevalence of *mecA*, while the expression levels of *ftsZ, femA,*

hla, pvl, and *icaA* were significantly elevated in the Iraqi population. The substantial presence of these genes in MRSA isolates from burn wound infections underscores the significant role of these virulence and resistance factors in the pathogenicity and antibiotic resistance of the strains.

Conclusion

Notably, our staphylococcal isolates showed an exceptionally high prevalence of *mecA*, while the expression levels of *ftsZ, femA, hla, pvl,* and *icaA* were significantly elevated in the Iraqi population. The substantial presence of these genes in MRSA isolates from burn wound infections underscores. Our study demonstrates that nanocurcumin exerts inhibitory effects on several virulence and resistance genes in *S. aureus*, including *icaA*, *hla*, *pvl*, *femA*, and *mecA*. These findings are consistent with previous studies showing that nanocurcumin reduces biofilm formation, toxin production, and enhances bacterial sensitivity to antibiotics. On the other hand, the lack of effect on *fnbA* and *ftsZ* suggests that nanocurcumin may have selective effects depending on the targeted genetic mechanism, requiring further research to determine its comprehensive impact.

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Authors' Contribution

A.A, M.S, and B.A.A: Conception and design, as well as data collection, analysis, and interpretation, contributed to the work's drafting or critical revision for significant intellectual content. All authors approved the final draft for publication and committed to taking responsibility for all parts of the work, making sure that any concerns about its integrity or correctness are thoroughly looked into and addressed.

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