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Molecular Evaluation of Clove Extract-synthesized Iron Nanoparticles and Their Impact on hla Gene Expression in Staphylococcus aureus Isolates from Athletes: A review

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Abstract

The increasing incidence of Staphylococcus aureus infections in athletes has attracted a great deal of attention, as these populations are at higher risk for skin trauma, shared equipment and intense physical contact that favor bacterial colonization and person-to-person transmission. Specifically, the major virulence factor for S. aureus is alpha-hemolysin which is encoded by *hla* gene and a pore forming toxin that leads to tissue damage and an enhanced capability to cause disease. Concurrently, an upcoming utilized processing method of green-synthesized nanoparticles especially iron nanoparticles derived from clove (Syzygium aromaticum) extract have been displayed as a sustainable antimicrobial alternative. The molecular effect of iron nanoparticles synthesized from clove extract on *hla* gene expression reported S. aureus isolates among athletes. The plant phytochemicals cap and stabilize iron nanoparticles during clove-mediated synthesis which is believed to result in stronger antibacterial and anti-virulence activity. Current evidence suggests that such nanoparticles interfere with cell membrane integrity, they induce oxidative stress, disrupt the regulatory signaling pathways and may modulate *hla* expression. This inhibition of alpha-hemolysin represents a non-antibiotic approach to controlling virulence expression in athletic environment, which may further reduce the potential spread of infection. Standardization of nanoparticle synthesis, mechanisms for long-term molecular interactions and clinical applications require further research.

Keywords: Staphylococcus aureus, athletes, *hla* gene, iron nanoparticles, clove extract, virulence regulation

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Introduction

Staphylococcus aureus is one of the most frequent bacterial pathogens involved in cutaneous and soft-tissue infections, especially bothersome in athletic populations that are repeatedly exposed to situations promoting bacterial colonization. Increased risk of S. aureus transmission, including methicillin-resistant (MRSA), due to crowding and close contact[1]. Virulence determinants such as the alpha-hemolysin toxin encoded by the *hla* gene have a significant impact on the severity of these infections. In addition to environmental factors such as oxidative stress, nutrient availability, and iron concentration, this toxin acts by the formation of pores in host cell membranes leading to cytotoxicity, immune evasion and tissue destruction making *hla* a key molecular factor for pathogenesis[2].

Nanotechnology has recently emerged as a viable strategy to fight bacterial infections and target virulence pathways, especially iron oxide nanoparticles (IONPs) due to their biocompatible, low cost and eco-friendly properties[3]. Plant-extract–mediated biosynthesis of nanoparticles is a promising green synthesis method because plant extracts contain many bioactive compounds like eugenol and flavonoids that serve as natural reducing and stabilizing agents, generate high stability, and produce biologically active iron nanoparticles[4]. To synthesize iron nanoparticles using different resources will bring great benefits for green nanotechnology approach in treatment process.

These iron nanoparticles produced from cloves have potential as an antibacterial agent and could affect virulence mechanisms in bacteria, such as the *hla* gene that is significant for the pathogenicity of *Staphylococcus aureus*[5]. Current evidence suggests that the potential pathways through which these nanoparticles might modulate genes that encode for bacterial communication (quorum sensing) as well as toxin production and the virulence regulation in general. Thus, this review discusses epidemiology of S. aureus in athletes, synthesis and properties of clove-derived iron nanoparticles, their antibacterial mechanisms, the molecular pathways involved in *hla* transcription regulation, followed by evidence-based records for potential nanoparticle-induced downregulation of virulence determinants, whilst concluding with prospects to utilize them as alternative or adjunctive measures to control *S. aureus* infections arising from sporting contexts[6].

Review methodology

A comprehensive literature search was carried out in major scientific databases (PubMed, Scopus, Google Scholar and ScienceDirect) between 2019 and 2025 pertaining to Staphylococcus aureus, *hla* gene expression, iron oxide nanoparticles, green synthesis, clove (Syzygium aromaticum) extract antimicrobial nanoparticles bacterial virulence regulation as well as athlete-associated infections.

The following keywords and their combinations were entered throughout the search process: Staphylococcus aureus, *hla* gene, alpha-hemolysin, iron oxide nanoparticles, green synthesis, clove extract, Syzygium aromaticum, virulence genes, MRSA, antimicrobial nanoparticles, athletes.

Only relevant peer-reviewed publications written in English were included based on their relevance to nanoparticle synthesis, antibacterial activity, virulence regulation, and molecular mechanisms associated

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with *hla* expression. Studies without sufficient methodological detail, articles not related to bacterial virulence, duplicate publications.

The current evidence supporting the anti-virulence effect of clove extract-synthesized iron nanoparticles on Staphylococcus aureus, with specific emphasis on *hla* gene regulation and athlete-associated infections were critically reviewed and summarized.

Overview and Epidemiology of Staphylococcus aureus Among Athletes

Staphylococcus aureus infections have become increasingly prevalent in athletes, especially in contact sports such as wrestling, judo, football and basketball. Athletes have been highlighted as an at-risk group by multiple epidemiological surveys due to repetitive skin abrasions, contact with contaminated surfaces and increased microbial transmission via shared equipment and locker-room environments. This setting favors skin and nasal colonization, leading to recurrent infections[7].

The prevalence of nasal carriage of S. aureus in athletes varied from 30%–60%, which is a higher ratio than that in general population according to previous Studies. The alarming rates of methicillin-resistant S. aureus (MRSA) strains in the contact sports setting posed major public health concerns because of treatment difficulties and rapid spread. Current studies indicate that athletes with a significant amount of time spent in high-density training camps are more likely to acquire MRSA as recreational athletes[8].

The virulence genes, particular *hla* have been closely associated with the severity of infection and repeat outbreaks in sports teams. These results underscore the importance of alternative antimicrobials strategies, in environments where antibiotic misuse or overuse[2].

Pathophysiology and Virulence Mechanisms of *S. aureus*

Staphylococcus aureus harbors an array of virulence factors that allow it to adhere, invade and damage host cells and tissues. Of these virulence components, the alpha-hemolysin encoded by the *hla* gene is especially important because of its key role in determining bacterial pathogenesis[9].

Role of the *hla* Gene and Alpha-Hemolysin

Alpha-hemolysin is a secreted, pore-forming cytotoxin produced by Staphylococcus aureus that assembles into heptameric complexes on the host cell membrane and lyse cells through transmembrane pores resulting from imbalance in osmosis[9]. This toxin is an important factor in the pathogenesis of infection because it causes tissue damage to epithelial and endothelial tissues, allows expression of molecular and invasion in various types of immune cells, assists biofilm formation, aids bacterial colonization processes after intracellular delivery within the host cells, and also raises severity of skin and soft-tissue infections[10]. The expression of the *hla* gene, encoding alpha-hemolysin, is tightly regulated by multiple regulatory systems including quorum-sensing mechanisms such as the agr system, oxidative stress and bacterial cell density. Higher levels of *hla* expression have been shown to correlate with more aggressive and invasive infections, especially in settings of repetitive skin trauma such as those seen among athletes[11].

Clove Extract as a Green Reducing Agent for Nanoparticle Synthesis

Green nanotechnology relies on using plant extracts as natural reducing and stabilizing agents. Due to their high concentration of phenolic compounds, clove (*Syzygium aromaticum*) is one of the most potent botanical sources available for nanoparticle biosynthesis[12].

Phytochemical Composition of Clove Extract

Clove buds are a well-known source of several bioactive compounds, the main active compound is eugenol along with several others, such as flavonoids, tannins, phenolic acids and terpenoids. In the case of nanoparticle synthesis, these constitute natural reducing agents that convert metal ions into nanoparticles, promote nucleation and growth. Moreover, they serve as stabilizing agents (inhibition of particle aggregation) and capping agent to enhance the biological activity of nanoparticles formed[13].

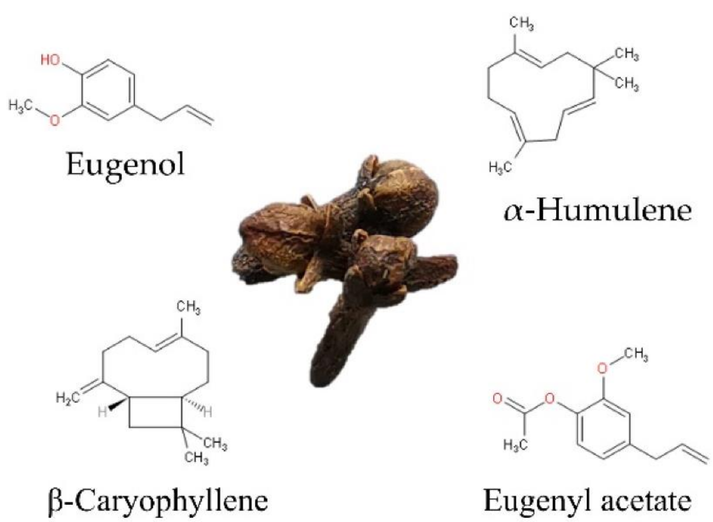

Figure 1. Major Phytochemical Constituents of Clove (*Syzygium aromaticum*)

Advantages of Clove-Mediated Nanoparticles

Iron oxide nanoparticles clove-mediated are a convenient and safe. They show more antimicrobial activity and better biocompatibility which makes them good candidates for biomedical applications. Moreover, these nanoparticles retain stable particle morphology and ensure uniformity and high efficiency. With a distinctive surface chemistry that improves their reactivity towards bacterial cells, they represent a new opportunity to interfere with virulence pathways[14].

Synthesis and Characterization of Iron Oxide Nanoparticles Using Clove Extract

Iron nanoparticles are commonly synthesized using green co-precipitation or hydrothermal methods using clove extract to synthesize iron nanoparticles. In this process, eugenol and other phytochemicals in the extract reduce Fe^{2+} and Fe^{3+} salts. "This process promotes nanoparticle nucleation and growth, leading to the formation of stable Fe_3O_4 particles. In addition, bioactive compounds of clove act as stabilizing agents that cover the surface of nanoparticles thus increasing stabilization[15].

Typical Characteristics

The synthesized nanoparticles typically range in size from 10 to 60 nm, depending on the synthesis conditions, and they commonly exhibit a spherical or nearly spherical shape. They possess a superparamagnetic nature and have a surface chemistry rich in functional groups such as hydroxyl (OH) groups and aromatic rings. These physicochemical properties play a crucial role in enhancing their antibacterial activity as well as their potential effects on gene regulation[١٦].

Antibacterial Activity of Clove-Synthesized Iron Nanoparticles

Iron oxide nanoparticles have also exhibited broad-spectrum antibacterial action, which can be enhanced through synergistic effect when synthesized with clove extract[17].

Generation of Reactive Oxygen Species (ROS)

Fe_3O_4 nanoparticles may catalyze Fenton-like reactions that generate reactive oxygen species (ROS), especially hydroxyl radicals. These reactive species then damage critical cellular targets such as DNA, proteins, lipids and cell membranes eventually leading to rapid bacterial cell death[18].

Disruption of Cell Membrane Integrity

Nanoparticle interaction with bacterial cells leads to several structural and functional disruptions, including membrane thinning and pore formation. This results in the leakage of cytoplasmic contents and ultimately causes a collapse of the proton motive force, compromising cellular integrity and leading to cell death[19].

Interference with Biofilm Formation

Nanoparticles prevent bacterial adhesion to surfaces and inhibit early biofilm development critical in sports equipment contamination[20].

Inhibition of Essential Metabolic Pathways

Iron nanoparticles can bind to proteins and enzymes essential for bacterial survival[12].

Molecular Impact on *hla* Gene Expression

The most of findings in the previous studies indicate that iron oxide nanoparticles may not just inhibit bacterial growth but also affect S. aureus virulence through the induction of oxidative stress and disruption of bacterial regulatory pathways. The *hla* gene, encoding α-hemolysin, is primarily regulated by global regulators including the Agr quorum-sensing system and SarA. Thus, nanoparticle-induced ROS, membrane damage and metabolic stress may indirectly down-regulate *hla* transcription and α-hemolysin production. Several anti-virulence effects have been documented for metal-based nanoparticles, including that nanoparticles are able to reduce the production of α-hemolysin and the expression virulence-related genes in S. aureus[21].

Table 1: proposed mechanism of clove-mediated iron nanoparticles against staphylococcus aureus virulence.

Proposed mechanism	**Expected biological effect**
Reactive oxygen species	Oxidative damage to the components of bacterial cell
Disruption of membrane integrity	Increased the permeability of membrane
Interference with agr quorum-sensing system	Reduce the production of toxin and altered the virulence regulation
Downregulation of hla gene expression	Reduce alpha-hemolysin
Inhibition of biofilm formation	Reduced bacterial adhesion and colonization
Metabolic stress induction	Bacterial survival pathways impairment
Surface interaction with bacterial cells	Enhance antibacterial activity

Relevance to Athlete-Associated Infections

Due to skin abrasions, the use of shared gym facilities and equipment, inadequate hygiene before are during training (particularly in team sports), heavy sweating and humidity within athletics environments as well as repeated close physical contact, athletes face increased risk of bacterial infections. suppression of virulence factors like *hla*, which may reduce wound severity and reduces the likelihood of secondary infection or spread within team members[17]. Consequently, targeting the *hla* gene with Clove-iron nanoparticles could be further explored to provide a non-antibiotic treatment option, which is particularly relevant considering the increase in MRSA worldwide.

Conclusion

Clove extract-synthesized iron nanoparticles have emerged as an innovative alternative to Target Staphylococcus aureus Infections in Athletes. However, methicillin-resistant S. aureus has proliferated extremely quickly, and MRSA poses an increasing challenge to conventional antibiotic therapy. Thus, in recent years significant efforts have been directed towards alternative strategies that target bacterial virulence factors directly rather than bacterial viability itself.

In this context, the *hla* gene encoding alpha-hemolysin is regarded as a major virulence determinant related to tissue injury, increased colonization and infection severity. Early indications suggest that iron oxide nanoparticles may be involved in the modulation of *hla* expression, reducing bacterial pathogenicity and lacking a selective pressure to induce antibiotic resistance.

The magnetic properties of the Fe_3O_4 nanoparticles may also complement their antimicrobial potential and support the development of novel translational biomedical applications such as targeted drug delivery, antimicrobial wound dressings, and nanoparticle surface coatings on sports equipment to minimize bacterial transmission in physical activity setting.

In Summary, current findings suggest clove-mediated iron nanoparticles may represent a new complementary approach to antibiotic treatment for the modulation of S. aureus virulence. However, further studies are necessary to standardize nanoparticle synthesis, assess long-term toxicity and biocompatibility, and elucidate their molecular mechanisms on virulence genes regulation in clinical and sport environments.

Conflict of interest

The authors declare no conflict of interest.

Findings

No external fundings was received for this study.

Data Availability

No datasets were generated or analyzed during the current study

Author’s Declaration

- Abeer Hameed Abbas: Conducted the literature search, organized and analyzed the collected studies, and drafted the initial version of the manuscript.
- Noora A. Hadi: Contributed to the conceptualization and design of the review, supervised the work, critically revised the manuscript, and approved the final version.

References

1- Pidwill, G. R., Gibson, F.L, Cole, J., Renshaw, S. A., & Foster, S. J. (2021). The role of macrophages in *Staphylococcus aureus* infection. *Frontiers in Immunology*, (11), 620339.

2- Divyakolu, S., Chikkala, R., Ratnakar, K., & Sritharan, V. (2019). Hemolysins of *Staphylococcus aureus*: An update on their biology, role in pathogenesis and as targets for anti-virulence therapy. *Advances in Infectious Diseases, 9(2),* 80–104.

3- Mohan, A., Rajendran, S., Palani, N., Syed Altaf, R. R., Mendonce, K. C., Nithya, T. G., Nagendran, S., Surya, P., Srinivasan, M., Kannan, R., Sundaresan, M., & Rajadesingu, R. (2025). Sustainable synthesis of iron oxide nanoparticles using phytochemicals: Mechanisms, functionalization strategies, applications and future perspectives. *Materials Science and Engineering International Journal, 9*(2).

4- Mohamed, A., Atta, R. R., Kotp, A. A., Abo El-Ela, F. I., Abd El-Raheem, H., Farghali, A., Alkhalifah, D. H. M., Hozzein, W. N., & Mahmoud, R. (2023). Green synthesis and characterization of iron oxide nanoparticles for the removal of heavy metals (Cd^{2+} and Ni^{2+}) from aqueous solutions with antimicrobial investigation. *Scientific Reports, (13),* 10112.

5- Butrico, C. E., & Cassat, J. E. (2020). Quorum sensing and toxin production in *Staphylococcus aureus* osteomyelitis: Pathogenesis and paradox. *Toxins, 12*(8), 516.

6- Wójcik-Bojek, U., Rózalska, B., & Sadowska, B. (2022). *Staphylococcus aureus*: A known opponent against host defense mechanisms and vaccine development—Do we still have a chance to win? *International Journal of Molecular Sciences, 23*(2), 948.

7- Jiménez-Truque, N., Saye, E. J., Soper, N., Saville, B. R., Thomsen, I., Edwards, K. M., & Creech, C. B. (2017). Association between contact sports and colonization with *Staphylococcus aureus* in a prospective cohort of collegiate athletes. *Sports Medicine, 47*(5), 1011–1019.

8- Karanika, S., Kinamon, T., Grigoras, C., & Mylonakis, E. (2016). Colonization with methicillin-resistant *Staphylococcus aureus* and risk for infection among asymptomatic athletes: A systematic review and meta-analysis. *Clinical Infectious Diseases, 63*(2), 195–204.

9- Berube, B. J., & Bubeck Wardenburg, J. (2013). *Staphylococcus aureus* α-toxin: Nearly a century of intrigue. *Toxins, 5*(6), 1140–1166.

10- Otto, M. (2014). *Staphylococcus aureus* toxins. *Current Opinion in Microbiology, (17)*, 32–37.

11- Tavares, A., Nielsen, J. B., Boye, K., Rohde, S., Paulo, A. C., Westh, H., Schønning, K., de Lencastre, H., & Miragaia, M. (2014). Insights into alpha-hemolysin (Hla) evolution and expression among *Staphylococcus aureus* clones with hospital and community origin. *PLoS ONE, 9*(7), e98634.

12- Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022). Green nanotechnology: Plant-mediated nanoparticle synthesis and application. *Nanomaterials, 12*(4), 673.

13- Edis, Z., Haj Bloukh, S., Ashames, A. A., Al-Tabakha, M. M., Shahwan, M. J. S. A., Abu Sara, H., Boddu, S. H. S., Khan, S. N., Bloukh, I. H., Eladdasy, M., Sadeghi, S., Alkubaisi, H., Bloukh, I. H., & Hassan, N. A. G. M. (2025). *Syzygium aromaticum* extract mediated, sustainable silver nanoparticle synergetic with heterocyclic antibiotic clarithromycin and their antimicrobial activities. *Frontiers in Chemistry, (13*), 1513150.

14- Zhang, Y., Wang, Y., Zhu, X., Cao, P., Wei, S., & Lu, Y. (2017). Antibacterial and antibiofilm activities of eugenol from essential oil of *Syzygium aromaticum* (L.) Merr. & L. M. Perry (clove) leaf against periodontal pathogen *Porphyromonas gingivalis*. *Microbial Pathogenesis, (113*), 396–402.

15- Yadav, J., Chauhan, P., Rawat, R., Pathak, S., & Srivastava, S. (2024). *Syzygium aromaticum*-mediated green synthesis of iron oxide nanoparticles for efficient heavy metal removal from aqueous solutions. *Journal of the Indian Chemical Society, (101*), 101201.

16- Attia, N., El-Monaem, E., El-Aqapa, H., Elashery, S., Eltaweil, A., Kady, M., Khalifa, S., Hawash, H., & El-Seedi, H. (2022). Iron oxide nanoparticles and their pharmaceutical applications. *Applied Surface Science Advances, (11),* 100284.

17- Singh, H., Desimone, M. F., Pandya, S., Jasani, S., George, N., Adnan, M., Aldarhami, A., Bazaid, A. S., & Alderhami, S. A. (2023). Revisiting the green synthesis of nanoparticles: Uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications. *International Journal of Nanomedicine, 18*, 4727–4750.

18- Rinna, A., Magdolenova, Z., Hudecova, A., Kruszewski, M., Refsnes, M., & Dusinska, M. (2015). Effect of silver nanoparticles on mitogen-activated protein kinases activation: Role of reactive oxygen species and implication in DNA damage. *Mutagenesis, 30*(1), 59–66.

19- Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., & Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters, 7*(3), 219–242.

20- Qayyum, S., & Khan, A. U. (2016). Nanoparticles vs biofilms: A battle against another paradigm of antibiotic resistance. *MedChemComm, 7(2),* 1479–1498.

21- Jabir, M. S., Rashid, T. M., Nayef, U. M., Albukhaty, S., AlMalki, F. A., Albaqami, J., AlYamani, A. A., Taqi, Z. J., & Sulaiman, G. M. (2022). Inhibition of *Staphylococcus aureus* α-hemolysin production using Cur-Au@ZnO nanoparticles. *Bioinorganic Chemistry and Applications, (2022*), 2663812.