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Assessing the Therapeutic Potential of ZnO-NPs: Effects on Antibiotic Resistance and Biofilm Formation in Staphylococcus Aureus

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Abstract

Introduction: *Staphylococcus aureus*, known for its extensive genetic resistance elements and biofilm-forming capabilities, poses a significant challenge in clinical settings. This study aimed to investigate the prevalence of antibiotic resistance genes, the occurrence of biofilm formation genes, and the interrelation between zinc oxide nanoparticles (ZnO-NPs) and gene expression in S. aureus.

Materials and Methods: Clinical isolates were procured from samples in Tehran, Iran, and identified through biochemical tests. Antibiotic susceptibility profiles were determined, and multidrug-resistant (MDR) strains were selected. The presence of resistance genes (vanA, mecA, and tetC) and biofilm formation genes (fnbA, fibA, clfA, and clfB) was assessed. Microdilution methods were employed to determine the minimum inhibitory concentration (MIC) using ZnO-NPs, and real-time PCR monitored the relationship between nanoparticle treatment and gene expression.

Results: Results indicated high resistance among isolates to tetracycline (100%), amoxicillin (91%), ciprofloxacin, and oxacillin (85%), with low resistance to vancomycin (1%) and linezolid (2%). Profiling of resistance genes revealed a high prevalence of tetC (100%) and mecA (57%), while vanA exhibited a 0% prevalence. Biofilm formation genes were prevalent in 98% of strains, including clfA (98%), clfB (85%), fib (75%), and fnbA (0%). The MIC of iron oxide nanoparticles inhibiting S. aureus growth was recorded at 750 µg/mL. Real-time PCR results demonstrated a significant decrease in the expression of mecA (74%) and biofilm formation gene clfB (76%).

Conclusion: This study underscores the potential efficacy of ZnO-NPs in mitigating bacterial resistance in both methicillin-resistant S. aureus (MRSA) and less-resistant strains (LRSA), impacting the expression of resistance and biofilm formation genes. The utilization of ZnO-NPs presents a promising strategy for managing MRSA/LRSA-associated diseases while minimizing antibiotic use.

Keywords: Antibiotic resistance, Biofilm formation, Staphylococcus aureus, Zinc oxide nanoparticles

1. Introduction

Staphylococcus aureus (S. aureus) manifests as both a commensal microorganism and pathogenic agent in humans. The colonization rate of *S. aureus* in the human population is estimated at around 30% [1]. Concurrently, it serves as a prominent etiological factor in bacteremia and infective endocarditis (IE), in addition to instigating infections in osteoarticular, cutaneous, and soft tissue, pleuropulmonary, device-related, and nosocomial infection. Antibiotic resistance in *S. aureus* poses a formidable challenge to global health, necessitating a thorough understanding of the mechanisms driving this phenomenon. Among the multifaceted strategies employed by *S. aureus* to evade traditional antimicrobial treatments, the formation of biofilms emerges as a pivotal factor contributing to heightened resistance. [2]. *S. aureus*, a Gram-positive bacterium, is well-known for its adaptability and resistance to antibiotics. The rise of antibiotic-resistant forms of *S. aureus*, such as methicillin-resistant Staphylococcus aureus (MRSA), has pushed *S. aureus* to the forefront of worldwide health concerns. The capacity of these bacteria to diminish the effectiveness of many antibiotics necessitates extensive study. Antibiotic resistance genes in *S. aureus* are a significant concern in the medical community because they contribute to the emergence of antimicrobial resistance (AMR) and circulation of resistant strains. Resistant *S. aureus* strains, such as methicillin-resistant *S. aureus* (MRSA), are becoming increasingly prevalent within medical settings and are linked to increased mortality and morbidity [3]. These resistant bacteria frequently possess particular genes that confer antibiotic resistance. For example, the *mecA* gene is connected to methicillin resistance, whereas the *tetC* gene is linked to tetracycline resistance [4]. [Table 1].

Table.1. *S. aureus* antibiotic resistance genes and their functions

Gene	Function
mecA	The *mecA* gene encodes an extra penicillin-binding protein (PBP2a) that has low affinity to virtually all beta-lactam antibiotics. This gene is carried by a mobile genetic element and is the primary determinant of resistance in methicillin-resistant S. aureus [5].
VanA	The *VanA* gene is associated with resistance to vancomycin, a last-resort antibiotic used to treat MRSA. The gene is typically acquired from enterococci and can lead to the emergence of vancomycin-resistant *S. aureus* [6].
tetC	The *tetC* gene is associated with resistance to tetracycline antibiotics. the resistance mechanisms of this gene typically involve altering the antibiotic's target site, actively pumping the antibiotic out of the cell, or enzymatically modifying or degrading the antibiotic [7].

Biofilm formation in *S. aureus* is a complex process that plays a crucial role in the virulence and persistence of this bacterium. *S. aureus* is a Gram-positive bacterium that is a common human pathogen, causing a range of infections from mild skin and soft tissue infections to severe systemic diseases.

Below is a comprehensive synopsis of biofilm formation in *S. aureus*. 1. Initial Attachment: The process begins with the reversible attachment of planktonic (free-floating) *S. aureus* cells to a surface. This initial attachment is mediated by various surface proteins and adhesions [8]. 2. Irreversible Attachment: Once attached, the bacteria undergo a transition to irreversible attachment, which involves the production of polysaccharide intercellular adhesion (PIA). PIA is a key component of the extracellular matrix that holds the biofilm together [2]. 3. Accumulation and Maturation: As bacteria continue to divide and adhere to the surface, the biofilm accumulates and matures. The extracellular matrix, composed of PIA, proteins, and extracellular DNA (eDNA), contributes to the structural integrity of the biofilm. 4. Three-Dimensional Structure: Mature biofilms develop a three-dimensional structure with water channels that allow the exchange of nutrients and waste products. This structure enhances the resilience of the biofilm to environmental stresses and immune responses. 5. Quorum Sensing: Quorum sensing is a communication system used by bacteria to coordinate gene expression in

response to cell density. S. aureus employs quorum sensing mechanisms to regulate the expression of genes involved in biofilm formation [9, 10]. 6. Genetic Regulation: The *ica* operon, comprising *icaADBC* genes, is crucial for the synthesis of PIA. Other regulatory elements, such as global regulators and two-component systems, also play roles in controlling biofilm formation in *S. aureus* [11]. 7. Resistance to Antibiotics: Biofilms provide protection to *S. aureus* against antibiotics and host immune responses. The extracellular matrix acts as a physical barrier, preventing the penetration of antimicrobial agents. This protective enclave not only fosters bacterial survival but also enhances the horizontal transfer of resistance genes, exacerbating the challenge of combating *S. aureus* infections [12, 13]. (Table 2) Understanding the molecular mechanisms behind *S. aureus* biofilm formation is essential for developing strategies to prevent and treat biofilm-associated infections. Disrupting biofilm formation or promoting biofilm dispersal are potential approaches to combat *S. aureus* infections, especially those associated with medical devices and indwelling catheters.

Table 2. *S. aureus* biofilm formation genes and their function

Gene	Function in Biofilm Formation
fnbA	- Encodes fibronectin-binding protein A - Mediates adhesion to host cells and extracellular matrix (ECM) - Promotes intercellular adhesion in biofilm formation
fibA	- Encodes fibrinogen-binding protein A - Mediates adhesion to fibrinogen, a component of blood clots - Enhances biofilm formation by promoting attachment to surfaces
clfA	- Encodes clumping factor A - Binds to fibrinogen and promotes clumping of bacterial cells - Contributes to biofilm formation by facilitating cell-cell adhesion
clfB	- Encodes clumping factor B - Similar to *clfA*, it binds fibrinogen and promotes cell clumping - Contributes to the formation of biofilms

Zinc oxide nanoparticles (*ZnO-NPs*) have been observed to exert inhibitory effects on the growth and biofilm formation of *S. aureus*, encompassing vancomycin-resistant *S. aureus* (VRSA) and MRSA. Numerous investigations have corroborated the antibacterial efficacy of *ZnO-NPs* against *S. aureus*, attributing it to mechanisms such as the generation of reactive oxygen species (ROS) and the inhibition of biofilm formation. The antibacterial attributes of *ZnO-NPs* have undergone in vitro assessments, revealing notable constraints on *S. aureus* proliferation and biofilm development. Furthermore, *ZnO-NPs* have demonstrated a synergistic effect with antibiotics, diminishing *S. aureus* virulence and exhibiting heightened antimicrobial activity against MRSA [14-16].

The intricate relationship between antibiotic resistance and the formation of biofilms in *S. aureus* necessitates a thorough investigation to untangle the complex pathways that bolster bacterial resilience. As conventional treatment methods experience diminishing effectiveness, it becomes imperative to uncover the molecular intricacies that govern antibiotic resistance within biofilm communities. This exploration not only guides the development of innovative therapeutic strategies but also provides a glimpse into the future landscape of tackling *S. aureus* infections.

ZnO-NPs show promise in mitigating bacterial resistance, particularly MRSA and less resistant strains. They can potentially alter the expression of resistance and biofilm formation genes, contributing to reduced virulence and enhanced susceptibility of bacteria to treatment. By leveraging *ZnO-NPs*, it may be possible to manage MRSA/LRSA associated diseases effectively while minimizing the reliance on antibiotics, thereby addressing the challenge of antibiotic resistance. In this study, we employ the real-time PCR method to analyze the genes responsible for antibiotic resistance within *S. aureus* biofilms, with a specific emphasis on understanding the impact of zinc oxide

nanoparticles. The subsequent sections offer a detailed account of our methodology, results, and conclusions, contributing to the collective effort to address this urgent challenge.

2. Materials and Methods

In this descriptive cross-sectional study, a total of 1450 samples, collected between 1400 and 1401 (Iranian calendar) from hospital around Tehran, were randomly obtained by a technician using swabs from various sources, including infectious abscesses, bedsores, burns, esophagus, opiate secretions, bronchial tubes, catheters, and other areas, from hospitalized patients. Out of these, 150 strains initially identified as *S. aureus* were further investigated in the laboratory. Following collection, the samples were introduced into a broth medium and transported to the laboratory. To classify the strains in accordance with microbiological standards, they underwent gram staining tests, as well as assessments for catalase, oxidase, coagulase, DNase, growth in mannitol salt agar, and urease activity. Among the 150 samples, 116 *S. aureus* isolates were conclusively identified through standard methods. The confirmed *S. aureus* isolates were preserved in Trypticase Soy Broth (TSB) with 20% glycerol and sterilized at -20°C.

Identification of methicillin-resistant strains (MRSA) was done by disc infusion method, which was used for quality control of *S. aureus* isolate as positive control *ATCC33592* and negative control *ATCC29213*. To confirm these results, the frequency of *mecA* gene was also checked by PCR method. Clinical isolates resistant to vancomycin (VRSA) were determined by disc method and MIC was determined by microdilution method, and the obtained data was confirmed by the frequency of Van gene. From antibiotics Cefoxitin (30 micrograms), Vancomycin (2 micrograms), linezolid (30 micrograms), Oxacillin (1 microgram), Erythromycin (15 micrograms), Clindamycin (2 micrograms), Tetracycline (30 micrograms), Trimethoprim-sulfamethoxazole (1.25-23.75 micrograms), Gentamicin (10 micrograms), Amoxicillin (10 micrograms) and Ciprofloxacin (5 micrograms) were used in this study [16, 17].

To check the minimum concentration of antibiotics to inhibit bacterial growth, the method of MIC was used by broth microdilution method, Enterococcus Fecalis strain *ATCC52192* was used as control agent and resistant to Vancomycin and *S. aureus* strain *ATCC29213* was used as control agent and sensitive strain for quality control. According to the CLSI standard, the minimum inhibitory concentration of *Enterococcus Faecalis* resistant strains to the antibiotic vancomycin is greater than or equal to 32 micrograms/ml.

Biofilm production was measured by the microtiter method of 96-well plates. Thus, 120 microliters of bacterial suspension (equivalent to 0.5 McFarland) was inoculated three times in the wells of a 96-well plate. After 24 hours of incubation at 37°C, the wells were washed and methanol was added to fix them and dried at room temperature. Then 100 microliters of crystal violet (10%) was added to each one, washed and dried after 15 minutes. Then it was dissolved with ethanol and its turbidity was measured by ELISA at a wavelength of 490 nm. The absorption obtained was compared with the negative control (containing dye) and the results were reported as strong, medium and weak biofilm producers [18]. [Table 3].

Table 3. Interpretation of ELISA results to investigate biofilm formation

OD>4ODc	**strong biofilm producer**
OD>4ODc	moderate biofilm producer
ODc<OD<2ODc	poor biofilm producer
OD<<ODc	no biofilm producer
TSB	as negative control

DNA extraction was performed by boiling method in TE buffer, and for PCR, primers for desire genes were obtained from articles. PCR reaction conditions and PCR program to detect

antibiotic resistance genes (methicillin, vancomycin and tetracycline) are provided in supplementary file 1. In this study, *S. aureus* strains *ATCC 33592* and *ATCC52199* were used as positive controls for the genes, and distilled water served as the negative control.

PCR reaction conditions and PCR program for detection of adhesion genes biofilm producer are provided in supplementary file 1. In this study, *S. aureus* strains *ATCC 33592* and *ATCC52199* were used as positive controls for the genes, and distilled water served as the negative control.

The zinc oxide nanoparticles (*ZnO-NPs)* used in this research were obtained from US Research Nanoparticles with a purity of 99%. In order to determine the optical properties, purity and nature of zinc oxide nanoparticles used in this research, Fourier-transform infrared spectroscopy (FT-IR) analysis, size analysis and zeta potential analysis were performed at room temperature. These analyses were conducted by Bim Gostar Taban company as an outsourced service; this knowledge-based company has a certificate of national qualification of Iran with code NACI/Lab/1497. The minimum inhibitory concentration (MIC) tests were performed using ZnO-NPs (see supplementary File 2).

In this research, in order to investigate the expression of the methicillin resistance gene (*mec*) and the biofilm producing gene (*clfB*) of the selected strains of this study that were treated with zinc nanoparticles, reverse transcriptase real-time PCR was used, and the expression of the genes before and after the treatment was examined to assess the relationship between the effect of zinc oxide nanoparticles and the expression of target genes. The results are interpreted using the ΔCT method [17].

3. Results

In this study, 116 isolates of *S. aureus* were examined morphologically, biochemically, and for antibiotic resistance using the disc infusion method. From these, 60 strains were selected, and the frequency of resistance genes against Methicillin, Tetracycline and Vancomycin was checked by the PCR method. Biofilm production was assessed by the microdilution method, and abundance of adhesin genes was determined by PCR. Finally, the selected strains were treated with zinc oxide nanoparticles and the expression of methicillin resistance genes and the biofilm-producing gene was measured by real-time PCR.

As can be seen in the results, *S. aureus* strains in this study showed a high antibiotic resistance pattern. Resistance to Tetracycline (100%), Amoxicillin (91%), Oxacillin (85%) and Ciprofloxacin (85%) was the highest, while resistance to Vancomycin (1%) and Linezolid (2%) was low. [Chart 1].

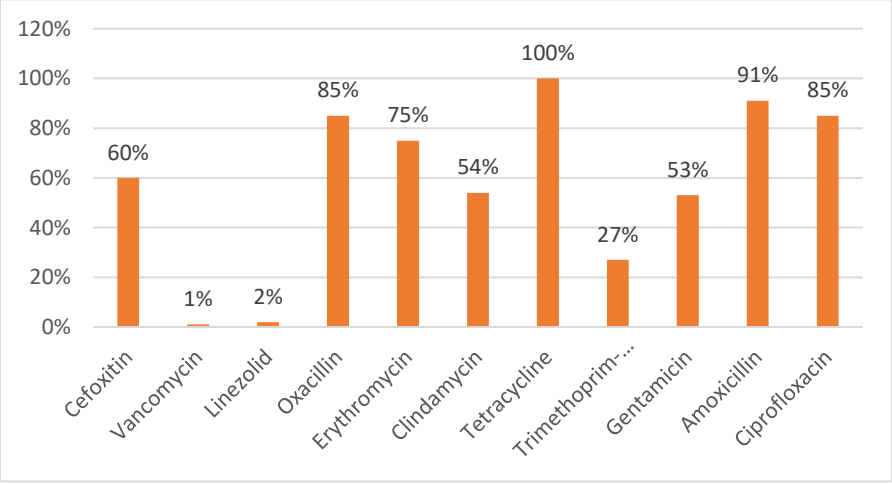

Chart 1. Frequency chart of antibio

DNA was extracted from 60 selected isolates and the existence of methicillin, vancomycin and tetracycline resistance genes (*mec*, *van*, and *tet*) was checked by specific primers. The results are shown in [Figure 1]. DNA was extracted from 60 selected isolates and the presence of biofilm-producing genes (*clfA* ،*clfB* ،*fib* and *fnbA*) was checked by specific primers, with results shown in [Figure 2].

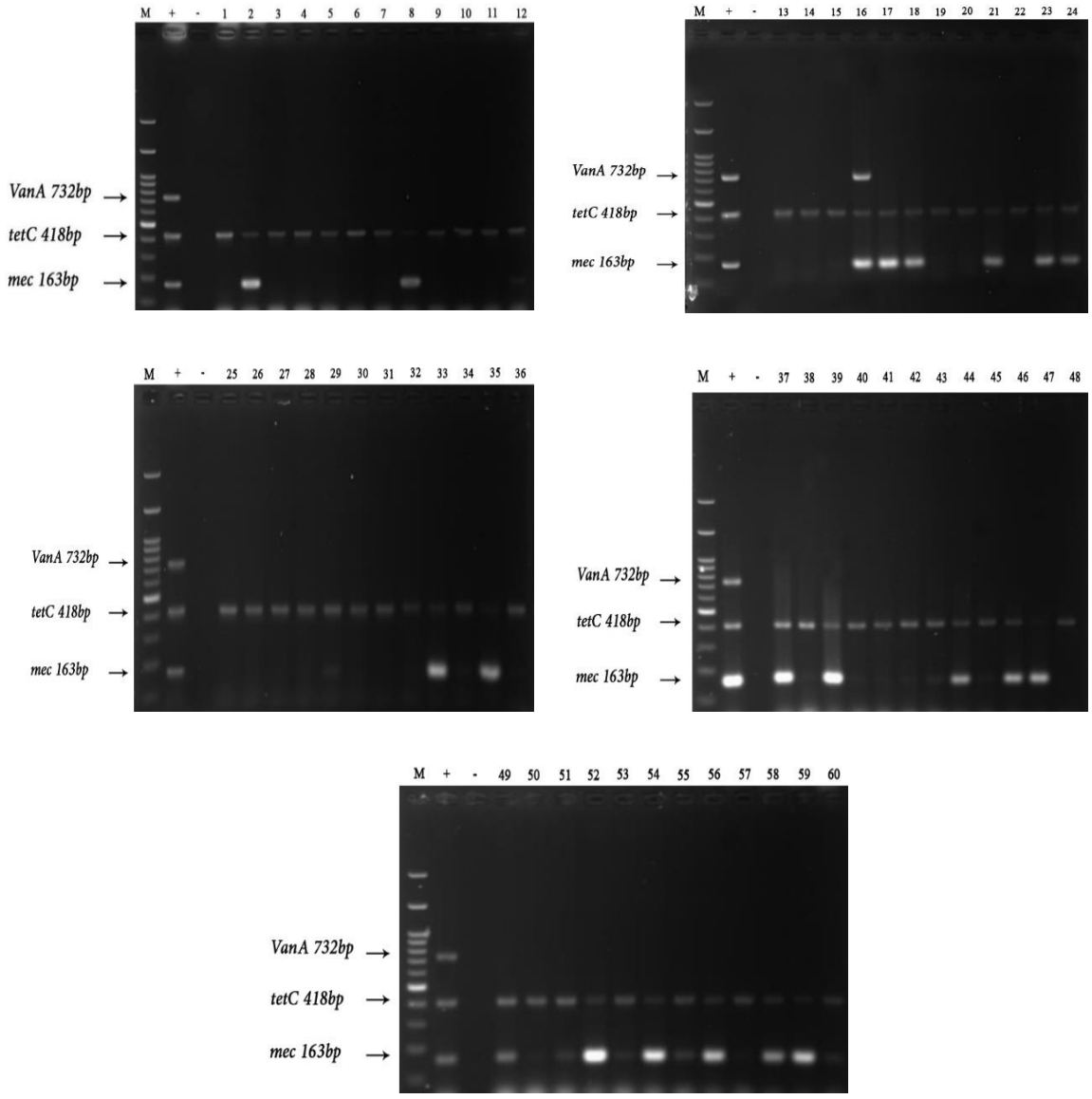

Figure 1. Electrophoresis results of PCR products for mec, tetC and vanA genes, for isolates 1 to 60, M gene marker, + positive control and - negative control

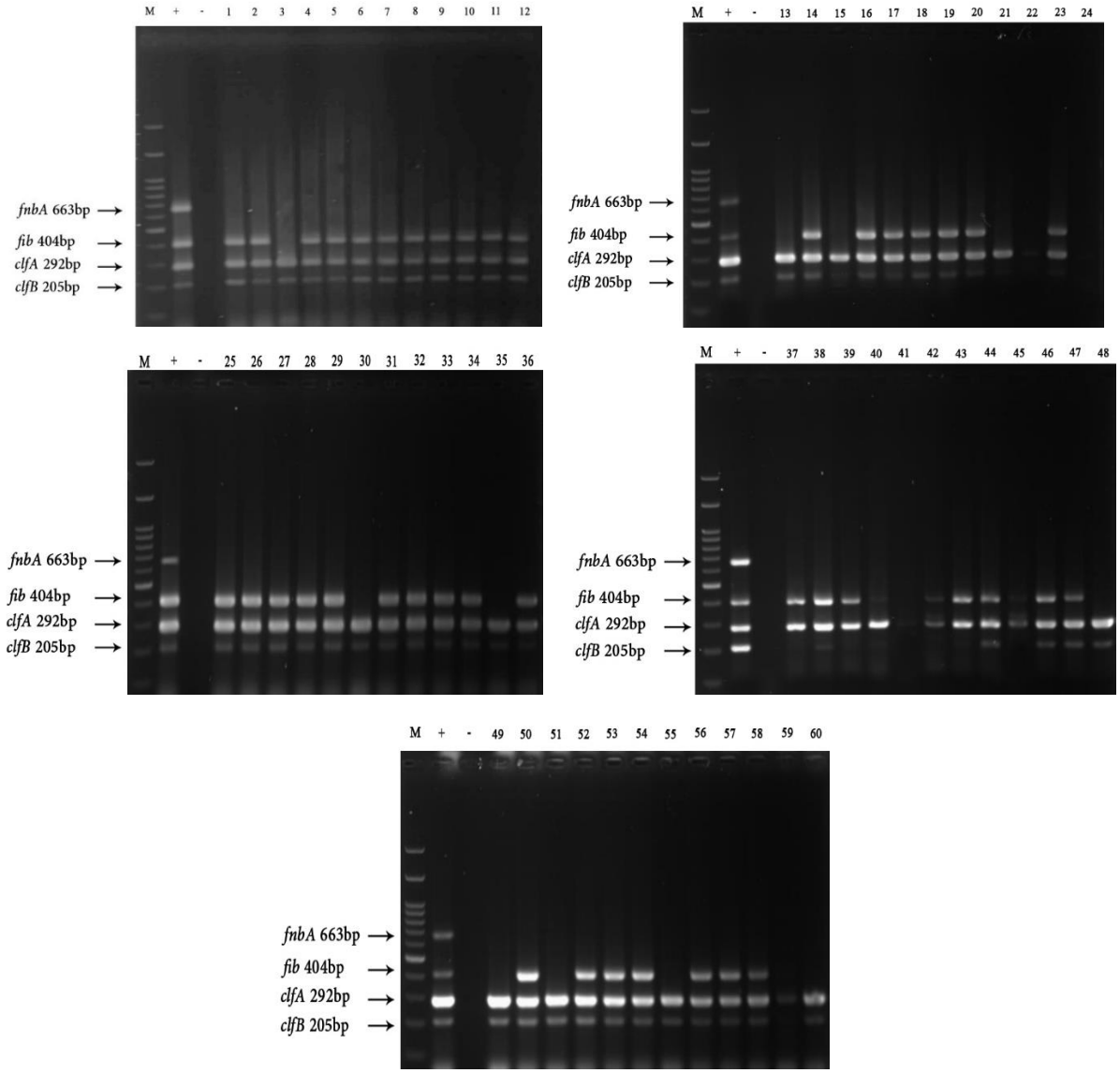

Figure 2. Electrophoresis results of PCR products for *clfA*, *clfB*, *fib* and *fnbA* genes, for isolates 1 to 60, M gene marker, + positive control and - negative control

The results show that 100% of the strains carried the *tetC* gene, 57% carried the *mec* gene, and only 2% of the strains contained the *vanA* gene. For this reason, only 2% were classified as MRSA and VRSA, while all the strains were resistant to tetracycline, indicating the high prevalence of this gene among the staphylococci of this study. The results of biofilm producing genes were as follows: the abundance of *clfA* gene (98%), *clfB* gene (85%), fib gene (75%) and *fnbA* gene (0%). Therefore, 98% of the strains harbored at least one biofilm-producing gene, while the frequency of *fnb* gene was very low. [Chart 2].

Following the initial tests, to further investigate biofilm production, a random selection of strains was made based on antibiotic resistance profiles, gene frequencies, and biofilm production. Biofilm formation and the MIC values of oxacillin-resistant strains for vancomycin and linezolid antibiotics were determined. The results are summarized in [Table 4].

Chart 2. Frequency chart of antibiotic resistance and biofilm producing genes in the strains of this research

Table 4. Results of biofilm production and the minimum inhibitory concentration for selected strains resistant to oxacillin against vancomycin and linezolid antibiotics.

	Mean relative expression	Standard Deviation (SD)
Control *mec* gene	1	0.237
Treatment *mec* gene	0.279968	0.099
Control *clfB* genes	1	0.323575
Treatment *clfB* genes	0.238434	0.118509

The data obtained from the biofilm production test and genetic expression indicate that biofilm production is one of the important characteristics of *S. aureus* strains, as 98% carried at least one biofilm-producing gene, and the biofilm production test was also reported negative in only one case. This shows that in addition to the high frequency of resistance genes (100% resistance to tetracycline and 85% resistance to methicillin), the phenotype is biofilm production that protects the bacteria against a variety of antimicrobial substances, disinfectants and environmental esters, and it plays an important role in the spread of infection. The results of treatment with zinc oxide nanoparticles showed that the growth of bacteria was inhibited at a concentration of 750 µg/ml and this concentration was considered as MIC.

In order to calculate the average relative expression, the average of ΔΔCTs was first taken and the result was calculated with the formula of relative expression.

The expression rate of *mec* gene, which is a measure of ΔΔCT which was calculated as 0.260276 in this research, which shows that the expression of resistance genes decreased by 74% during treatment (P value <0.05), moreover the expression rate of *clfB* gene which was calculated as 0.246323 in this study, which shows that the expression of resistance genes decreased by 76% during the treatment (P value <0.05).

Frequency of antibiotic resistance and biofilm producing genes in the strains of this research show that 100% of the strains contain the *tetC* gene, 57% of the strains contain the *mec* gene, and only 2% of the strains contain the *vanA* gene. For this reason, only 2% were MRSA and VRSA, and all the strains contain resistance to tetracycline, which shows the high frequency.

The results of biofilm producing genes were as follows: the abundance of *clfA* gene (98%), *clfB* gene (85%), *fib* gene (75%) and *fnbA* gene (o%). Therefore, 98% of the strains have at least one biofilm producing gene and the frequency of *fnb* gene was very low.

Statistical Analysis

To compare the outcomes between the treatment and control groups, an independent T-test was conducted on ΔCT and ΔΔCT values. For non-normally distributed data, the Mann-Whitney U test was applied.

Inclusion and exclusion criteria

Inclusion criteria included sample size, patient age, and geographic distribution. Samples were collected between 1400 and 1401 (Iranian calendar). Exclusion criteria were isolates not subjected to standard antibiotic resistance testing or with incomplete results.

4. Discussion

S. aureus has maintained its status as a prominent pathogen associated with hospital-acquired infections. Over the past two decades, strains of staphylococci displaying resistance to methicillin (MRSA) and various other antibiotics have proliferated significantly. In this study, we show that ZnO-NPs hold promise as effective agents against antibiotic-resistant strains of *S. aureus*. Furthermore, these bacteria have now become prevalent as the most common causative agents of community-acquired infections in numerous geographic regions, highlighting the global rise in multidrug resistance. Consequently, the significance of *S. aureus* pathogenicity is notably underscored by its heightened antibiotic resistance, its capacity for biofilm production, and its resilience to environmental stressors [19]. In this investigation, *S. aureus* exhibited the greatest degree of antibiotic resistance to tetracycline, amoxicillin, oxacillin, and ciprofloxacin. Conversely, the least resistance was observed towards linezolid and vancomycin, with rates of 2% and 1%, respectively. This resistance pattern was observed in several studies in Iran [20-23]. It appears that vancomycin remains a viable treatment option for *S. aureus* infections in hospital settings, particularly in instances characterized by a high prevalence of antibiotic resistance.

Clumping factor A (*ClfA*) and clumping factor B (*ClfB*) are significant virulence factors for *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), and play critical roles in the progression of infections. The *clfA* gene exists in almost all clinical strains of *S. aureus*. *ClfA* promotes bacterial attachment to the blood plasma protein fibrinogen, allowing bacteria to deal with the mechanical pressures imposed by the blood stream. *ClfB*, on the other hand, acts as a biofilm formation stimulant, which plays a vital role in the development of long-lasting infections. The *ClfB* gene is found in a large percentage of *S. aureus* strains [24-27]. Al-Badri et al. studied vancomycin and linezolid antibiotic resistance of *S. aureus* hospital isolates and their interaction with neutrophils. In this study, 300 isolates were isolated and identified from different hospitals in Baghdad. The results indicated that 67% (200 samples) were MRSA, among which 50.5% were strong producers and 22% were moderate biofilm producers. All MRSA strong biofilm producers contained *clfA, clfB, fnbA, fnbB, fib* and *eno* genes. Among MRSA, about 1.66% were VRSA and 3% were LRSA, which were isolated from skin and blood infection samples. *vanA* and *cfr* genes were observed in 2 and 3 MRSA strains. Three VRSA and 2 LRSA were strong biofilm producers that contained adhesin genes (*clfA, clfB, fnbA, fnbB, eno, fib, cna and ebps*). The data of this study are very similar to the data obtained from the current research, and the phenotypic pattern of antibiotic resistance and the pattern of resistance genes are very close and confirm each other [28].

The intricate interplay between antibiotic resistance genes and biofilm-producing genes in *S. aureus* has profound implications for the efficacy of traditional antibiotics. As *S. aureus*, including methicillin-resistant strains (MRSA), often harbor antibiotic resistance genes, the challenge of combatting infections becomes exacerbated. Notably, biofilm formation, mediated by genes such as *clfA* and *clfB*, adds an additional layer of complexity, as biofilms act as protective shields, rendering bacteria more resilient to antibiotics [12, 29]. In addressing this multifaceted issue, the utilization of metallic nanoparticles as potential antibiotics presents a promising avenue. Metal nanoparticles exhibit unique antimicrobial properties, acting through diverse mechanisms such as membrane disruption and generation of reactive oxygen species [30]. These properties could disrupt biofilm integrity and counteract the resistance mechanisms conferred by specific genes [31]. For example, silver and gold nanoparticles are well-known for their antimicrobial capabilities, which include antibacterial, antifungal, and antiviral activities. Their capacity to pass through bacterial cell walls induces structural changes to cell

membranes, potentially leading to cellular death [32, 33]. *AgNPs* or silver nanoparticles, and *AuNPs* or gold nanoparticles have antibacterial activity against bacteria, including those resistant to multiple antibiotics. Furthermore, they mimic a triclosan-like antibacterial action and inhibit biofilm formation [34]. *AgNPs*' antibacterial activity is attributed to a combination of several and concurrent modes of action, including the production of reactive oxygen species (ROS) and the release of silver ions [35].

Extensive research has been conducted on zinc oxide nanoparticles *(ZnO-NPs)* due to their noteworthy antibacterial properties, especially in combatting diverse bacterial pathogens. The antibacterial efficacy of *ZnO-NPs* is associated with their compact size and substantial surface-to-volume ratio, facilitating improved interaction with bacterial cells [36]. The primary mechanism contributing to the antibacterial impact of *ZnO-NPs* lies in the generation of reactive oxygen species (ROS). This process induces damage to the bacterial cell membrane, moreover The antibacterial action of *ZnO-NPs* may also be facilitated by the release of *Zn2+* ions and ultimately resulting in cell death [37-39]. *ZnO-NPs* have been documented to exhibit synergistic effects with antibiotics, thereby augmenting their efficacy in combating bacterial infections [40]. The antibacterial effects of *ZnO-NPs* extend beyond a specific bacterial type; they demonstrate broad-spectrum activity against a range of both Gram-positive and Gram-negative bacteria, including pathogens resistant to beta-lactam antibiotics [41]. This wide-ranging efficacy, coupled with the capacity to improve antibiotic effectiveness, positions *ZnO-NPs* as a promising contender for the development of novel antibacterial treatments, particularly in light of escalating antibiotic resistance.

In this study, the lowest concentration of *ZnO-NPs* that inhibited the growth of *S. aureus* (MIC) was recorded at 750 µg/mL. Real-time results show significant decrease on expression of resistance genes *mecA* (74%) and biofilm formation gene *clfB* (76%). The outcomes of this study are consistent with prior research, which have demonstrated that the utilization of zinc oxide nanoparticles effectively reduces the presence of antibiotic resistance genes and concurrently mitigates the expression of genes associated with biofilm formation [42-45]. The data of the current study shows the effectiveness of nanoparticles in higher concentrations, which can be pointed out due to the combined approach of using nanoparticles along with antibiotics, as a result, the minimum concentration of the antibacterial compound has decreased. The results of the current research also showed a decrease in the expression of biofilm-producing genes, and with a decrease in the expression of methicillin resistance genes, it can be predicted that the sensitivity of bacteria to antibiotics will decrease and the use of antibiotics will be effective. The result of this investigation and prior studies shows that in the not-too-distant future, nanoparticles along with antibiotics will be used in the combined treatment of diseases caused by *S. aureus*.

5. Conclusion

There has been noticeable impact of zinc oxide nanoparticles (*ZnO-NPs*) on methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). *ZnO-NPs* have significant antibacterial properties against these challenging bacterial strains, as indicated by their low MIC values and significant inhibition of biofilm formation. The ability of *ZnO-NPs* to successfully suppress MRSA and VRSA growth demonstrates their potential to be valuable assets in the ongoing battle against multidrug-resistant bacteria. Continued research efforts should focus on the potential synergistic effects of combining *ZnO-NPs* with existing antibiotics which pave the way for enhanced therapeutic interventions. The translation of these findings from laboratory studies to real-world applications represents a crucial avenue for future research. Optimizing the formulation of *ZnO-NPs* for practical use in clinical settings, medical devices, and other relevant applications is essential. This involves addressing challenges such as cytotoxicity concerns and fine-tuning nanoparticle characteristics to ensure both efficacy and safety in diverse contexts.

In summary, the current body of evidence suggests that *ZnO-NPs* hold promise as effective agents against antibiotic-resistant strains of *S.*

aureus. The continued exploration of their mechanisms of action, potential synergies with existing treatments, and practical applications will contribute to the ongoing quest for innovative solutions in the face of antimicrobial resistance.

Ethical Considerations

Compliance with ethical guidelines

This MSc Thesis was approved by approval number of 12345230450248623001616264754 5 in School of Science and Agriculture, Islamic Azad University, Roudehen Branch, Tehran, Iran.

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Author's contributions

All authors equally contributed to preparing this article.

Conflict of interest

All authors disclosure of any potential conflicts of interest.

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