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Article

Molecular Detection of Virulence Factors of Staphylococcus aureus and Their Relationship to Infection

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Abstract: Staphylococcus aureus is regarded as one of the most significant bacteria which infect human beings, causing various infections including simple skin infections, hospital-acquired infections, and severe systemic infections such as bacteremia. The pathogenicity of this bacterium is primarily attributed to its various virulence factors, such as adhesion factors, exotoxins and biofilm formation, which helps this bacterium to survive under different environmental conditions and evade the immune system of the host. The present study was designed to molecularly detect selected virulence genes of Staphylococcus aureus isolated from various clinical samples and the correlation of these virulence genes with the type of infection. Wounds samples were collected from patients, visiting the Kirkuk General Hospital in addition to blood samples and Urine samples. Culture methods and biochemical tests were used to identify the phenotype and then confirmed by Polymerase Chain Reaction (PCR) for the molecular confirmation. The nuc gene was selected as a diagnostic marker as well as virulence genes such as pvl and icaA. The results showed that the nuc gene was the most commonly found among the isolates, whereas the pvl gene was predominantly found in skin infections. Chronic and catheter-associated infections, however, had a higher prevalence of the icaA gene. In addition, the study identified multiple virulent strains containing more than one virulence factor, which could lead to more serious disease and hamper treatment. The study concluded that PCR is a rapid and accurate method for detecting virulence genes in Staphylococcus aureus, and its routine application in clinical laboratories could improve diagnosis and reduce complications associated with bacterial infections.

Keywords: Staphylococcus aureus, Virulence factors, PCR, Biofilm, pvl gene.

Introduction

Bacteria are microorganisms that play an important role in the environment and human health, as they may be either beneficial or pathogenic. Among the most important pathogenic bacteria that pose major threat to public health is *Staphylococcus aureus*, which is considered one of leading causes of human infections in both community and hospital [1]. The bacterium is highly prone to adaptation to

various environmental conditions and contains a wealth of virulence factors, which allow it to induce a wide variety of diseases ranging from skin infections to severe systemic infections like septicemia, endocarditis and pneumonia. Several times S. aureus colonizes the human skin and mucosal membranes without symptoms, especially in the nose. Under proper conditions, however, it can be a serious pathogen (such as immunocompromised patients or wound infection) [2]. This bacterium is one of the pathogens most frequently found in nosocomial infections due to its production of several toxins and enzymes that allow the microorganism to invade tissues and avoid the action of host defense system. [1].

1) **Research Problem**: Although many advances have been made in diagnostic and therapeutic options, infections caused by S. aureus remain a significant problem in clinical practice, especially because the incidence of complicated infections is rising and these become more difficult to treat. This is mostly due to the large number of virulence factors that this bacterium possesses, which cause fluctuations in virulence and clinical symptoms. The variability of different strains of S. aureus, which express multiple virulence genes such as toxin-associated genes and adhesion – related genes, complicates the ability of predicting the behavior a strain will exhibit during an infection or selecting the best treatment based on standard diagnostic techniques. Moreover, certain strains display both antibiotic resistance and other traits that would make them difficult to treat, such as the ability to grow in extreme environments.Additionally, some strains possess antibiotic resistance and other characteristics that would make the disease difficult to manage and can result in failure to treat. Further, traditional diagnostic methods only give a small amount of information about the molecular traits of bacterial strains and therefore do not facilitate the comprehension of the link between virulence factors and particular types of infection [3]. This restriction thus poses a barrier to the success of therapeutic and preventive measures and heightens the possibility of the spread of highly virulent strains in both the community and hospital. Thus, more sophisticated molecular tools, including polymerase chain reaction (PCR) are required to identify virulence associated genes, and to study their association with various infections. This could potentially enhance the precision of diagnosis, and aid in the creation of more effective treatment options and in the minimisation of the dissemination of complicated S. aureus infections. [2,3].
2) **Significance of The study**: The importance of our study is that we found that it is possible to detect S. aureus, one of the more important human pathogens, at a molecular level in the very powerful technique of polymerase chain reaction (PCR). The study also adds to the process of identification of virulence genes such as nuc and pvl, which helps understanding of the behaviour of bacteria and severity of the infection associated with them. In addition, it offers a scientific foundation for better early diagnosis, elimination of reliance on traditional approaches, and assisting physicians in treatment selection. Epidemiological surveillance programs in hospitals could be improved by the results of this study, and the spread of dangerous strains could be reduced if the results of this study are taken into consideration. [4].

3) **Objectives of the Study**: The aim of this study is to elucidate the molecular level characteristics of S. aureus with special emphasis on the virulence factors and their role in infection by the following objectives **:**

1. To identify S. aureus in the clinical sample using molecular methods, such as the polymerase chain reaction (PCR **.(**
2. **.** To examine the virulence genes present in different bacterial isolates and their presence in the samples studied **.**
3. To test the significance of virulence factors in determining levels of disease severity, and to see if certain virulence genes are linked with increased pathogenicity **.**

4 To evaluate the accuracy and speed of molecular detection techniques compared with traditional diagnosis techniques **.**

5 To help advance the scientific knowledge of the pathogenesis used by S. aureus, to help facilitate the development of better diagnostic and therapeutic options.

4) **Definitions of the Bacterium**: S. aureus is a gram-positive (GV+) bacterium (also referred to as a staph bacterium) having a spherical shape, which normally appear in grape-like clusters when viewed under a microscope. It is a part of the genus S. aureus and is known for its adaptability and survival abilities and its growth in a wide range of environmental conditions, including high salt concentrations on the media. S. aureus colonizes the human nasal cavity and mucosal surfaces as part of the normal flora and can remain non-pathogenic in healthy humans [5]. S. aureus is an opportunistic pathogen, however, because it can be a serious pathogen when host defenses are lowered (immunosuppression, wounding, use of medical devices), enabling it to breach host barriers and initiate infection. This bacterium is one of the most significant pathogens of man due to the variety of infections it can cause including skin and soft tissue infections (boils and abscesses), more serious infections such as pneumonia, osteomyelitis, septicemia and infective endocarditis. It is also a significant contributor to nosocomial infections, especially in intensive care units (ICUs), enhancing its clinical importance. The pathogenicity of S. aureus is due to the presence of several virulence factors that allow adhesion to host cells, toxin production and escape from the immune system. Furthermore, it can be a challenge to manage in the modern era because of the emergence of antibiotic resistant strains, particularly methicillin resistant S. aureus (MRSA). [6].

5) **Importance of Molecular Detection**: In recent decades, enormous progress has been made in the area of molecular diagnostics of pathogenic agents in medical sciences. One of the most popular and important techniques in medical microbiology is the polymerase Chain Reaction (PCR). This method is based on the amplification of particular fragments of the genetic material deoxyribonucleic acid (DNA) and can detect the presence of bacteria and accurately identify the genes that are responsible for virulence factors. [7]. The importance of using PCR in the diagnosis of *S. aureus* lies in many key aspects, the most importance of which is speed, as results can be obtained within a few hours compared with conventional methods that may require 24-72 hours or longer. This technique is also characterized by high sensitivity, as it can detect very small quantities of genetic material, even in cases where bacterial numbers are low or difficult to culture in the laboratory. In addition, PCR provides high diagnostic accuracy because it is based on the direct detection of specific genetic sequences, thereby reducing the possibility of error or cross- reactivity among different bacterial species. Furthermore, this technique enables the identification of highly virulent strains through the detection of genes responsible for virulence, such as toxin-encoding or adhesion genes. The ability to do this is not commonly available using conventional methods which usually only identify the bacterial species without determining the molecular properties of the bacteria. Conventional tests like bacterial culture and biochemical tests, on the other hand, rely on bacterial growth on culture media, which can take a considerable amount of time, and may also have lower accuracy in certain situations, especially where slow-growing bacteria are involved or with contamination in the sample. Thus, molecular techniques like PCR have become crucial in recent research due to the speed and accuracy of the information that can be obtained and which can help with better diagnosis and proper treatment decisions. [7].

6) **Virulence Factors:** Virulence factors are molecular and biochemical properties which allow bacteria to cause disease in the host. These include a range of proteins, enzymes and toxins which facilitate bacterial attachment to host cells, invasion of host tissue, proliferation within the host, and the ability to evade the immune system. Some of the

most significant factors that account for strain-to-strain variation in infection severity are these factors. S. aureus is one of the most virulent bacteria because, in this species, there is a large variety in the virulence factors. These factors include adhesion factors such as fibronectin-binding proteins and collagen-binding proteins which help the bacterium bind to cells and tissues. This bacterium also produces a group of exotoxins, such as PVL toxin and TSST-1, which play an important role in destroying immune cells and inducing a severe inflammatory response. S. aureus also produces enzymes, including coagulase and protease, which help spread the bacteria in tissues and prevent the immune system from attacking it.

7) The study of virulence factors is of importance because of understanding of the mechanism of pathogenesis. The use of molecular diagnostic tools like PCR has become crucial to the identification of the genes responsible for these factors, and the linkage of these genes with other specific forms of infection, which will help facilitate better disease management and decrease complications. Virulence factors are characteristics or components that bacteria have that allow them to make the host sick. These include surface proteins that mediate cell adhesion, toxins that lyse cells and enzymes that mediate intra-tissue spread of the bacteria. Virulence factors are very variable in S. aureus. [7,8].

8) **Classification of Virulence Factors**: S. aureus virulence factors can be categorized into a number of major groups:

The first step in the establishment of infection is for bacteria to attach to host cells, called adhesion factors. S. aureus has a set of surface proteins which help it attach to tissue components including collagen and fibronectin. The genes involved in the following factors are the most important: (fnbA): Involved in binding to fibronectin. (can): Attaches to collagen and is involved in deep infections.

2. Exotoxins: S. aureus produces a number of toxins which are responsible for much of the inflammation and cell destruction. The most obvious toxins are: PVL (Panton-Valentine Leukocidin): A toxin that targets the white blood cells. (TSST-1): Causes toxic shock syndrome. (Hemolysis): Cause lysis of blood cells.

3. Enzymes: The enzymes that the bacterium produces help it spread in tissues. The most significant enzymes are: (Coagulase): Converts fibrinogen into fibrin, thus protecting the bacterium. (Protease): Cleaves proteins and aids in tissue invasion.

Materials and Methods

1) **Sample Collection:** The study was performed at Kirkuk General Hospital, in addition to being one of the larger hospitals in Kirkuk Governorate, it has received various clinical cases, such as the complex cases and severe infections. This hospital was chosen due to the wide variety of clinical cases that are treated at the hospital, a condition that made it ideal for studying the prevalence of Staphylococcus aureus and its virulence factors. The study comprised collection of clinical samples from the hospital during a defined time period (20/2/2026-21/4/2026). Patients with signs or symptoms suggestive of possible bacterial infection including wound infection, blood stream infection or urinary tract infection were included. Samples have been collected in a sterile manner and in conjunction with the specialized medical personnel, in which all ethical aspects have been taken into consideration, as well as all necessary approvals.

2) **Table 1.** *Distribution of the Total Samples Used and the Targeted Groups According to Age and Gender*

Category	Male	Female	Average
1 year ≥	8	7	15
20-40 years	20	165	35
41-60 years	16	14	30

60 years ≤	12	8	20
Total	56	44	100

Note: The frequency distribution of all the clinical samples in the study. The largest number of samples were wound swabs, due to the association of Staphylococcus aureus with skin and soft tissue infections. Blood samples were added to examine isolates that were part of systemic infections, urine samples were added to see if the bacterium was present in urinary tract infections, especially those of inpatients or those using urinary catheters.

3) **Wound Swabs**: Wound swabs were taken from skin infections, purulent wounds and post-surgery infections. The external contamination and superficial exudates were gently removed from the wound surface before the sample was taken. A sterile cotton swab was then used to take the swab from the wound depth or the inflamed wound edge. Ideally sampling should be taken from pus or areas of active inflammation because these are the locations in which the bacteria causing infection are likely to be present. The swab was collected and placed immediately in a sterile transport tube with an appropriate transport medium (Amies transport medium or Stuart culture tube). The transport medium did not adversely affect the survival of the bacteria until the time of arrival in the laboratory. Samples were delivered to the laboratory within 1-2 hours and in limited cases stored at an appropriate temperature till culture. The swabs of wounds were considered the most significant specimen in this study as S. aureus is known to be strongly related to skin or soft tissue infections, abscesses and purulent wound. This specimen type is also crucial for investigating adhesion genes and toxin genes like pvl, and also for investigating genes associated with biofilm like icaA, since these genes are linked to the severity of infection and persistence. [9].

Figure 1. *Sterile wound swab used for microbiological culture and molecular detection.*

4) **Blood Samples**: Blood samples were taken from patients with systemic infection or suspected bacteremia (fever, chills, hypotension, elevated inflammatory markers, see Fig. 2). Sample was drawn from a vein and strict aseptic technique was used. The site for the collection was disinfected with alcohol and suitable antiseptic before the insertion of the needle to minimise the risk of contamination from normal skin flora. Blood was immediately inoculated into special blood culture bottles that contain culture media which will allow the growth of bacteria if they are in the blood stream. Depending on the protocol used, two bottles might have been used, one for aerobic and the other for anaerobic conditions. The bottles were then sent to the laboratory and were incubated following approved procedures to determine bacterial growth. Blood samples are particularly significant because blood samples are the most severe cases and if the organism S. aureus is present in the blood stream it is possible that there is a systemic infection or septicemia. Thus, molecular identification of virulence genes in blood isolates can

aid in identification of the most dangerous ones, particularly the genes encoding toxins and immune evasion. [10].

Figure 2. *Blood sample collection for culture and molecular detection.*

5) **Urine Samples**: Patients who had presented with symptoms of UTI included dysuria, urinary frequency, lower abdominal pain or any inflammatory parameters in routine urinalysis had their urine collected. Sterile plastic containers were used for sample collection and patients were asked to collect a midstream urine sample, which minimizes the contamination by skin bacteria and bacteria found on the external genital area. Fig. 3. The container was then tightly capped and clearly labeled with the sample identification code and was then sent to the lab as soon as possible. If the sample was not examined immediately, then it was kept at a low temperature for a short time to prevent the growth of unwanted bacteria. Bacterial culture and preliminary diagnosis were then carried out and isolates were subsequently confirmed and molecular analysis was carried out on the bacteria. S. aureus is not the most prevalent UTI of Gram-negative bacteria, but it can be clinically significant, especially in the hospital setting, in patients using a urinary catheter or in immunocompromised patients. Hence, urine samples were added and the prevalence of this bacterium in this infection was verified and compared with virulence genes. [3].

Figure 3. *Midstream urine sample collected in sterile container.*

Table 2. Distribution of Total Samples According to Sample Source and Purpose of Collection.

Sample Type	Total Number of Samples	Percentages %	Source (Hospital)	Purpose of collections
Wound Swabs	50	50%	Surgery, Emergency departments, wound wards	Isolation of S. aureus from skin and soft-tissue infections.

Blood	25	25%	Internal medicine, Emergency Departments, Intensive Care Unit.	Detection of isolates associated with bacteremia
Urine	25	25%	Laboratory, Internal Medicine , Urology Departments	Investigation of bacterial presence urinary tract infection
Total	100	100%	-	-

Note: Laboratory procedures for sample collection and transportation were performed as a fundamental stage to ensure the reliability of the results. Any error in the collection or transportation method may lead to sample contamination or bacterial death, which directly affects culture and molecular examination results. Therefore, sterile tools and appropriate transport media were used, with adherence to a short transportation time to the laboratory.

Table 3. *The relationship between the applied criteria and sample details.*

Criteria	Details
Inclusion criteria	Patients presenting with signs of infection, infected wound, septicemia, or urinary tract infection symptoms
Inclusion criteria	Samples collected under sterile conditions and delivered to the laboratory within the specified time
Exclusion criteria	Contaminated samples or those incorrectly labeled
Exclusion criteria	Delayed samples for a prolonged period or improperly preserved samples
Exclusion criteria	Duplicate samples from the same patient unless there is a clear clinical indication

Note: It presents inclusion and exclusion criteria, where only correctly collected samples were included and contaminated or unsuitable samples were excluded. This approach reduces experimental errors and improves the accuracy of the relationship between sample type and virulence gene detection results. Table 4 presents the clinical data recorded for each sample, which are essential for subsequent statistical analysis and for linking genes with the type of infection.

6) Sample transport and preservation: The samples were transported to the laboratory within a short time not exceeding (1–2 hours) to maintain bacterial viability and prevent contaminant growth. When analysis delayed, the samples stored at an appropriate temperature (4 °C) until testing. All patient's data were recorded, including age, gender, and type of infection, for later statistical analysis. The consider sample collection a critical stage of the study because it directly influences result accuracy, proper collection and transport increase the reliability of laboratory findings, especially when using sensitive techniques such as PCR [3].

Figure 4. *Sample collection from different areas in Hospital.*

Table 4. Types of clinical samples and their collection methods.

Sample type	Collection tool	Collection method	Transport method	Recommended transport time
Wound swabs	Sterile cotton swab	From the wound depth after surface cleaning	Amies or Stuart transport medium	Within 1–2 hours
Blood	Sterile syringe or collection system	From venous blood after skin disinfection	Blood culture bottle	Immediate transfer to laboratory
Urine	Sterile container	Midstream urine sample	Sterile closed container	Within 1–2 hours

7) **Phenotypic identification**: Phenotypic identification of *S. aureus* relies on conventional methods, including bacterial culture and biochemical tests, used as a preliminary step before molecular assays.

1) ***Culture methods***: Clinical samples (wound swabs, blood, and urine) were cultured on appropriate media such as: (Blood agar and Mannitol salt agar). Mannitol salt agar is a selective and differential medium. It supports *S. aureus* growth due to its high salt concentration and differentiates *S. aureus* through its ability to ferment mannitol, resulting in a color change from red to yellow. On blood agar, *S. aureus* colonies appear circular, smooth, and golden-yellow, and may exhibit beta-hemolysis in some cases [11].

Figure 5. Growth characteristics of *Staphylococcus aureus* on Blood agar and Mannitol salt agar.

2) *Biochemical tests*: After culturing, a series of biochemical tests were performed to confirm bacterial identity, including:

1. **Catalase test**: Produces a positive result with bubble formation after the addition of H_2O_2, indicating the presence of the catalase enzyme.
2. **Coagulase test**: Represents one of the most important tests and yields a positive result in *S. aureus*, indicating the bacterium's ability to coagulate plasma.
3. **Gram stain**: The bacteria appear as Gram-positive cocci arranged in clusters.

Table 5. Observed findings and their relationship to the tests.

Test	Result in S. aureus	Significance
Gram stain	Positive	Gram-positive bacterium
Catalase	Positive	Differentiates it from *Streptococcus*
Coagulase	Positive	Differentiates it from other *Staphylococcus* species
Mannitol fermentation	Positive	Medium color change

Figure 6. Biochemical identification tests for *Staphylococcus aureus*.

3) *DNA Extraction:* DNA was extracted from bacterial isolates using one of the following methods [12]:

A. **Manual Method**: This method relies on: Cell lysis, Removal of proteins and impurities, DNA precipitation using alcohol. This method is less expensive but requires more time and expertise, and may provide lower purity compared with commercial methods.

B. **Using a Commercial Kit**: Commercial DNA Extraction Kits were used, which rely on silica columns for DNA separation and purification. This method is characterized by: Rapid processing, High DNA purity, More accurate PCR results.

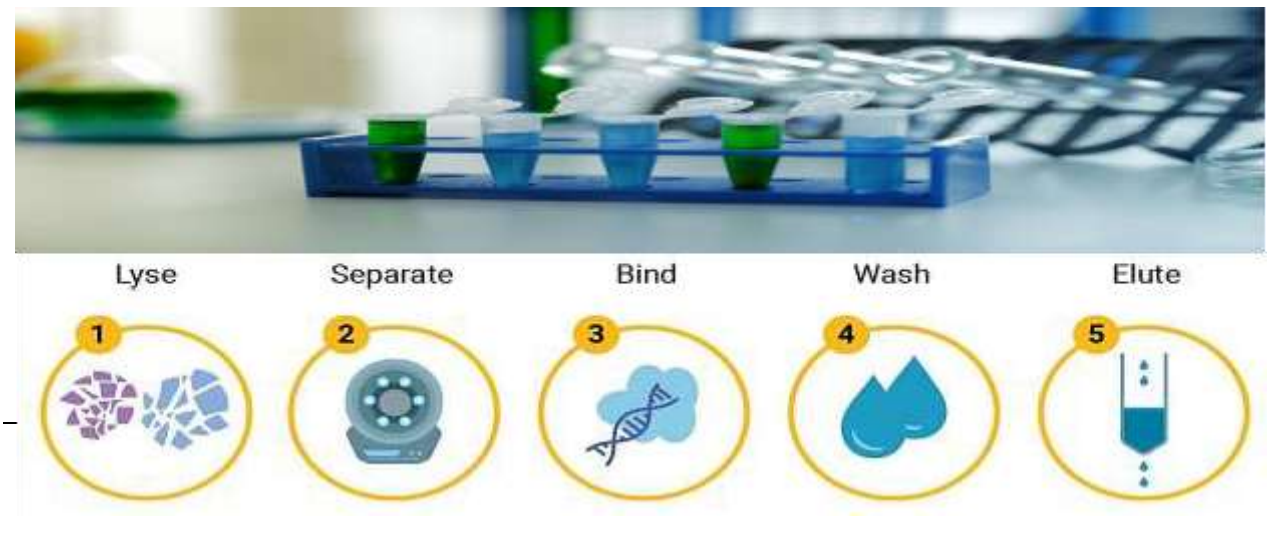

Figure7: DNA extraction procedure using commercial kit.

8) **Results Analysis**: PCR products were analyzed using gel electrophoresis, where bands appear according to gene size. See fig 8.

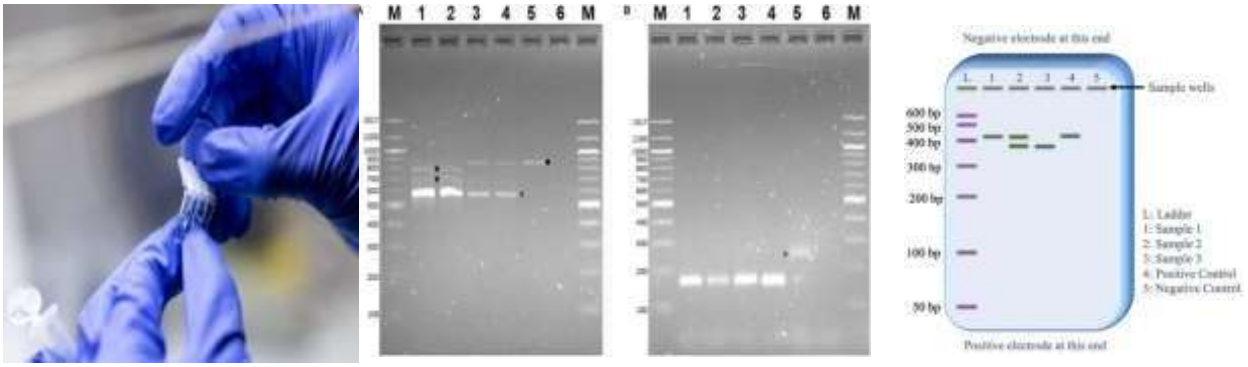

Figure 8. PCR amplification results showing DNA bands on agarose gel.

Results

Distribution of Samples: Clinical samples were analysed in total, 100 samples. Wound swabs, blood and urine samples were collected from Kirkuk General Hospital. The distribution of the samples and the percentage of positive isolation of Staphylococcus aureus from each sample source are shown in table 6.

Table 6. Distribution of Samples and percentage of positive isolation of *Staphylococcus aureus* from each source.

Sample Type	Total Number	Number of Positive *S. aureus* Isolates	Isolation Percentage (%)
Wound swabs	50	40	80%
Blood samples	25	20	80%
Urine samples	25	15	60%
Total	100	75	75%

The results revealed that the highest isolation rate was wound swabs (80%), blood samples (80%) and the lowest was urine samples (60%). This is indicative of the close relationship that S. aureus has with skin and soft tissue infections. [13].

Molecular Detection by PCR: For the detection of the target genes (nuc for identification and pvl and icaA as virulence factors), PCR was done. After running on agarose gel the results revealed the presence of DNA bands of the predicted size. [14].

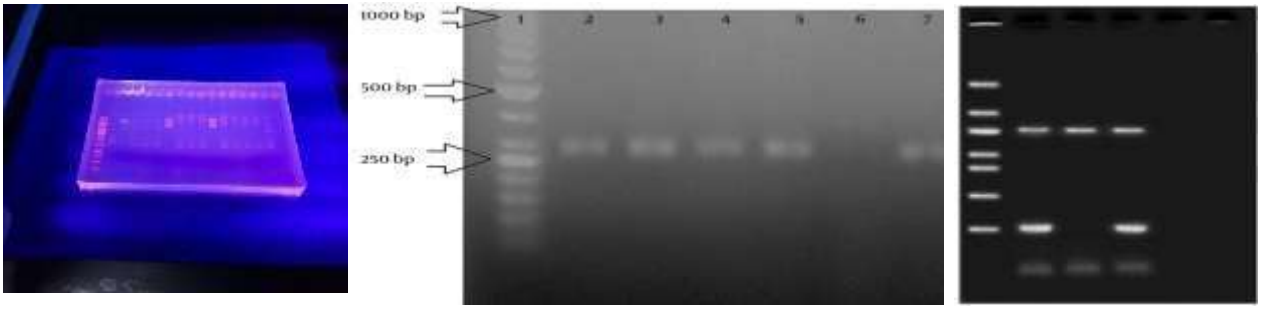

Figure 8. Agarose gel electrophoresis showing PCR products. L: DNA ladder; lanes 1–5 positive for *nuc* gene (279 bp); lanes 6–8 positive for *pvl* gene (433 bp).

Association with Type of Infection: The presence of the genes was associated with the type of sample/infection. Table (7) presents the correlation of the pvl gene with the type of infection and correlation of the icaA gene with catheter/device-associated infections.

Table 7. Association between the *pvl* Gene and Type of Infection.

Sample Type	pvl (+)	pvl (-)	Percentage within Sample (%)
Wound Swabs	20	20	50%
Blood Samples	8	12	40%
Urine Samples	2	13	13.3%

A higher frequency of the pvl gene was found in skin and soft tissue infections samples (50%) versus urine samples (13.3%) which supports its association to skin and soft tissue infections. It was found that the rate of isolation of Staphylococcus aureus from wound swab and blood sample was high as revealed in the results. The nuc gene was the most commonly occurring one, while the virulence genes (pvl and icaA) were associated with the type of infection. The pvl gene was linked to skin infections and the icaA gene to catheter and chronic infections. These results imply the value of PCR for the identification of the high risk strains [15].

Discussion

The findings of this study indicate that S. aureus still continues to be the important cause of clinical infections including those related to wound and hospital-associated infections [16]. Use of PCR facilitated the accurate identification of diagnostic and virulence genes and helped to gain a better insight into the nature of the circulating strains and the relationship between type and severity of infection. The results of this study suggest that there is a genetic variability in the virulence factors of S. aureus and these differences influence the nature and severity of disease. The other advantage was the use of PCR which facilitated the precise identification and the relation of these genes to the clinical condition [17]. The results confirm the importance of focusing on multi-virulent strains because of the real threat they pose to patient health, especially in clinical settings. Additionally, the study reinforces the importance of using more sophisticated molecular approaches to ensure more precise diagnosis and better treatment efficacy, while minimizing patient complications [18].

Conclusions

Based on the findings of the present study, Staphylococcus aureus is one of the leading causes of clinical infections especially in the case of wound infections and hospital infections. The results showed that the nuc gene was the most prevalent among the isolates studied and thus it was important to use it as an accurate and reliable diagnostic marker for this bacterium which is commonly applied in molecular studies to confirm S. aureus identity. The prevalence of virulence genes pvl and icaA was variable, in contrast to those mentioned above. The pvl gene appeared to be linked to severe infections while the icaA gene seemed more linked to chronic infection and biofilm formation, especially in medical catheters and long-term wounds. This is similar to recent research showing that virulence factors help the bacteria to attach to tissues, avoid detection by the immune system, and cause tissue damage. Given these results, molecular detection via PCR is a non-negotiable tool and should be used alongside conventional methods, as it affords high accuracy in virulence gene detection and also contributes to a prediction of infection behaviour and severity [19,20].

Recommendations

Because of their speed and accuracy, routine use of PCR assays in clinical laboratories, especially in severe or complicated infections.

2 Collection of strains of S. aureus in hospitals for surveillance activity.

3 Future use of Multiplex PCR which would allow detection of multiple genes in one test, thereby saving time and money and making the test more efficient.

4 Increasing the study of gene expression (not just gene presence) to understand the activity of these genes and what they do in the development of disease.
5 Further studies with more samples and various geographical areas of Iraq to get a more representative figure of the distribution of virulence factors.
6 Molecular findings correlate with patient's clinical condition to design pathogen based therapeutic strategies (Precision Medicine).

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