The Use of Nanomaterials to Detect Virulence Genes in Staphylococcus Aureus Isolated from Bullous Impetigo, Impetigo and Ecthyma Skin Infections

Nawras Bahaa Manje[1], Abeer Fawzi AL- Rubaay[1], Amal Merza Hasan[2]

[1]Biology Department, College of Science for Women, University Babylon, Babylon Province, Hilla, Iraq
[2]Babylon, Babylon Province, Hilla, Iraq
Email: nawrasbahaa1@gmail.com

Nanobiotechnology is the practical investigation of biological materials and elements at the nano scale. At the nanoscale, everything has a new feature regardless of what it is, and two nanoparticles with different sizes show distinctly different behavior. In nanotechnology, by exploiting new features and phenomena in the length scale of nanometers, new nanoparticles are produced with very high efficiency, which are used to label different biomolecules. Skin diseases are among the infection spread around the world that largely affect children. Skin infections occur when bacteria infect the skin and sometimes the deep tissues of the skin. Staphylococcus aureus is one of the most common types of bacteria that cause skin infections, especially in children, such as impetigo, ecthyma Bullous impetigo, boils, furuncles. A total of 25 S. aureus isolates were molecularly tested for the presence of mecA and spa genes after being collected from Bullous impetigo, Impetigo and Ecthyma skin infections. The aim of this study is to describe the presence of genes encoding virulence factors conferred by the mecA gene in beta-lactam-resistant Staphylococcus aureus bacteria and spa gene isolated from Bullous impetigo, Impetigo and Ecthyma skin infections.

Keywords: Nanomaterials, Staphylococcus aureus, skin infections, mecA gene, spa gene.

1. Introduction

The skin is the largest organ of animal and human bodies, is the outermost and first line of defense against infectious agents and is easily exposed to physical and chemical agents and different pathogens that cause a wide variety of infections and wounds (Mala et al .,2021) . Generally, skin diseases are among the most frequently occurring illnesses in humans. Skin and subcutaneous disorders were the fourth leading cause of nonfatal disease burden

worldwide in thy last decade (Hay et al., 2014; Seth, 2017).

Bacterial skin infections represent one of the major healthcare issues affecting people worldwide (Tognetti et al 2012). S. aureus and S. epidermidis may inhabit the human skin and other microbes(Skowron et al 2021).
Common infections of the skin such as impetigo and scabies represent a large burden of disease globally, being particularly prevalent in tropical and resource-limited settings(Taiaroa et al.,2021). The typical organisms that colonize the skin above the waist are usually Gram-positive species such as Staphylococcus epidermidis, Corynebacterium species, S aureus and Streptococcus pyogenes .

Staphylococcus aureus which can be detected by nanomaterials, is a nosocomial bacterium causing different infectious diseases, ranging from skin and soft tissue infections to more serious and life-threatening infections such as septicaemia . S. aureus forms a complex structure of extracellular polymeric biofilm that provides a fully secured and functional environment for the formation of microcolonies, their sustenance and recolonization of sessile cells after its dispersal (Muhammad et al.2021).

The pathogenesis of S . aureus is caused by many virulence factors and their mechanisms into invasion and inflammation, which include colonization, synthesis of extracellular molecules, which promote adherence, and the ability to avoid host defenses; secreted virulence factors such as toxins (Al-Mebairik et al., 2016). S. aureus causes several forms of human infections and syndromes, especially infections of the skin and soft tissue (Kobayashi et al.2015).
Staphylococcus aureus have thirty-six common virulence genes of S. aureus were detected by PCR amplification, including 11 adhesion associated genes. (bbp, clfA , clfB, can , ebps, eno, fib, fnbA, fnbB, icaA, and icaD),12 enterotoxin genes sea , seb , sec , sed, see, seg, seh, sei, sej , sem, sen, and seo), and 13 other , virulence genes (hla, hlb, hld, hlg, hlgv, lukM, lukED, pvl, psmα, tst, eta, etb and edin) (Jiang et al ., 2017).

The mec gene complex contains one of two genes that have been identified as encoding methicillin resistance . The mec gene complex contains mecA, a gene that encodes an altered penicillin binding protein (PBP2a) in the vast majority of MRSA lineages (Ward et al ., 2016).

The mecA gene is a 2.1 kb exogenous DNA fragment carried on the staphylococcal cassette chromosome mec (SCCmec), a mobile genetic element that inserts at site-specific positions on the staphylococcal chromosome and is acquired through horizontal gene transfer (Piette and Verschraegen, 2009).The identification of the mecA gene is the most reliable method for detecting methicillin-resistant strains of S. aureus (Becker et al ., 2018; Javanshir & Zarepour 2021).

The spa gene is the most widely used for S. aureus typing , which is based on repeats of the hypervariable X region in the spa gene (Mayerhofer et al ., 2015). Spa typing is based on the polymorphism of the gene encoding protein A (spa).

The spa is one of the surface proteins of S. aureus. In addition to being a virulence factor of the bacterium, it is used to determine the specific identity of S. aureus. With the molecular typing of this protein , it is possible to prevent epidemics, reduce the number

of infections , and reduce the cost of nosocomial infections (Foster et al ., 2014). The gene encoding this protein (spa) consists of two regions, one encodes the Fc- binding domain, and the other encodes X region (Harmsen et al. 2003).

2. Material and Methods

1- Demography of the study population's

A total 120 clinical specimens were collected in the current study from patients attending the medical consultation department, dermatology unit at (Marjan Medical City) in Babylon Province. 66.7% infection were in less than 20 years and 15.0% in age between 20-30 years as show in Table (1). The infection rate was in males (40.70%) while it was in females (53.30%).

The results in figure (1) Table (3) show that the higher percentage (28.3%) of sample area were from foot Impetigo (school sores), a common superficial skin infection (Cole and Gazewood, 2007). The first signs of impetigo can usually be seen around the mouth and nose in the form of an itchy reddish rash with small blisters. The blisters are filled with water or pus and burst easily. Once they have burst, yellowish crusts form. These fall off after some time without scarring. As well as on the face, impetigo can occur on the arms and legs (Galli et al.2019).

Table (2) show that less than two third (65%) of types of disease were impetigo as represented in Figure (2).

Table 1: descriptive statistic of age in patients with bacterial skin infections

		Frequency	Percent
Age	Less than 20	80	66.7
	20-30	18	15.0
	31-40	10	8.3
	41-50	8	6.7
	More than 50	4	3.3
	Total	120	100.0
	Min-Max	1-60	
	Mean (SD)	15.98 (15.36)	

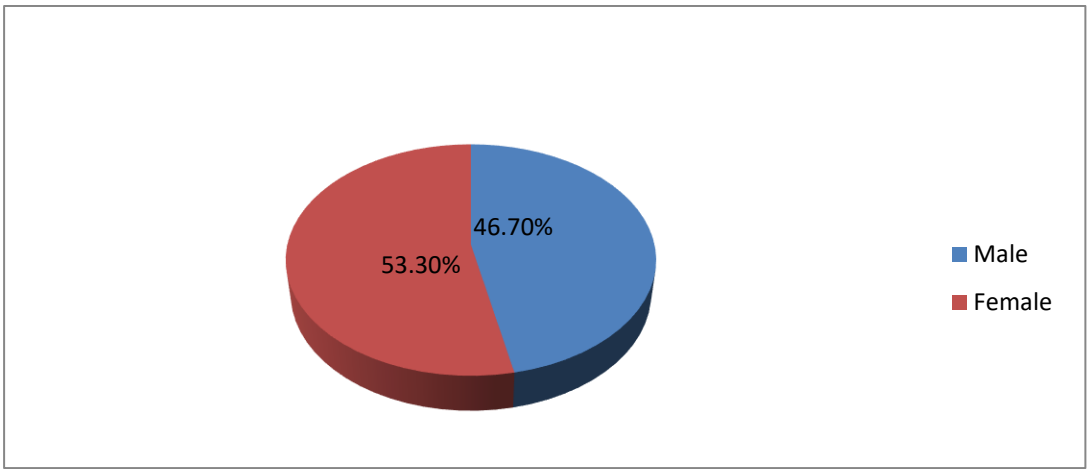

Figure 1: Distribution of gender .

Table 2: Frequency and percentage of types of disease

		Frequency	Percent
Types of disease	Impetigo	78	65.0
	Ecthyma	36	30.0
	Bullous	6	5.0
	Total	120	100.0

Table 3: Frequency and percentage of sample area

		Frequency	Percent
Sample area	Foot	34	28.3
	Face	28	23.3
	Gluteus	8	6.7
	Femoral	22	18.3
	Hand	18	15.0
	Head	10	8.3
	Total	120	100.0

2- Bacterial strains

A (120) skin infection isolates taken from clinical samples were examined by sterile cotton swab from subjects suffering from impetigo from Al-Murjan Hospital in Babil Province between September and October 2022. Demographic data such as gender, age, and sample isolation location were obtained from patients attending the department skin diseases.

All specimens and samples were transferred in sterile transport swabs and inoculated

on culture of selective medium, namely Blood and Mannitol salt agar, using the direct method of inoculation. They were then inoculated at 37°C for 18-24 hours (Cheesbrough,2010).

According to MacFaddin (2000), isolates were identified and diagnoses based on cell and colony shape toward growing bacteria on media, as well as several crucial biochemical assays. Biochemical assays were used to identify suspected bacterial isolates that were Gram – positive, negative to oxidase, and coagulase tests, as well as non-motile.

3- Bacterial DNA extraction:

The S. aureus isolates after being cultured on mannitol salt agar and Brain Heart Infusion broth . The Genomic DNA Extraction Kit (G-spinTM Genomic DNA Extraction Kit) was used for DNA extraction by manufacturers protocol .

3. Results and Discussions

1. Molecular detection of mecA

A total of 25 S. aureus isolates were molecularly tested for the presence of spa genes after being collected from various skin infections

The spa protein, found on the surface of S. aureus bacteria , serves as both a virulence factor and a distinguishing marker for the bacterium. Utilizing molecular typing of this protein can help prevent epidemics, reduce the incidence of illnesses, and decrease the financial burden of nosocomial infections.

The present study involves identifying the specific genes responsible for the resistance to commonly used medications against S. aureus isolates in the country. One such gene is mecA, which plays a role in conferring Methicillin resistance in S. aureus. Methicillin resistance in S. aureus occurs when a penicillin-binding protein, encoded by a chromosome, undergoes a mutation. This type of resistance can be transferred among S. aureus organisms through bacteriophages (Lakhundi et al., 2018).

MRSA is the major cause of nosocomial mortality and morbidity; it is commonly found in the community and hospital environment especially in the ICUs (Montesinos et al., 2002).

In this research, a comprehensive analysis was conducted on 25 S. aureus isolates collected from skin infection (Impetigo). The aim was to detect the presence of mecA genes. Figure (2) visually represents the distribution of these genes in the isolates as percentages, with all the clinical isolates showing a 100% (25) occurrence.

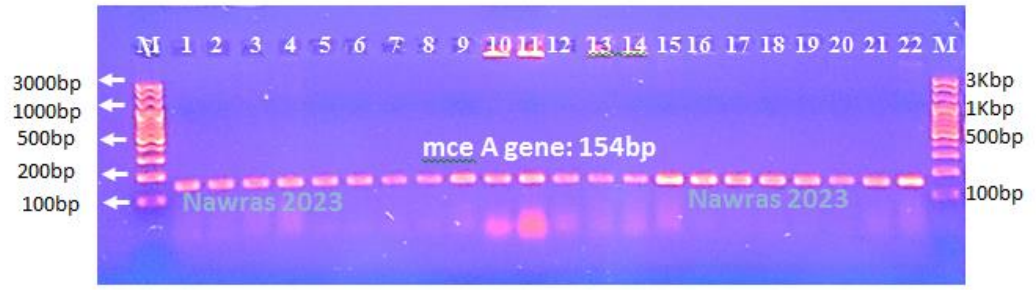

Figure (2) Amplification of mceA gene: : 2% Agarose gel electrophoresis analysis of PCR amplification products of mecA gene of 310 bp, extracted from S. aureus all S.aureus isolate posses mceA gene the electrophoresis was run at 70 volts. lane (L), DNA Molecular Size Marker (100 bp ladder) . Gene mecA produces positive results in all Lanes.

The findings from our study regarding clinical isolates align with the observations made by Yurtsever et al. (2020) and Jowad and Yousif (2013). They reported a 100% presence of the mecA gene in all of their isolates. However, our results differ from those of Alhamadani and Tuwaij (2020) and Koosha et al. (2016), who found a mecA gene ratio of 80% and 87.3%, respectively, in S. aureus isolates.

The results of clinical isolates are comparable to that (Jowad and Yousif , 2013) that found all S. aureus isolates possess spa genes. While this finding differs from that of (Rezashateri et al ., 2021) that showed the spa gene distribution was present in (82%) of the samples and (Kareem et al ., 2020 ; Ali ,2020) who showed that spa gene variation was detected in (63.5 %) of the isolates.

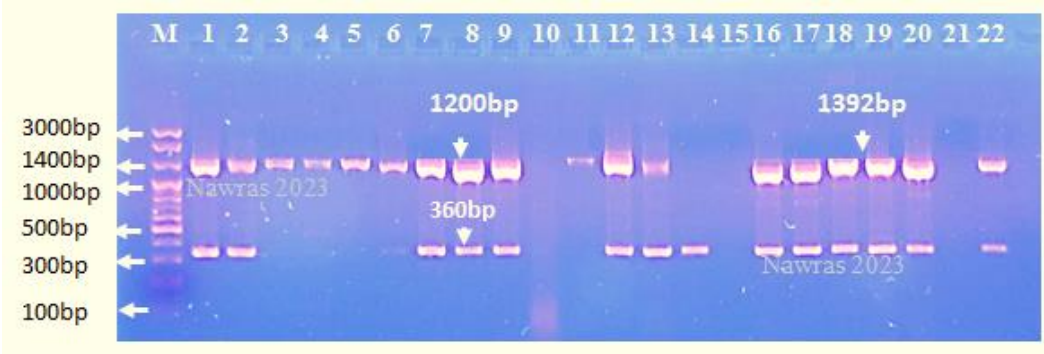

Figure (.3) Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of 25 S. aureus isolates Primer spa gene with Product (350bp). For 1.5 hours , the electrophoresis was run at 70 volts) Gene spa produces positive results in 14 isolates.

2-Molecular Detection of spa Genes

A total of 25 S. aureus isolates were molecularly tested for the presence of spa genes after being collected from various skin infections.

The spa protein, found on the surface of S. aureus bacteria, serves as both a virulence factor and a distinguishing marker for the bacterium. Utilizing molecular typing of this protein can

help prevent epidemics, reduce the incidence of illnesses, and decrease the financial burden of nosocomial infections.

Gene frequency in the isolates under investigation as a percentage:56% (14) clinical isolates as shown in Figure (4).

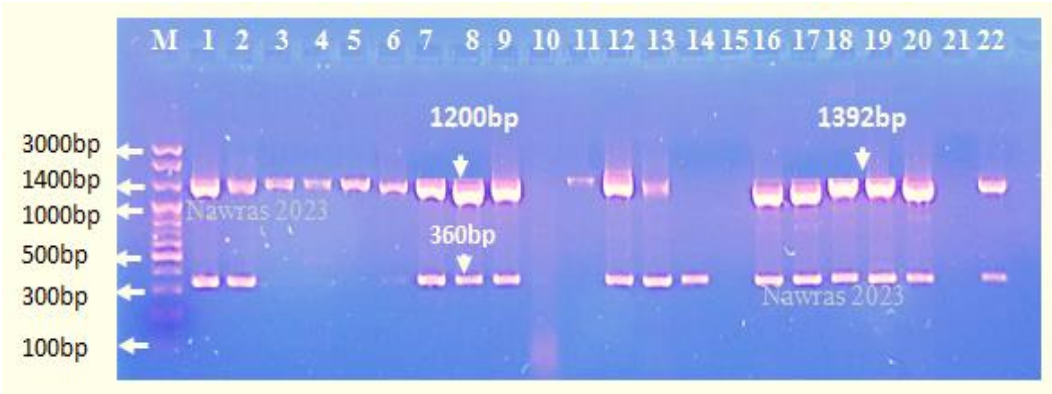

Figure (4) Electrophoresis diagram of Monoplex PCR generated products for extracted DNA of 25 S. aureus isolates Primer spa gene with Product (350bp). For 1.5 hours , the electrophoresis was run at 70 volts) Gene spa produces positive results in 14 isolates

The results of clinical isolates are comparable to that of (Jowad and Yousif, 2013) which found that all S. aureus isolates possess spa genes. While this finding differs from that of (Rezashateri et al ., 2021) that showed the spa gene distribution was present in (82%) of the samples and (Kareem et al ., 2020 ; Ali et al., 2020) who showed that spa gene variation was detected in (63.5 %) of the isolates.

4. Conclusions

The use of gold nanoparticles in the detection of point mutations and isolation and detection of target proteins in genetic diseases can be mentioned. In addition, in RNA interference technology, or RNAi, by designing small RNA biomolecules, pathogenic gene products are specifically targeted. The main findings of this research can be listed as:

1-Detection the virulence factors in clinical isolates of S. aureus using PCR techniques: mecA and Spa gene.

2- Specific genes responsible for resistance to commonly used drugs against Staphylococcus aureus isolates have been identified in the country.

References

1. Alhamadani, R . M . I . and Tuwaij , S.S.N. (2020) . Genetic Diversity of Vancomycin - Resistant Staphylococcus aureus Isolated From Different Clinical Specimens in Al-Najaf Province. M.Sc. Genetics Department / Faculty of Biology/ University of Sofia (2013), Ph.D. in Science/Biology/ Microbiology / University of Kufa (2020) .
2. Al-Mebairik , N. F., El-Kersh, T. A., Al-Sheikh, Y. A., & Marie, M. A. M. (2016). A

review of virulence factors, pathogenesis, and antibiotic resistance in Staphylococcus aureus. Reviews in Medical Microbiology, 27(2), 50-56.

3. ALI, J. F., AL-SAADI, Z. N., & SAEED, Y. S. (2020). Spa Gene Polymorphism Based Molecular Typing of Clinical MRSA Strains Isolated from Wasit City/Iraq. International Journal of Pharmaceutical Research.
4. Becker , K ., van Alen, S., Idelevich, E. A., Schleimer, N., Seggewiß, J., Mellmann, A., ... & Peters, G. (2018). Plasmid-encoded transferable mecB-mediated methicillin resistance in Staphylococcus aureus. Emerging infectious diseases, 24(2), 242.
5. Chessbrough , M. (2010). District Laboratory Practice in Tropica Countries, Part-2, New York, USA, Cambridge University, : 184- 186.
6. Cole, C., & Gazewood, J. D. (2007). Diagnosis and treatment of impetigo. American family physician, 75(6), 859-864.
7. Foster, T. J., Geoghegan, J. A., Ganesh, V. K., & Höök, M. (2014). Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nature reviews microbiology, 12(1), 49-62.
8. Galli L, Venturini E, Bassi A, Gattinara GC, Chiappini E, Defilippi C et al. Common Community-acquired Bacterial Skin and Soft-tissue Infections in Children: an Intersociety Consensus on Impetigo, Abscess, and Cellulitis Treatment. Clin Ther 2019; 41(3): 532-551.e517.
9. Ghorbani Tajandareh , S. and Imani Fooladi, A. A. (2016). Distribution of tsst-1 and mecA Genes in Staphylococcus aureus Isolated From Clinical Specimens. Jundishapur journal of microbiology, 9(3), e29057.
10. Harmsen , D., Claus, H., Witte, W., Rothganger, J., Claus, H., Turnwald, D., & Vogel, U. (2003). Typing of methicillin-resistant Staphylococcus aureus in a university hospital setting by using novel software for spa repeat determination and database management. Journal of clinical microbiology, 41(12), 5442-5448.
11. Hay, R. J., Johns, N. E., Williams, H. C., Bolliger, I. W., Dellavalle, R. P., Margolis, D. J., ... & Naghavi, M. (2014). The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. Journal of Investigative Dermatology, 134(6), 1527-1534.
12. Javanshir I, Zarepour G. (2021). Vibration analysis of fluid-structure interaction using tuned mass dampers. Journal of Applied Engineering Science. 19(2):318-26.
13. Jiang , B., Wang, Y., Feng, Z., Xu, L., Tan, L., Zhao, S., ... & Hu, X. (2017). Panton-Valentine leucocidin (PVL) as a potential indicator for prevalence, duration, and severity of Staphylococcus aureus osteomyelitis. Frontiers in microbiology, 8, 2355.
14. Jowad ,R . M. and Yousif G.M. (2013). Detection of some Virulence Factors and Antibiotic Resistance Genes in Staphylococcus aureus Isolated from Clinical Cases in Al-Diwanyia City. M.S.C. in biology \ College of Science, Al-Qadisiya University.
15. Kareem , S. M.; Aljubori, S. S. and Ali, M. R. (2020). Novel determination of spa gene diversity and its molecular typing among Staphylococcus aureus Iraqi isolates obtained from different clinical samples. New Microbes and New Infections, 34, 100653.
16. Kobayashi S D, Malachowa N, DeLeo F R.,2015, Pathogenesis of Staphylococcus aureus abscesses. The American journal of pathology. 185(6): 1518-1527.
17. Lakhundi , S. and Zhang, K. (2018). Methicillin-Resistant Staphylococcus
18. Mala, L., Lalouckova, K., & Skrivanova, E. (2021). Bacterial Skin Infections in Livestock and Plant-Based Alternatives to Their Antibiotic Treatment. Animals, 11(8), 2473.
19. Montesinos I., Salido E., Delgado T., Cuervo M. and Sierra, A. “Epidemiologic genotyping of methicillin-resistant Staphylococcus aureus by pulsed-field gel electrophoresis at a University Hospital and comparison with antibiotyping and protein A and coagulase gene polymorphisms,” Journal of Clinical Microbiology, vol. 40, no. 6, pp. 2119–2125, 2002

20. Muhammad Idrees , Sheeba Sawant, Nazira Karodia, and Ayesha Rahman.2021. Staphylococcus aureus Biofilm: Morphology, Genetics, Pathogenesis and Treatment Strategies
21. Rezashateri , M.; Ahrabi, M. and Salehi, M. (2021). Molecular Analysis of the Presence of pvl, spa, and mecA Genes and Their Correlation with a Range of Antibiotics in Staphylococcus aureus Collected from Burn Patients. Iranian Journal of Medical Microbiology, 15(6), 625-637.
22. Skowron , K., Bauza-Kaszewska, J., Kraszewska, Z., Wiktorczyk-Kapischke, N., Grudlewska-Buda, K., Kwiecińska-Piróg, J., ... & Gospodarek-Komkowska, E. (2021). Human skin microbiome: impact of intrinsic and extrinsic factors on skin microbiota. Microorganisms, 9(3), 543.
23. Taiaroa , G., Matalavea, B., Tafuna'i, M., Lacey, J. A., Price, D. J., Isaia, L., ... & Jack, S. (2021). Scabies and impetigo in Samoa: a school-based clinical and molecular epidemiological study. The Lancet Regional Health-Western Pacific, 6, 100081.
24. Tognetti , L., Martinelli, C., Berti, S., Hercogova, J., Lotti, T., Leoncini, F., & Moretti, S. (2012). Bacterial skin and soft tissue infections: review of the epidemiology, microbiology, aetiopathogenesis and treatment: a collaboration between dermatologists and infectivologists. Journal of the European Academy of Dermatology and Venereology, 26(8), 931-941
25. Ward , M. J., Goncheva, M., Richardson, E., McAdam, P. R., Raftis, E., Kearns, A., ... & Fitzgerald, J. R. (2016). Identification of source and sink populations for the emergence and global spread of the East-Asia clone of community-associated MRSA. Genome biology, 17(1), 1-13.
26. Yurtsever , S. G.; Aygül, A.; Öztürk, İ.; Nemli, S. A.; Kaya, S. and Ermertcan, Ş. (2020). Investigation of Various Virulence Factors and SCCmec Types in the Health care-associated and Community-associated Methicillin Resistance Staphylococcus aureus Strains. European Journal of Therapeutics, 26(2), 113-118.
27. Zarei Koosha , R.; Mahmoodzadeh Hosseini, H.; Mehdizadeh Aghdam, E.;Ghorbani Tajandareh, S. and Imani Fooladi, A. A. (2016). Distribution of tsst-1 and mecA Genes in Staphylococcus aureus Isolated From Clinical Specimens. Jundishapur journal of microbiology, 9(3), e29057.