Molecular analysis of *Staphylococcus aureus* isolated from clinical samples and natural flora

Amal A. Abdulbaqi*, Ashraf Sobh Ibrahim
Jazan University, Saudi Arabia

**Introduction**

Gram-positive, coagulase-positive, catalase-positive *Staphylococcus aureus* (*S. aureus*) is an anaerobic bacterium that frequently results in nitrate reduction (1). *S. aureus* contributes to the normal flora of human skin and is one of the main sources of pyogenic illnesses (2). *S. aureus* is typically found in the upper respiratory tract and on the skin. They can act as a common pathogen of bloodstream infections, pneumonia, soft tissue and skin infections, postoperative wound infections, or device-related infections (3) by entering deep within the bloodstream, joints, bones, lungs or heart (4). Additionally, *S. aureus* infects a host by secreting dozens of virulence factors which influence the host’s immune responses. Such virulence factors include the release of exotoxins which account for around 10% of the entire secretome. Overall, *S. aureus* produces more than 40 recognized exotoxins having similar activities and strong structural similarities, but a deeper look says otherwise (5). Pathogenic *S. aureus* strains often promote infections by producing virulence factors such as potent protein toxins and extracellular enzymes such as lipase, urease, DNase, hemolysin (hly), β-lactamase, coagulase (coa), and lecithinase (6, 7).

As a result, in consideration of the facts previously mentioned, the productivity of some virulence factors of the extracellular enzymes (urease, hemolysin, coagulase, and catalase) from various clinical *S. aureus* isolates is examined. Additionally, the genes for the enzymes coagulase (coa) and hemolysin (hly), which are encoded by PCR using specialized primers that target Co-specific of these genes, were also examined in 20 isolates.

**Materials and Methods**

**Sampling**

This study was carried out in the Marjan Teaching Hospital from March 2019 to June 2019. Ethical approval was received at the beginning of the research. Samples were collected from wounds (n=30), abscessed skin (n=35), and healthy people’s normal flora (n=35). The clinical specimens have been adequately labelled, transported, and prepared in accordance with the aerobic bacterial culture’s requirement.

**Isolation and diagnosis**

The samples were processed using established microbiological protocols, and suspected *S. aureus* colonies were examined extensively (8). Samples were cultured at 37 °C for 24 h on both blood agar and mannitol salt agar (MSA), and isolates were analyzed consequently for phenotypic features, microscopy and biochemical tests by the reported protocol (9). All strains were grown as per the manufacturer’s instructions and sterilized for 15 minutes at 121°C 1 bar. The plates were examined for staphylococci morphology, and suspect colonies were recognized using standard microbiological procedures (Gram staining, catalase reactions, and coagulase reactions) (10).

**Investigation of some virulence factors**

The isolate which has the ability to produce coagulase and hemolysin enzyme as virulence factors were selected for further investigations. Using various laboratory culture mediums, the efficiency of virulence factors of the extracellular enzymes (urease, hemolysin, coagulase, and catalase) was examined. For such purpose *S. aureus* isolates,

---

* Corresponding author. Email: aabdulbaqi@jazanu.edu.sa

Cellular and Molecular Biology, 2023, 69(1): 145-149
including ten normal flora as families sample (coagulase-negative (5) and coagulase positive (5)), and ten clinical samples were selected to evaluate the presence of genes encoding the enzymes coagulase and hemolysin through polymerase chain reaction (PCR) by utilizing specialized primers that target Co-specific genes (coa and hly), (Table 1).

Results

Diagnosis of Staphylococcus spp.

One hundred samples from the wound, abscess skin and normal human flora were collected to investigate the presence of S. aureus. The samples were cultured in nutrient agar containing either blood agar medium, nutrient broth, or MSA medium, respectively. Purification was done on several sub-culturing on corresponding media. Initially, Staphylococcus spp were diagnosed based on phenotypic characteristics and biochemical tests. When Staphylococci spp were viewed under a microscope, grape-like clusters having large round colonies were observed, as reported previously (11). Staphylococcus spp was further identified based on Gram staining, catalase, hemolysin, coagulase, sugar fermentation, and urea test.

Staphylococcal spp colonies obtained from selective media were all Gram-negative and catalase positive. In Figure 1, the growth of bacteria in the blood medium illustrates clear zones of β-hemolysis. Still, the small difference between the zone of β-hemolysis in the pathogenic and the normal flora sample is because of the environment in which bacteria grow.

S. aureus isolates and extracellular enzymes.

Overall, it was found that in 40 samples, S. aureus isolates were present. Most S. aureus strains were isolated from healthy samples (50.0%) followed by wound (37.5%) and burn samples (12.5%) (Table 2). S. aureus can typically be found in humans in the skin, upper respiratory tract, and gut mucosa as a part of the normal microbiota. This explains the variation in the presence of S. aureus isolates in the sample (18). On the other hand, because S. aureus is categorised as a pathobiont, it can spread disease under specific host and environmental conditions (19, 20). Moreover, isolated S. aureus strains were tested for the production of extracellular enzymes (a wide variety of exoenzymes) as virulence factors.

The result revealed that S. aureus isolates from clinical samples could produce all four tested enzymes i.e., catalase, coagulase, urease and hemolysin–ß. In a similar manner S. aureus isolates from families (mean strains) as normal flora samples were also able to produce enzymes, i.e., catalase, urease and hemolysin–ß, except coagulase 70% (14 isolates) (Table 3).

Correlation between bacterial enzymes production

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>S. aureus (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy people</td>
<td>20</td>
<td>50%</td>
</tr>
<tr>
<td>Wound</td>
<td>15</td>
<td>37.5%</td>
</tr>
<tr>
<td>Burns</td>
<td>5</td>
<td>12.5%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3. The ability of S. aureus to produce some extracellular enzymes as virulence factors.
On MSA, medium pathogenic S. aureus produces small colonies surrounded by yellow zones due to mannitol fermentation which clearly distinguishes S. aureus from S. epidermidis (Figure 2) (12, 13). 90% of S. aureus strains are known for urease production (14) because it is important for bacteria in environmental adaptation, pathogenicity, and protection against human immunity. As a result, urease synthesis was indicated by a vivid pink colour on the slant that extended into the butt after a few hours of incubation. A vivid pink colour showed urease production in Stuart’s urea broth throughout the broth (15).

Fibrinogen can be turned to fibrin by the protein enzyme known as coagulase, which is produced by several bacteria. It is used in the lab to differentiate between different Staphylococcus isolates. S. aureus is coagulase-positive generally, which is significant since it signifies that

In Table 3, it was noticed that 30% of the S. aureus isolated from the normal flora (from the hosts) did not produce coagulase, while all pathogenic samples produced selected enzymes at 100%. These findings remain consistent when repeating the examination after two weeks in the same samples and from the same people, which means that these bacteria are the bacterial fingerprint of these people and their inability to produce this enzyme can be relied upon as a distinguishing mark (Figure 3).

Molecular detection of coa and hly gene
A total of 20 S. aureus isolates, including normal flora as family’s sample (10) (coagulase-negative (5) and coagulase positive (5)), and clinical samples (10) were selected based on their ability to produce coa and hly enzyme as virulence factors. To evaluate the presence of genes encoding the enzymes coa and hly, a PCR test was performed. The result showed the presence of the hly gene in all studied isolates, while the coa gene was present in all clinical samples (10) but not present in 6 families isolation, as shown in Figures 4-6.

Correlation between bacterial production of enzymes and gene analysis
The obtained results signified the S. aureus ability to produce extracellular enzymes that express virulence factors. Still, there are some bacterial isolates from normal flora samples which did not produce coagulase enzymes even after repeating the examination more than once. Therefore, genes responsible for the production of these enzymes were examined, and it was found that only those isolates which were negative for the coagulase assay do not possess coa gene (Table 4).

Discussion

Table 4. The presence of coa and hly genes.

<table>
<thead>
<tr>
<th>S. aureus isolates</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>P(10)</td>
<td>N(5)</td>
</tr>
<tr>
<td>coa gene</td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>hly gene</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*P= Pathogenic. *N= Normal flora.

On MSA, medium pathogenic S. aureus produces small colonies surrounded by yellow zones due to mannitol fermentation which clearly distinguishes S. aureus from S. epidermidis (Figure 2) (12, 13). 90% of S. aureus strains are known for urease production (14) because it is important for bacteria in environmental adaptation, pathogenicity, and protection against human immunity. As a result, urease synthesis was indicated by a vivid pink colour on the slant that extended into the butt after a few hours of incubation. A vivid pink colour showed urease production in Stuart’s urea broth throughout the broth (15).

Fibrinogen can be turned to fibrin by the protein enzyme known as coagulase, which is produced by several bacteria. It is used in the lab to differentiate between different Staphylococcus isolates. S. aureus is coagulase-positive generally, which is significant since it signifies that
a positive coagulase test indicated that S. aureus was present in the determined sample (16, 17). A negative coagulase test reveals the presence of organisms like S. epidermidis or S. saprophyticus that are coagulase negative. More typically, opportunistic infection is associated with coagulase-negative Staphylococci species. (1). In most cases, isolated cultures were purified by serial dilution on reputable media, and the acquired pure cultures were maintained at 37°C for 24-48 h, then stored at 4°C in a refrigerator for further investigation. The final results are in accordance with the Abdul-Kareem et al., findings who report the presence of hly gene (virulence factor of hemolysine enzyme) in S. aureus isolate from burns, wounds and skin flora (21). Similarly, it is also reported that most strains of S. aureus are the producer of the coa enzyme as a significant virulence factor of pathogenicity (22-24). This result helps to link the existence of a relationship between people and isolated bacteria, which helps in the diagnosis and identification of bacterial fingerprints for families.

The S. aureus isolated from pathological conditions and a healthy person can produce some virulence factors extracellular enzymes such as coagulase enzyme, catalase, hemolysin, and urease. While not all S. aureus isolated from normal persons produced coagulase enzymes. From PCR analyses, it was revealed that this difference is due to the absence of the cos gene in S. aureus isolates from normal flora in comparison to clinical isolates where cos genes are detected in all selected samples.

Acknowledgements
The author extends there appreciation to Jazan University President / Mar’ei Bin Hussein Mohammed AlQuhtani for continuous support to researchers, and to Dr/Omyma Radarwan, the Dean of the University College in Al-Darb.

Authorship
Every writer is recognized as an author because they have all completed the requirements for authorship and approved the final product.

Funding
The financing sources are not disclosed.

Normative Consent
From March to June 2019, this study was conducted at the Marjan Teaching Hospital. The research was given the go-ahead after receiving ethical permission.

Competing interests
There are no conflicts of interest for the authors.

Data accessibility declaration
The manuscript that has been submitted contains data.

References
20. Jassim YA, Al-Amery SMHJDIT. Purification and characterization of protease and lipase from Pseudomonas aeruginosa isolated from some wound and burn infection. Drug Invention Today 2019;11(10).
