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MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF A NOVEL STAPHYLOCOCCUS AUREUS STRAIN ISOLATED FROM BURN INFECTIONS IN IRAQ

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ABSTRACT
Background: *Staphylococcus aureus* is a major opportunistic pathogen associated with burn infections due to its ability to produce virulence factors and form biofilms that enhance infection persistence. The increasing prevalence of antibiotic-resistant strains, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), has created a growing challenge for effective treatment. Consequently, attention has shifted toward alternative antimicrobial agents, including medicinal plant extracts and nanomaterials. *Taraxacum officinale* has been reported to possess antibacterial properties, while zinc oxide nanoparticles (ZnO NPs) have demonstrated promising activity against various pathogenic bacteria. Therefore, this study aimed to isolate and identify *Staphylococcus aureus* from burn infections and evaluate the antibacterial activity of *Taraxacum officinale* extract and ZnO nanoparticles against the isolates.
Methodology: Clinical isolates (n=112) were collected from burn patients in Baghdad and identified using Mannitol Salt Agar and biochemical tests. Antimicrobial resistance and virulence profiles were determined via Kirby-Bauer disk diffusion (CLSI standards) and PCR for *mecA*, *icaD*, and *crtN* genes. Simultaneously, *Taraxacum officinale* extract and Zinc Oxide Nanoparticles (ZnO NPs) were prepared. Their antibacterial efficacy was evaluated using the agar well diffusion method. The chemical composition of the plant extract was further characterized via High-Performance Liquid Chromatography (HPLC).
Results: The results showed that several isolates of *Staphylococcus aureus* were successfully identified from burn infections. Molecular analysis confirmed the presence of the *mecA*, *icaD*, and *crtN* genes in some isolates. The tested isolates exhibited varying patterns of antibiotic resistance. Both *Taraxacum officinale* extract and zinc oxide nanoparticles (ZnO NPs) demonstrated notable antibacterial activity against the bacterial isolates.
Conclusion: These findings suggest that *Taraxacum officinale* extract and ZnO nanoparticles may serve as promising alternative or complementary agents for controlling antibiotic-resistant *Staphylococcus aureus*.

KEYWORDS: *Staphylococcus aureus*, Burn infections, *Taraxacum officinale*, ZnO NPs, Virulence genes, Antibiotics

INTRODUCTION
Burn injuries are considered serious conditions that pose a major challenge to healthcare systems worldwide due to the complications they may cause, which can lead to increased morbidity and mortality rates, particularly when secondary bacterial infections occur. The loss of the natural skin barrier as a result of burns exposes the underlying tissues to microbial colonization, making burn wounds a favorable environment for the growth and proliferation of microorganisms. In addition, the weakened immune response in burn patients increases the likelihood of developing bacterial infections and serious complications such as bacteremia and sepsis, which are among the leading causes of death in burn patients [(5)(20)].
Staphylococcus aureus is considered one of the most common pathogens associated with burn infections, as it is characterized by its high ability to colonize the skin and damaged tissues, in addition to possessing numerous virulence factors that enable it to initiate and sustain infection. These factors include the production of exotoxins, adhesion to host tissues, and the formation of biofilms, which contribute to increased bacterial resistance to harsh environmental conditions and antibiotics [(19)(13)].

Moreover, the increasing spread of antibiotic-resistant strains, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), has complicated the treatment of these infections and made them one of the major challenges in the control of infectious diseases [10].

The antibiotic resistance of *Staphylococcus aureus* is associated with the presence of several genes responsible for this resistance. Among these, the *mecA* gene is considered one of the most important, as it confers resistance to β-lactam antibiotics through the production of a low-affinity penicillin-binding protein (PBP2a). In addition, this bacterium possesses other genes associated with virulence factors, such as *icaD*, which plays an important role in biofilm formation, and *crtN*, which is responsible for the biosynthesis of the pigment staphyloxanthin that helps the bacterium resist oxidative stress and enhances its survival within the host [7][14].

Therefore, molecular diagnosis using techniques such as the polymerase chain reaction (PCR) has become an important tool for detecting these genes and understanding the mechanisms of virulence and resistance in this bacterium.

In light of the continuous increase in antibiotic resistance rates, the search for effective and safe alternative therapeutic strategies has become a major research priority in the field of bacterial infection control. Among the promising alternatives is the use of nanomaterials, which have demonstrated broad-spectrum antimicrobial activity. Zinc oxide nanoparticles (ZnO NPs) are among the most widely studied nanomaterials in recent years due to their notable antibacterial properties and their ability to inhibit the growth of various bacterial pathogens. Studies suggest that the antibacterial activity of these nanoparticles is attributed to several mechanisms, including the generation of reactive oxygen species (ROS), disruption of the bacterial cell membrane, and the release of zinc ions that interfere with essential cellular processes within bacterial cells [15][21].

In addition to nanomaterials, plant extracts have gained increasing attention in recent years as natural sources of bioactive compounds with antimicrobial properties. *Taraxacum officinale* (dandelion) is a well-known medicinal plant containing numerous bioactive compounds such as flavonoids, phenolics, and terpenoids, which exhibit antibacterial and antioxidant activities. Several studies have demonstrated that extracts from this plant are capable of inhibiting the growth of various pathogenic bacteria, including *Staphylococcus aureus*. This activity is associated with the presence of bioactive compounds that can compromise bacterial cell membrane integrity and disrupt essential enzymatic systems within the bacterial cell [8][11].

METHODOLOGY

Study design

A total of one hundred and twelve samples were collected from burn wound patients and placed in sterile containers at Medical City, Al-Kindi Teaching Hospital, and Al-Wasiti Hospital in Baghdad, Iraq, from both genders during the period from September to November 2025.

Sample collection and microbiological culture

This study involved culturing clinical samples obtained from burn wounds on appropriate culture media, including Blood Agar and Mannitol Salt Agar, followed by incubation at 37 °C for 24 hours. Bacterial isolates were initially identified based on the morphological characteristics of the colonies, such as color and shape, in addition to microscopic examination using Gram staining, which revealed Gram-positive cocci arranged in clusters. Furthermore, several standard biochemical tests were performed to confirm the identification, including the catalase test and the coagulase test, as well as the ability of the isolates to ferment mannitol on Mannitol Salt Agar. The identification of *Staphylococcus aureus* was further confirmed using additional standard diagnostic methods when necessary.

Antibacterial activity Assay

The antibacterial activity of *Taraxacum officinale* extract and zinc oxide nanoparticles (ZnO NPs) against *Staphylococcus aureus* isolates was evaluated using the agar well diffusion method on Mueller–Hinton agar plates. Bacterial suspensions were prepared and adjusted to 0.5 McFarland standard, then evenly spread over the surface of the agar plates. Wells with a diameter of approximately 6–7 mm were made in the agar, and different concentrations of the plant extract and ZnO nanoparticles were added to the wells. The plates were incubated at 37 °C for 24 hours, after which the diameters of inhibition zones (mm) were measured to assess the antibacterial activity.

Ethical Approval

The present study was conducted in accordance with the ethical principles of medical research based on the Declaration of Helsinki. Informed consent was obtained from the patients prior to sample collection. The study protocol was approved by the relevant institutional ethics committee, and all clinical samples were handled confidentially and used solely for scientific research purposes.

Molecular Detection of Resistance and Virulence Genes

Molecular analysis was performed to detect the *mecA*, *icaD*, and *crtN* genes in *Staphylococcus aureus*. Bacterial DNA was extracted using commercial kits, and PCR was carried out with gene-specific primers under standard thermal cycling conditions. PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with Ethidium bromide, and visualized under UV light using a gel documentation system [16].

Preparation of Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles (ZnO NPs) were used in this study to investigate their antibacterial effect. The nanoparticle suspension was prepared at different concentrations using sterile distilled water. The suspension was then subjected to ultrasonication to achieve a homogeneous dispersion of the nanoparticles prior to their use in the antibacterial assays [15].

Preparation of *Taraxacum officinale* Extract

Wild *Taraxacum officinale* plants were collected from roadsides and abandoned gardens in Baghdad, Iraq, during the study period. The plant was identified and its botanical identity was confirmed at the Department of Biology, College of Science, University of Baghdad. The plant material was air-dried at room temperature and then ground into a fine powder. The plant extract was prepared using the solvent extraction method, in which a measured amount of plant powder was mixed with the solvent (70% ethanol) and left for a specific period with continuous agitation. The extract was subsequently filtered and concentrated using a rotary evaporator to obtain the crude extract. Finally, the extract was stored at a low temperature until further use in the antibacterial assays [9][11].

Phytochemical Analysis of *T. officinale* Extract Using HPLC

The bioactive compounds in the *Taraxacum officinale* extract were analyzed using High-Performance Liquid Chromatography (HPLC). Prior to analysis, the extract was filtered through a 0.45 µm membrane filter. The HPLC analysis was performed using an HPLC system equipped with a UV detector and a C18 reverse-phase column. The mobile phase consisted of a mixture of methanol and water at an appropriate ratio with a flow rate of 1 mL/min, and the injection volume was 20 µL. Chromatograms were recorded at a suitable wavelength to detect phenolic and flavonoid compounds. Compounds were identified based on their retention times and by comparing the peaks with standard references of known compounds, in order to determine the bioactive constituents potentially responsible for the antibacterial activity of the plant extract [17].

Evaluation of Antibacterial Activity

The antibacterial activity of both zinc oxide nanoparticles and *Taraxacum officinale* extract was evaluated against *Staphylococcus aureus* isolates. The agar well diffusion method was used, in which wells were made in Mueller-Hinton agar plates inoculated with the bacterial suspension, and specific volumes of the plant extract or nanoparticle suspension were added into the wells. The plates were incubated at 37°C for 24 hours, after which the diameters of inhibition zones around the wells were measured to assess the effectiveness of the tested agents against the bacterial isolates [3].

RESULTS AND DISCUSSION

Isolation and Identification of *Staphylococcus aureus*

A total of 112 clinical samples were collected from burn patients from various body sites. Bacterial culture on selective and differential media yielded 52 bacterial isolates. Some isolates exhibited characteristic morphological features on Mannitol Salt Agar, producing yellow colonies due to mannitol fermentation, while on Blood Agar, they formed smooth, circular colonies with beta-hemolysis. Microscopic examination revealed that the bacterial cells were Gram-positive cocci arranged in clusters. The isolates also tested positive for Catalase, Coagulase, and DNase tests, confirming their identity as *Staphylococcus aureus*, as shown in Table 1. Identification was further confirmed using the VITEK 2 system.

Table (1): Morphological characteristics of *Staphylococcus aureus* isolated from burn infections.

Test	Result
Gram stain	Gram-positive cocci in clusters
Catalase test	Positive

Coagulase test	Positive
DNase test	Positive
Mannitol fermentation	Positive

These results demonstrate the feasibility of isolating and identifying *Staphylococcus aureus* from burn wounds using both phenotypic and molecular methods, which is consistent with numerous studies indicating that this bacterium is one of the most common pathogens associated with burn infections. This is attributed to its ability to colonize damaged skin and tissues, as well as its possession of a wide range of virulence factors that enable it to initiate and sustain infection [(19)]. Moreover, the moist and nutrient-rich environment of burn wounds provides a suitable medium for the growth and proliferation of this bacterium, increasing the likelihood of secondary bacterial infections in burn patients [(5)].

Antibiotic Susceptibility Testing

The susceptibility of *Staphylococcus aureus* isolates to ten antibiotics was tested using the Kirby–Bauer disk diffusion method according to CLSI guidelines. The results showed varying levels of sensitivity and resistance among the bacterial isolates, with some exhibiting multidrug resistance, as presented in Table 2.

Table (2): Antibiotic susceptibility patterns of *Staphylococcus aureus* isolates.

Antibiotic	Code	Susceptibility(%)	Resistance (%)
Penicillin G	PEN	0.0%	100%
Methicillin	MET	0.0%	100%
Azithromycin	AZM	44.0%	54.0%
Amoxicillin	AMX	0.0%	100%
Levofloxacin	LEV	75.0%	25.0%
Gentamicin	GEN	65.38%	26.92%
Vancomycin	VAN	92.30%	5.76%
Ceftriaxone	CRO	13.0%	75.0%
Clindamycin	DA	69.23%	13.46%
Chloramphenicol	CHL	86.53%	11.53%

These findings indicate different resistance patterns among the *Staphylococcus aureus* isolates, with some resistant to multiple antibiotics. This result is consistent with recent studies reporting a continuous increase in antibiotic resistance rates in *Staph. aureus*, particularly methicillin-resistant strains (MRSA), which pose a significant challenge for the treatment of bacterial infections in hospitals [(10)]. The emergence of such resistance is associated with specific genes, such as *mecA*, which confers resistance to β-lactam antibiotics through the production of low-affinity penicillin-binding proteins [(7)].

Molecular Detection of Target Genes

Polymerase chain reaction (PCR) was used to detect resistance- and virulence-associated genes in *Staphylococcus aureus* isolates. The results revealed the presence of the *mecA* gene in all isolates, indicating that all isolates were methicillin-resistant (MRSA), reflecting the widespread prevalence of this resistance type among the burn wound isolates. Some isolates also carried the *icaD* and *crtN* genes at varying frequencies, as shown in Table 3 and Figure 1, indicating their potential for biofilm formation and carotenoid pigment production.

Table (3): Distribution of detected genes in *Staphylococcus aureus* isolates.

Gene	Function	No. of tested isolates	No. of positive isolates	Percentage (%)
mecA	Methicillin resistance	52	52	100%
icaD	Biofilm formation	52	41	78.8%
crtN	Staphyloxanthin production	52	45	86.5%

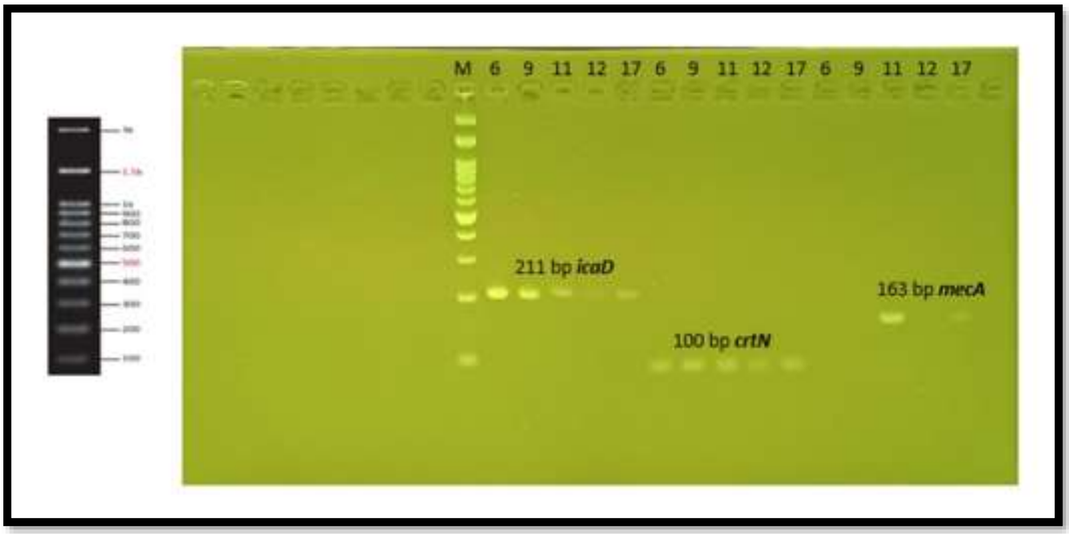

Figure (1): Agarose gel electrophoresis of PCR products for *mecA*, *icaD*, and *crtN* genes.

The molecular analysis in this study confirmed the presence of *mecA*, *icaD*, and *crtN* in some bacterial isolates, suggesting that the *Staph*. *aureus* strains possess a combination of virulence and resistance factors that may enhance their pathogenic potential [14].
Furthermore, *16S rRNA* gene sequencing and phylogenetic analysis revealed a distinct genetic variation in one of the studied isolates compared to previously registered strains in global databases, as shown in Figure 2. This isolate represents a novel strain of *Staphylococcus aureus* and has been registered in the NCBI GenBank under the accession number PZ028331 by the researcher of this study (Al-Qaisy, D.M.). This represents the first recording of this strain in Iraq and globally, highlighting the genetic diversity and geographical distribution of *Staph*. *aureus* and emphasizing the importance of molecular surveillance. This discovery may contribute to the development of new strategies for prevention and treatment.

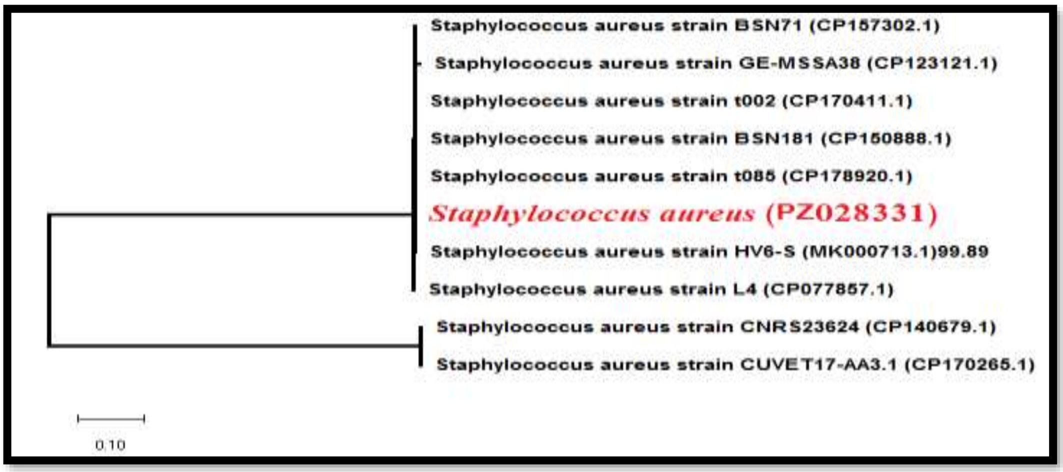

Figure (2): Phylogenetic tree of *Staphylococcus aureus* isolates based on gene sequence analysis.

Antibacterial Activity of ZnO NPs

Zinc oxide nanoparticles (ZnO NPs) exhibited significant inhibitory activity against *Staphylococcus aureus* isolates. The inhibitory effect increased with higher concentrations of the nanoparticles, as shown in Table 4.

Table (4): Diameters of inhibition zones produced by Zinc Oxide Nanoparticles (ZnO NPs).

Nanomaterial Concentration	Inhibition Zone Diameter (mm)
100%	38
75%	34
50%	30
25%	29

These findings are consistent with several studies reporting that metallic nanoparticles possess broad-spectrum antimicrobial properties. The antibacterial mechanism of ZnO nanoparticles is believed to involve the generation of reactive oxygen species (ROS), as well as damage to the bacterial cell wall and membrane, ultimately leading to growth inhibition or bacterial cell death [15][21].

HPlC analysis of the *T. officinale* Extract

HPLC analysis of the *Taraxacum officinale* extract revealed the presence of several phenolic and flavonoid compounds believed to be responsible for the plant's bioactivity. These compounds were identified based on their retention times and comparison with standard references. The chromatogram also showed several major peaks indicating the presence of bioactive plant compounds that may contribute to the antibacterial activity of the extract used in this study, as presented in Table 5 and Figure 3.

Table (5): Principal chemical compounds detected via HPLC technique

Compound	Retention Time	Peak Area	Concentration (ppm)
Caffeic acid	11.87	1650.98	98.8
Apigenin	4.25	1411.14	74.6
Quercetin	5.90	1987.07	124.6
Rutin	7.08	2145.87	99.8
Gallic acid	8.25	1887.98	162.6
Ferulic acid	3.05	1874.08	71.6
Kaempferol	10.15	2032.65	52.6

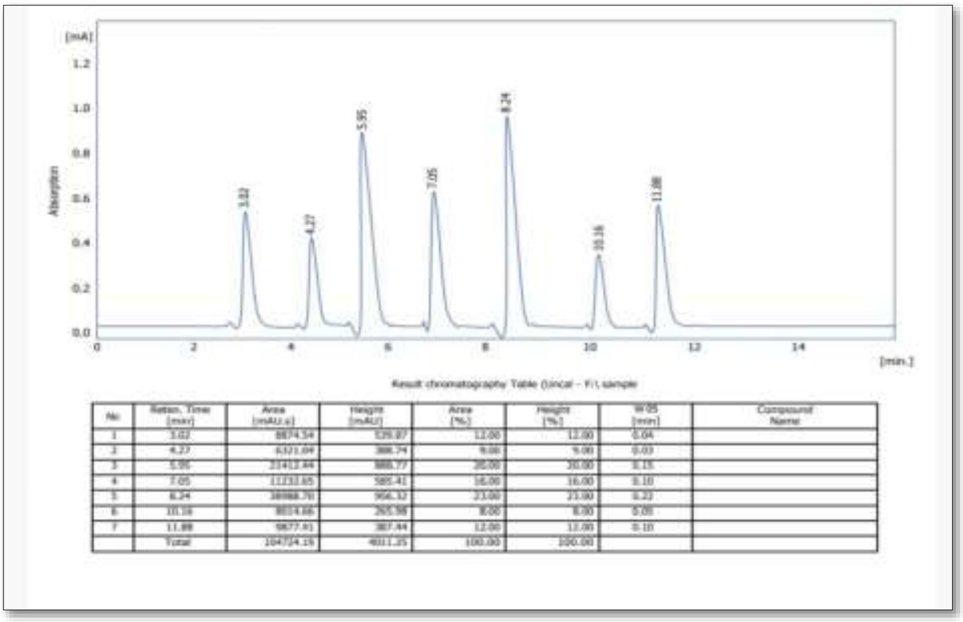

Figure (3): HPLC chromatogram of phenolic compounds identified in *Taraxacum officinale* extract.

The HPLC results in this study confirmed the presence of several bioactive compounds in the plant extract, which may explain the observed inhibitory effect of the extract against the tested bacteria [(2)].

Antibacterial Activity of *T. officinale* Extract

The *Taraxacum officinale* extract exhibited antibacterial activity against *Staphylococcus aureus* isolates. The diameters of the inhibition zones varied depending on the concentration of the extract used, as shown in Table 6 and Figure 4.

Table (6): Inhibition zone diameters produced by *Taraxacum officinale* (Dandelion) extract

Plant Extract Concentration	Inhibition Zone Diameter (mm)
100%	30
75%	27
50%	25
25%	21

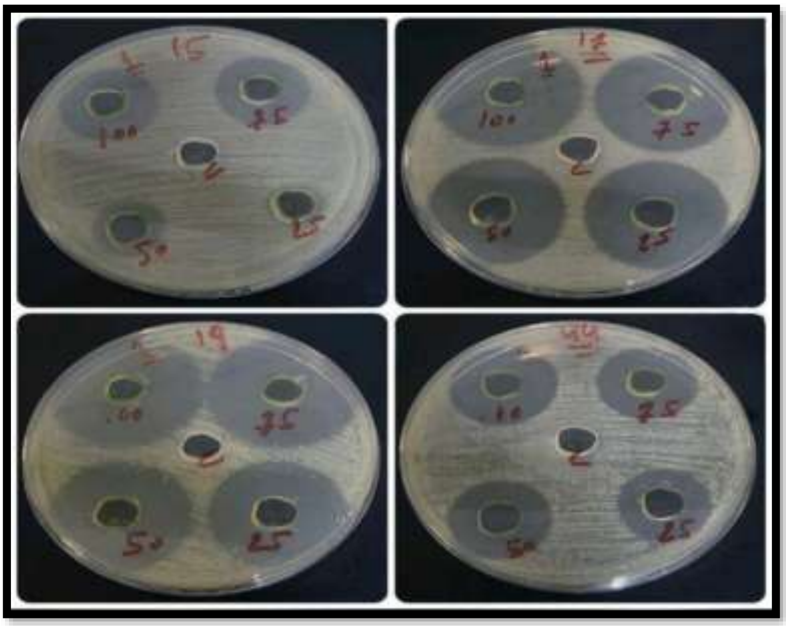

Figure (4): Inhibition zones against Staphylococcus aureus following treatment with *Taraxacum officinale* (Dandelion) extract.

These results indicate that *T. officinale* extract possesses antibacterial activity against *Staph. aureus* isolates. This activity can be attributed to the presence of various bioactive compounds in the plant, such as phenolic compounds and flavonoids, which have antioxidant and antimicrobial properties. These findings are consistent with previous studies reporting that *Taraxacum* extracts exhibit antibacterial activity against a range of human pathogens [(8)(17)(1)(11)].

CONCLUSION

1. *Staphylococcus aureus* was successfully isolated and identified from burn wound infections using both phenotypic methods and molecular techniques.
2. The results revealed the presence of *mecA*, *icaD*, and *crtN* genes associated with antibiotic resistance and virulence in some bacterial isolates.
3. Antibiotic susceptibility testing showed variability in resistance among the isolates, indicating the prevalence of resistant strains in the clinical samples.
4. Zinc oxide nanoparticles and *T. officinale* extract exhibited significant inhibitory activity against the studied isolates, suggesting their potential use as adjuvant or alternative agents to antibiotics in combating resistant bacteria. Further studies are recommended to evaluate the therapeutic efficacy of these agents in medical applications.
5. The discovery of a distinctive *Staph. aureus* strain in this study highlights the importance of molecular investigations in understanding the genetic diversity and geographical distribution of pathogenic bacteria.

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