

Evaluation of Gene Expression of *Staphylococcus Aureus* Biofilm (*ica*) by the Effect of Iron Oxide Nanoparticles by Real Time PCR method

Nasser Hussein Nasser ¹, 

¹ *Department of Biology, Azad Islamic University, Tehran, Iran*

* **Corresponding Author:** nasserhussian55@gmail.com

Abstract: Biofilm formation is one of the key virulence factors of *Staphylococcus aureus*, contributing significantly to its ability to cause infections. Recent research has demonstrated that biofilms act as protective barriers, reducing the efficacy of antibiotics and complicating treatment efforts. The use of nanoparticles, particularly as alternatives to conventional drugs, has gained attention for their potential to inhibit biofilm formation. In this study, 60 *S. aureus* strains were isolated from wound samples and confirmed through morphological identification. Biofilm production was assessed using the Microtiter Plate (MTP) method. The presence of target genes was determined using PCR, and the minimum inhibitory concentration (MIC) of iron oxide nanoparticles was measured. To evaluate the impact of sub-MIC concentrations on gene expression, the expression level of the *icaC* gene—associated with biofilm formation—was analyzed using Rotor-Gene and REST software. Among the isolated strains, 16.66% were weak biofilm producers, 48.33% moderate, and 35.01% strong producers. The efficacy of iron oxide nanoparticles was tested on the 21 strains classified as strong biofilm formers. Compared to untreated controls, all treated strains exhibited increased Ct values, and the fold change in *icaC* gene expression was -3.88, indicating significant downregulation. Given the inhibitory effect of iron oxide nanoparticles on *icaC*, a key gene in biofilm formation, these nanoparticles show promise as potential therapeutic agents to combat *Staphylococcus aureus* infections and suppress biofilm-associated virulence.

Keywords: Biofilm, Iron oxide nanoparticles, *Staphylococcus aureus*, Gene expression.

1. Introduction

Staphylococcus aureus was first identified and described in 1880 by Alexander Ogston, a surgeon who isolated the bacterium from surgical abscess [1]. *S. aureus* is a Gram-positive bacterium and a facultative anaerobe with a low genomic G + C content [2], [3]. It is commonly found as a commensal in humans and animals, colonizing various body sites such as the nares, axilla, perineum, and skin. However, nasal carriage is of greater concern as it is the most common source of MRSA for disease spread [4]. It is estimated that approximately 15% of the population persistently carries *S. aureus* in the anterior nares, with higher rates observed in certain populations, including healthcare workers and hospitalized patients. Individuals harboring this bacterium are at a greater risk of developing *S. aureus* infections. In humans, *S. aureus* can infect most organs and is a leading cause of death from infection [6]-[10]. It is a major contributor to morbidity and mortality in hospitals, partly because it can form biofilms on indwelling devices

such as catheters and medical implants. Detachment of bacterial cells from these biofilms can result in secondary and disseminated INFECTIONS [11]. *S. aureus* is considered one of the most successful pathogens due to its extensive arsenal of virulence factors, including anchor proteins, secreted toxins and enzymes, polysaccharides, and immune system modulators. Many of these virulence factors are located on mobile genetic elements (MGEs), which facilitate the diversification of virulence and resistance genes through horizontal gene TRANSFER [12]. *Staphylococcus aureus* is one of the most common causes of hospital- and community-acquired infections. The importance of biofilm production in pathogenesis of *S. aureus* and development of MDR strains has been DOCUMENTED [13]. Apart from other adhesion factors, a polysaccharide intercellular adhesion (PIA) which is encoded by *ica* operon is essential for biofilm formation in staphylococci [14]. The intercellular adhesion (*ica*) locus consists of *ica*ADBC operon which contains four genes encoding the main proteins required for the generation of PIA. The first two genes, including *icaA* and *icaD* perform principal role in the synthesis of exopolysaccharides. The product of *icaA* gene is a transmembrane protein with N-acetyl-glucosaminyl transferases enzymatic activity which led to synthesis of the poly-N acetyl glucosamine polymer. It has been documented that the product of the *icaD* gene is essential for the most favorable enzymatic activity of the product of *icaA* gene.

2. PROPLAM OF STUDY

A biofilm is a well-organized population of bacteria that forms a membrane-like extracellular matrix (ECM) through the adhesion of bacterial colonies and the secretion of extracellular polymeric substances (EPS), including polysaccharides, nucleic acids, and proteins. These substances are produced by bacteria during their growth process. The interaction between EPS and bacterial aggregates gives the biofilm its cohesive and viscoelastic properties, allowing bacteria to attach to both living and non-living Surfaces [15]-[17]. The formation of pathogenic biofilms plays a crucial role in the development of chronic persistent infections. It is widely accepted in the scientific community that bacterial biofilms mediate more than 80% of chronic infections. *Staphylococcus aureus* (*S. aureus*) is commonly found in hospital environments, where it can attach to and persist on host tissues and medical devices. This can lead to various infections such as skin and soft tissue infections, osteomyelitis, endocarditis, pneumonia, and bacteremia. Treating these infections is challenging due to the protective biofilm formed by *S. aureus*, which enhances its resistance to antibiotics. Moreover, biofilm formation is recognized as a protective growth strategy for bacteria to adapt to harsh environments [17]-[20]. The biofilm serves as a barrier, creating a stable internal environment for bacterial cells and shielding them from unfavorable conditions such as extreme temperatures, nutrient scarcity, dehydration, and even antibacterial agents. As a result, bacteria can quickly establish themselves, evade host defense mechanisms, and promote long-term infections by enhancing adhesion to host surfaces. The biofilm essentially serves as the primary line of defense for bacteria, ensuring their self-protection. It is well-established that biofilm-forming bacteria exhibit resistance to most antibiotics [21]. Clinical antibiotics are primarily designed to target planktonic microbial cells. Antibiotics targeting planktonic cells can inadvertently exert

selective pressure on microorganisms, providing them with a survival advantage over susceptible competitors. Consequently, treating biofilm-related infections typically necessitates prolonged use of high-dose antibiotics. However, chronic administration of such antibiotics may elevate the risk of antibiotic resistance development and drug toxicity [22]. Given the highly intricate and adaptable nature of biofilm populations, gaining a comprehensive understanding of the mechanisms underlying biofilm formation can potentially offer new insights for the development of effective strategies to control biofilm-related infections [23][24].

3. OBJECTIVES:

3.1 Overall Objective:

- Determining the expression of *Staphylococcus aureus* biofilm-associated genes (*ica*) under the influence of iron oxide nanoparticles using Real-Time PCR method.

3.2 Minor goals:

- Isolation and identification of *Staphylococcus aureus* bacteria from human clinical samples.
- Investigation of biofilm formation using a phenotypic method.
- Determination of the prevalence of the biofilm-associated gene (*ica*) in *Staphylococcus aureus* bacteria.
- Synthesis of iron oxide nanoparticles.
- Examination of the expression of the *ica* gene under the influence of iron oxide nanoparticles.

4. MATERIALS AND METHODS

4.1 Sample collection

A number of 60 *Staphylococcus aureus* isolates were isolated from patients in Iraqi hospital, which included wound samples, and were transferred to the laboratory by Carry Blair transfer medium and cultured in blood agar medium, and if a colony was observed, specific characteristics and complete hemolysis, using diagnostic tests such as gram staining, catalase confirmed the presence of gram-positive cocci. All the isolates were subjected to various diagnostic tests, including Gram staining, catalase, and oxidase, in order to confirm their classification as belonging to the *Staphylococcus* genus. Isolates that showed positive results for catalase, were Gram-positive, and negative for oxidase were identified as *Staphylococcus*. Further examination was carried out on the isolates displaying *Staphylococcus* characteristics. This involved testing their ability to ferment mannitol on agar medium, as well as conducting DNase and coagulase tests. All strains identified as *Staphylococcus aureus* exhibited positive results for both DNase and coagulase tests, indicating the production of these respective enzymes. Additionally, they demonstrated the ability to ferment mannitol.

4.1 Biofilm production test

The investigation was carried out by the quantitative microtiter plate method. This is a quantitative method based on the amount of adhesion of bacterial colonies to the bottom of the wells and staining with crystal violet and reading their OD using an ELISA reader. In such a way that 20 microliters of dilution²-10 bacterial suspensions were inoculated in trypticase culture medium in a 96-well polyester microtiter plate and incubated for 24 hours at 37°C, and then each of these wells were washed twice with PBS solution. Then 200 microliters of ethanol%96 were added to each of the wells for 15 minutes, and then the contents of the wells were emptied again and dried at room temperature, the violet crystal solution was added. %1 was used for staining and then they were washed again with PBS solution and dried, and in the last step, the optical absorption (OD) of the biofilms at 570 nm wavelengths was measured using an ELISA reader device.

- ✓ Samples with OD less than 0.1: no biofilm (non-adhesive)
- ✓ Samples with OD between 0.1 and 0.2: weak biofilm (weak adhesive)
- ✓ Samples with OD between 0.2 and 0.3: medium biofilm (medium adhesive)
- ✓ Samples with OD more than 0.3: strong biofilm (strong adhesive)

4.2 DNA Extraction and PCR

DNA extraction was done by High Pure PCR Template Preparation Kit (Cinna Gene) according to manufacturer's instructions. The concentrations of all extracted DNAs were determined by Nanodrop.

Table 1: primers used in this research

Primers	Sequences (5'-3')
icaA forward	GAC CTC GAA GTC AAT AGA GGT
icaA reverse	CCC AGT ATA ACG TTG GAT ACC
icaB forward	ATC GCT TAA AGC ACA CGA CGC
icaB reverse	TAT CGG CAT CTG GTG TGA CAG
icaC forward	ATA AAC TTG AAT TAG TGT ATT
icaC reverse	ATA TOT AAA ACT CTC TTA ACA
icaD forward	AGG CAA TAT CCA ACG GTAA
icaD reverse	GTC ACG ACC TTT CTT ATA TT

Table 2: PCR program

Number Cycle	Time	Temp	Processes
1 cycle	3 mins	95 ^o C	Initial denaturation
	30 sec	95 ^o C	Denaturation
30 cycles	30 sec	50. ^o C	Annealing
	1 min	72 ^o C	Extension
	5 mins	72 ^o C	Final extension
1 cycle		4 ^o C	Stop

PCR products were electrophoresed on a 2% agarose gel in a 0.5X Tris-Borate-EDTA (TBE) buffer and stained in ethidium bromide.

4.3 Biosynthesis of Iron Nanoparticle

Iron oxide magnetic nanoparticles were synthesized using a supernatant culture of Bacillus bacteria, which was isolated from mineral samples. The aforementioned bacteria were cultured in a standard nutrient broth medium for 24 hours, and then the bacterial biomass was separated from the supernatant by centrifugation. To ensure the absence of microorganisms, the culture medium was passed through a 0.22 μm filter membrane. By adding iron (III) chloride to the filtered medium, a color change and increased turbidity of the solution were observed, followed by the formation of brown precipitates. The presence of iron oxide nanoparticles in the resulting suspension was initially identified using UV spectroscopy, and subsequently, dynamic light scattering (DLS) was employed to confirm the particle size. Furthermore, the particles in this suspension exhibited a reaction with a magnet and thus manifested magnetic properties.

Study of antimicrobial effect of the Iron Nanoparticle

The agar well diffusion method was employed to evaluate the antimicrobial activity of iron oxide nanoparticles. In this method, 100 μL of the prepared microbial suspension was uniformly spread on the surface of Muller-Hinton agar medium with a half McFarland turbidity. Then, wells with a diameter of 6 mm were created on the agar surface, and 100 μL of iron oxide nanoparticles at concentrations of 10, 100, and 1000 $\mu\text{g}/\text{mL}$ were added to their respective wells. Additionally, 100 μL of physiological serum and a pure solution of cephalexin antibiotic with a concentration of 100 $\mu\text{g}/\text{mL}$ were used as negative and positive controls, respectively. After 24 hours of incubation at 37 degrees Celsius, the diameter of the growth inhibition zone was measured.

4.4 Real Time-PCR

RNA extraction was performed from Staphylococcus aureus isolates before and after treatment with iron oxide nanoparticles using an RNA extraction kit. Following the kit's protocol, cDNA was synthesized from the extracted RNA using a cDNA kit. Subsequently, the quantitative gene expression level was measured as Ct values, which were then entered into a formula for gene expression interpretation. Real-time PCR was conducted in a two-step method using SYBR Green dye as the identifier and relative quantification. The results were interpreted using the $2^{-\Delta\Delta\text{CT}}$ method. In this study, the 16S rRNA gene was used as an internal control for normalization and as a reference gene for relative quantification.

Real Time-PCR Protocol

Cycle	Cycle Point
Hold @ 95°C, 10 min 0 secs	
Cycling (40 repeats)	Step 1 @ 95°C, hold 20

secs

Step 2 @ $TM^{\circ}C60$,
hold 25 secs

Step 3 @ $72^{\circ}C$, hold 20
secs,

Melt ($72-99^{\circ}C$), hold secs on the 1st step, hold 5 secs on next
steps, Melt A([Green][1][1])

5.RESULTS

Based on the biochemical and bacteriological tests performed on clinical samples sent to the microbiology laboratory, 60 strains of *Staphylococcus aureus* were isolated and identified from these samples.

The results of biofilm formation in the standard microtiter plate method

From 60 isolates, Biofilm formation ability:

- ✓ 10 isolates (16.66%) in a weak form
- ✓ 29 isolates (48.33%) in an average form
- ✓ 21 isolates (35.01%) with high potency
- The amount of biofilm production in the standard microtiter plate method
- The results of determining the minimum growth inhibitory concentration (MIC) of iron oxide

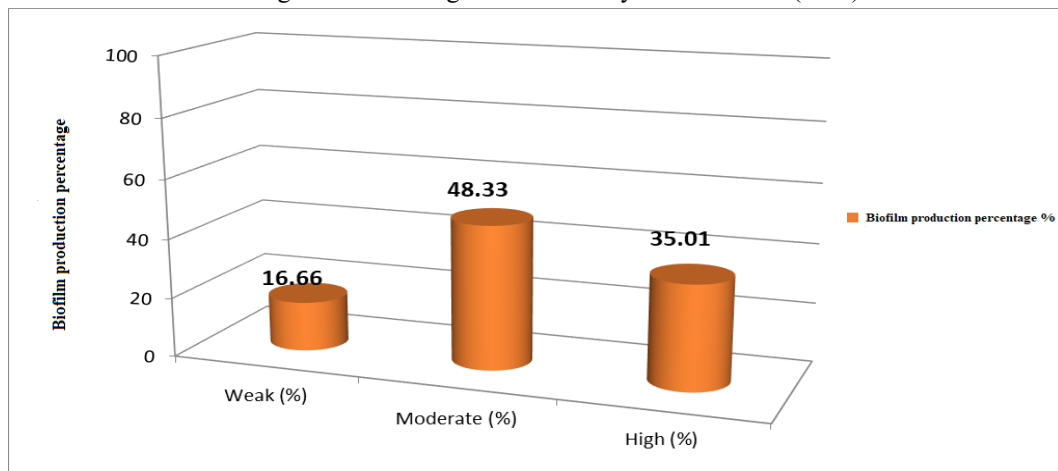
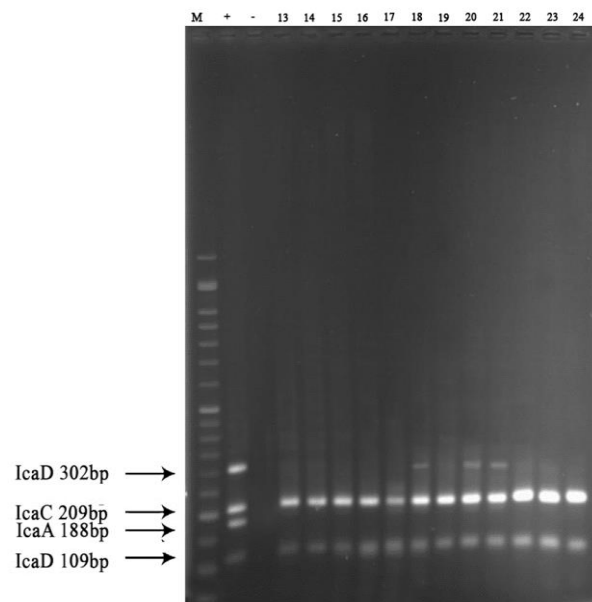
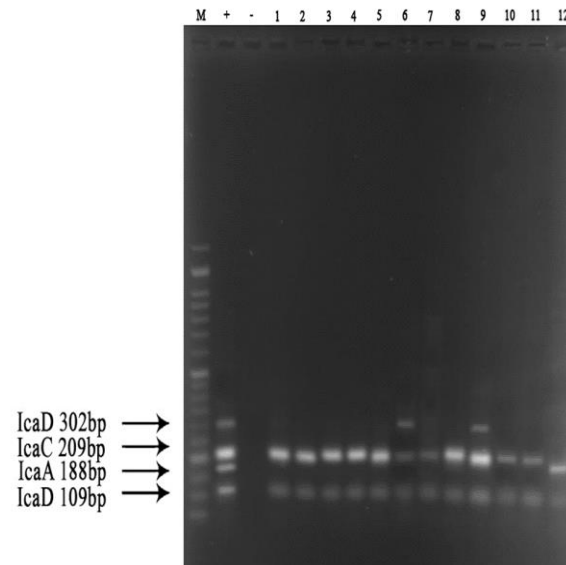


Figure 1. The MIC value for all strains and the positive control strain is the same, and it was MIC:1250 μ g/ml.

PCR results of the presence of genes

The results of the study on the prevalence of the studied genes showed that the *icaA* gene had the lowest frequency, with a prevalence of 1.66% (1 positive case), while the *icaC* gene had the highest frequency, with a prevalence of 96% (58 positive cases). The prevalence of the other studied genes was *icaB* at 90% (54 positive cases) and *icaD* at 13.6% (8 positive cases).

Evaluation of Gene Expression of Staphylococcus Aureus Biofilm (ica) by the Effect of Iron Oxide Nanoparticles by Real Time PCR method



Evaluation of Gene Expression of Staphylococcus Aureus Biofilm (ica) by the Effect of Iron Oxide Nanoparticles by Real Time PCR method

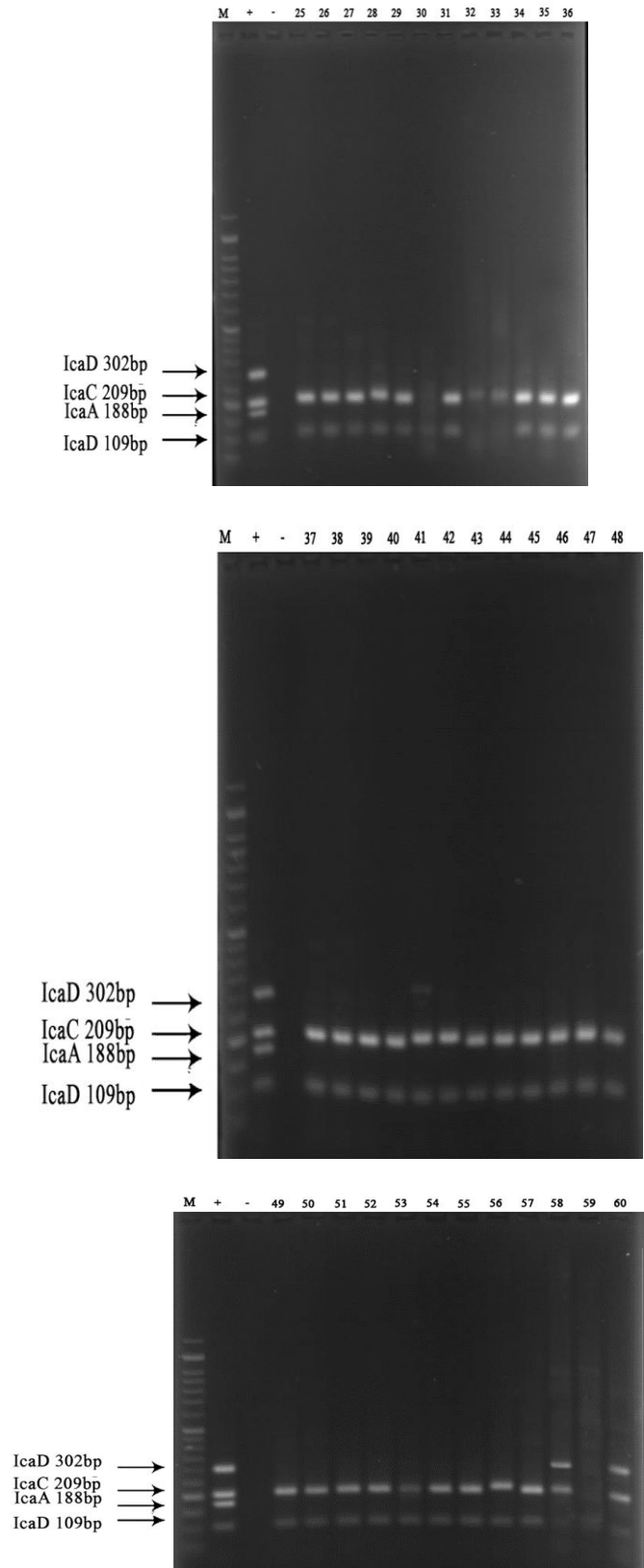


Figure 2. The results of the presence of studied genes. Well m marker 50bp, well +: positive control sample, well -: negative control sample, wells 1-60: studied samples.

Results from Real-Time PCR

- ✓ The expression level of *icaC* gene decreased in the presence of iron oxide nanoparticles

- ✓ fold change for this gene decreased by -3.88 times the ratio of the control gene.

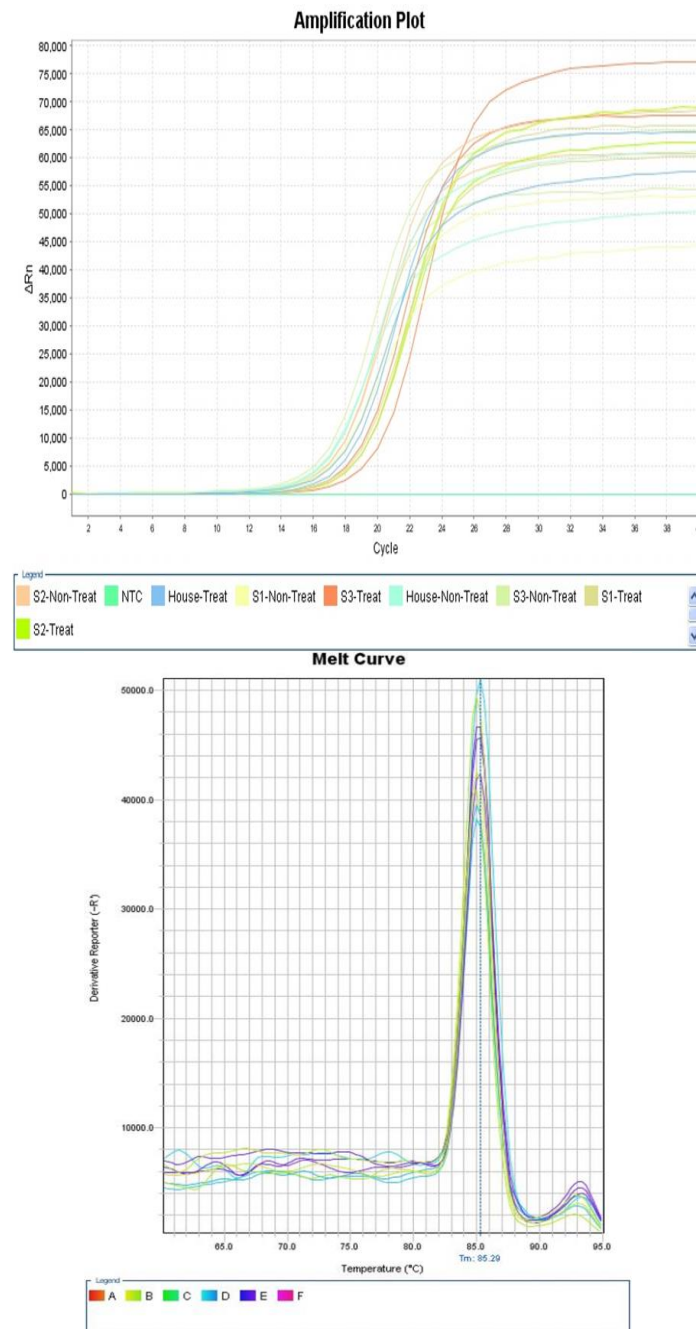


Figure 3. Logarithmic graph of acC gene expression and melt curve in Real Time PCR.

6.DISCUSSION

Staphylococcus aureus is one of the common pathogens associated with biofilm-related infections and is a major cause of healthcare-associated infections. This bacterium is known as a common pathogenic agent capable of causing infections related to external factors such as medical devices. Among other pathogens that have the ability to produce biofilms, the formation of biofilm in *Staphylococcus aureus* strains is recognized as a

virulence factor, aiding the bacterium in tolerating harsh conditions and developing resistance to antibiotics. In this study, which was conducted on 60 strains obtained from wounds, the prevalence of biofilm among the 60 studied strains was 100%. Of this prevalence, 16.66% of the strains had weak biofilm-forming ability, 48.33% had moderate ability, and 35.01% had strong ability to form biofilms. In a study conducted by Ouhadi Moghaddam et al. in 2014, it was found that among the 65 obtained strains, 97% of MRSA strains and 70% of MSSA strains studied had the ability to form biofilms. These findings are consistent with the results of this study. Furthermore, in another study conducted by Vasudevan et al. in 2010, out of 35 strains studied, 32 strains (91%) were found to be biofilm producers. These results also align with the findings of our study. Currently, nanotechnology is also beneficial in the field of nano antibiotics. This technology enhances the performance of these substances by manipulating their size and scale. Nanoparticles have a larger surface area compared to their volume, and materials at the nanoscale exhibit different properties compared to the same material at larger dimensions. One of the products of nanotechnology is iron oxide nanoparticles. It is likely that the released ions from nanomaterials react with thiol groups on the surface proteins of bacterial cells. Some of these membrane proteins are responsible for the transport of mineral substances from the cell wall, and nanomaterials can render these proteins inactive and the membrane impermeable. The inactivation of membrane permeability ultimately leads to cell death. Additionally, nanomaterials delay the attachment of bacterial cells and the formation of biofilms, preventing certain bacteria from adhering and proliferating. The antimicrobial changes that hinder the growth of pathogenic bacteria are considered a desirable goal. Infections can be caused by various factors, including the formation of colonies, bacterial growth, and the formation of dense microbial biofilm matrices, which protect bacteria against the host's immune system. Nanoparticles prevent the formation of these microbial defense factors against the host's immune system. Nanomaterials based on metal ions exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses.

In the conducted study, the MIC value for iron oxide nanoparticles was measured in 20 strains that were strong biofilm producers using the microdilution method. The MIC value was the same for all 21 strains, which was 1250 micrograms per milliliter. A concentration of 750 microliters per milliliter was used for real-time treatment. All 21 examined strains, which were treated with iron oxide, showed an increase in Ct and the fold change of the target gene was calculated in the treated samples compared to the untreated samples, resulting in a value of 3.88 for the *icaC* gene. In other words, after adding iron oxide nanoparticles, the expression of the *icaC* gene decreased by 3.88 times compared to the untreated samples.

Therefore, considering the drug resistance and the risk of biofilm formation, as well as the increasing resistance of *Staphylococcus aureus* in the medical system, compared to phenol, phenolic compounds, drugs, disinfectants, and the importance of this bacterium in nosocomial infections and medical devices, finding an effective and safe

preventive and therapeutic approach is necessary. Therefore, the identification of an effective compound that can inhibit the virulent genes of this bacterium is of great importance. Considering the inhibitory effect of iron oxide nanoparticles on the biofilm gene of *Staphylococcus aureus*, it is likely that these nanoparticles can be used as a complementary therapeutic agent against this bacterium and to inhibit its virulence genes.

7.CONCLUSION

The proteins encoded by the *ica* genes play a crucial role in the production and extension of biofilms. Among them, *icaC* has one of the most essential roles in biofilm production based on the PIA pathway by creating a receptor as a binding site for polysaccharides. Based on the results of this study and the examination of biofilm formation ability, as well as the impact of iron oxide nanoparticles on the expression of one of the important genes in biofilm formation, namely *icaC*, it seems that these nanoparticles can effectively reduce the expression of this gene and disrupt biofilm formation. Therefore, it is likely that these nanoparticles can be used as a complementary therapeutic agent against *Staphylococcus aureus* and inhibit its virulence genes, considering their potential in reducing biofilm formation ability and disrupting biofilm structure.

8.SUGGESTIONS

- Investigating the effectiveness of iron oxide nanoparticles on other genes involved in biofilm production:
- The assessment of the effectiveness of iron oxide nanoparticles on the expression of other genes involved in biofilm formation, specifically in the PIA and PSA pathways, can be conducted separately. These studies would involve examining the impact of the nanoparticles on the expression of additional genes that play a role in biofilm formation, such as *icaA* and *icaD*, and not limited to a single factor.
- The combination of iron oxide nanoparticles with other nanoparticles enhance synergistic effects can be investigated. The interaction between different nanoparticle compositions can be explored to strengthen their effectiveness in inhibiting biofilm formation and reducing bacterial resistance.
- Assessing the cellular toxicity of iron oxide nanoparticles and utilizing animal models can provide insights into potential side effects and systemic toxicity of the nanoparticles being used. These investigations can aid in determining safe dosage levels and the use of these nanoparticles in internal therapeutic applications.
- evaluating the effectiveness of iron oxide nanoparticles when coated on bandage alloys used in wounds or sprayed onto medical devices can contribute to assessing their potential use in controlling infections and improving the antimicrobial properties of medical equipment.

Funding

The authors had no institutional or sponsor backing.

Conflicts Of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

The authors have no acknowledgments to declare.

References

- [1] Andriole VT, Lyons RW. Coagulase-negative staphylococcus. *Annals of the New York Academy of Sciences*. 1970;174(2):533-44.
- [2] Ajello L, Hay RJ. *Topley & Wilson's microbiology and microbial infections*. Volume 4: Medical mycology: Arnold, Hodder Headline; 1998.
- [3] Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials*. 2012;33(26):5967-82. Jawetz EM, Adelberg I. *Review of medical microbiology*. 1980.
- [4] Brooks GF, Carroll KC, Butel JS, Morse SA. *Medical microbiology*. University Sultan Qaboos. 2007;7(3):273-5.
- [5] Batzilla CF, Rachid S, Engelmann S, Hecker M, Hacker J, Ziebuhr W. Impact of the accessory gene regulatory system (Agr) on extracellular proteins, codY expression and amino acid metabolism in Staphylococcus epidermidis. *Proteomics*. 2006;6(12):3602-13.
- [6] Bronesky D, Wu Z, Marzi S, Walter P, Geissmann T, Moreau K, et al. Staphylococcus aureus RNAPIII and its regulon link quorum sensing, stress responses, metabolic adaptation, and regulation of virulence gene expression. *Annual review of microbiology*. 2016;70:299-316.
- [7] Benson HJ. *MICROBIOLOGICAL APPLICATIONS: COMPLETE VERSION LABORATORY MANUAL IN GENERAL MICROBIOLOGY* 1994.
- [8] Blot SI, Hoste EA, Vandewoude KH, Colardyn FA. Staphylococcal septicaemia in burns. *Burns*. 2001;27(2):203.
- [9] Brígido MDM, Barardi CRM, Bonjardin CA, Santos CLS, Junqueira MDL, Brentani RR. Nucleotide sequence of a variant protein A of Staphylococcus aureus suggests molecular heterogeneity among strains. *Journal of basic microbiology*. 1991;31(5):337-45.
- [10] Bukowski M, Wladyka B, Dubin G. Exfoliative toxins of Staphylococcus aureus. *Toxins*. 2010;2(5):1148-65.
- [11] Dmitriev BA, Toukach FV, Holst O, Rietschel E, Ehlers S. Tertiary structure of Staphylococcus aureus cell wall murein. *Journal of bacteriology*. 2004;186(21):7141-8.
- [12] Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. *Clinical microbiology reviews*. 2000;13(1):16-34.
- [13] Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis*. 2002;8 (9).
- [14] Friend J. Mackie & McCartney *Practical Medical Microbiology*. Edinburgh: Churchill Livingstone; 1996.
- [15] Fischetti V, Pancholi V, Schneewind O. Conservation of a hexapeptide sequence in the anchor region of surface proteins from gram-positive cocci. *Molecular microbiology*. 1990;4(9):1603-5.
- [16] Garrity GM, Bell JA, Lilburn TG. Taxonomic outline of the prokaryotes. *Bergey's manual of systematic bacteriology*. Springer, New York, Berlin, Heidelberg. 2004.
- [17] Gün I, Ekinçi F. Biofilms: microbial life on surfaces. *GIDA-Journal of Food*. 2009;34(3):165-73.
- [18] Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *Jama*. 2007;298(15):1763-71.

- [19] Kloos WE, Schleifer KH. Simplified scheme for routine identification of human Staphylococcus species. *Journal of Clinical Microbiology*. 218-82:(1)1;975
- [20] Lowy FD. Staphylococcus aureus infections. *New England journal of medicine*. 1998;339(8):520-32.
- [21] Murray P, Rosenthal KS, Kobayashi GS, Pfaller MA, *Medical Microbiology*. Arthropods; 2002.
- [22] Murray EJ, Crowley RC, Truman A, Clarke SR, Cottam JA, Jadhav GP, et al. Targeting Staphylococcus aureus quorum sensing with nonpeptidic small molecule inhibitors. *Journal of medicinal chemistry*. 2014;57(6):2813-9.
- [23] Morello JA. *Bailey and Scott's Diagnostic Microbiology*. JAMA. 1982;24-.7-2056:(16).
- [24] Novick RP. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of Staphylococcus aureus. *Plasmid*. 2003;49(2):93-105
- [25] O'Riordan K, Lee JC. Staphylococcus aureus capsular polysaccharides. *Clinical microbiology reviews*. 2004;17(1):218-34.
- [26] Orrett FA, Land M. Methicillin-resistant S taphylococcus aureus prevalence: Current susceptibility patterns in Trinidad. *BMC infectious diseases*. 2006;6(1):1.
- [27] Pfaller MA, Herwaldt LA. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clinical Microbiology Reviews*. 1988;1(3):281-99.
- [28] Patti JM, Allen BL, McGavin MJ, Hook M. MSCRAMM-mediated adherence of microorganisms to host tissues. *Annual Reviews in Microbiology*. 1994;48(1):585-617.
- [29] Roche FM, Massey R, Peacock SJ, Day NP, Visai L, Speziale P, et al. Characterization of novel LPXTG-containing proteins of Staphylococcus aureus identified from genome sequences. *Microbiology*. 2003;149(3):643-54.
- [30] Winn WC, Koneman EW. *Koneman's color atlas and textbook of diagnostic microbiology*: Lippincott williams & wilkins; 2006-.