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Genetic Comparison of Some Virulence Genes in *Staphylococcus Aureus* bacteria Isolated from Clinical Samples and Normal Flora

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Abstract

A total of 100 samples were collected to investigate the bacteria of *Staphylococcus aureus*, distributed as follows (30 samples were collected from healthy people, 35 Wounds, and 35 burns were collected from patients admitted to Marjan Teaching Hospital.

It was found 40 isolates belonging to *Staphylococcus aureus* were distributed as follows(21 based on phenotypic, culture, and biochemical characteristics.

The study showed that bacteria isolated from pathological conditions may produce extracellular enzymes that contribute to pathogenicity, including urease, hemolysin, catalase, and coagulase at 100%, while isolated from normal persons produced coagulase enzymes by 70% and 100% of the rest of the enzymes including catalase, hemolysin, and urease. A total of 20 isolates of *S. aureus* bacteria (pathogenic and normal flora) were selected depending on virulence factors produce to investigate some virulence genes.

A molecular study of some virulence genes of *S. aureus*, which are the *coa*, which encodes the production of the coagulase enzyme, and the *hly*, which encodes for the production of the hemolysin enzyme, indicated that all of the chosen sick isolates had the genes present, but just six of the normal flora isolates had the *coa*.

Keywords: *coa*, *hly*, *S. aureus*, extracellular enzymes

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1. INTRODUCTION

About 30% of the population is asymptomatically colonized by the very efficient bacterium *Staphylococcus aureus.* It can, however, also result in a number of illnesses, ranging from mild skin and soft tissue infections to dangerous ones like sepsis and pneumonia. When *S. aureus* infects the host, it produces a number of virulence factors that aid in controlling the host immune reactions while ensuring bacterial survival. Exotoxins, which make up around 10% of the entire secretome, are one of these virulence factors. Although these bacteria are known to create over 40 different exotoxins, many of them share a lot of structural and functional similarities. These allegedly redundant exotoxins were examined more closely and each was found to have distinct characteristics (Harris et al 2002).

A typical component of the body's microbiota, Staphylococcus aureus is usually discovered on the skin and in the upper respiratory system. It frequently exhibits catalase and nitrate reduction, and because it is a facultative anaerobe, it may grow without oxygen (Jacquemyn et al, 2013). Although S. aureus typically functions as a commensal member of the human microbiota, it has the potential to develop into an opportunistic pathogen. It is a common cause of food poisoning, sinusitis, and a variety of skin diseases, including abscesses.

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Pathogenic strains typically contribute to the spread of diseases by producing virulence factors like toxins and enzymes like urease, coagulase, lecithinase, lactamase (Jaloud and Hassan, 2018; Jaloud et.al., 2022).

2. MATERIALS AND METHODS

Sample collection

A total of 100 samples from healthy individuals, burn victims, and wounds overall. Swabs were collected from people of all ages and genders between March 2021 and June 2021; there were 35 samples for wounds, 35 samples for burned tissue, and 30 samples for normal flora.

Isolation and diagnosis of *Staphylococcus* bacteria

Both blood agar and MSA (salt mannitol agar) were used to culture the samples. The plate was cultivated for 24 hours at 37°C, and isolates were identified in accordance with their phenotypic characteristics using microscopy and biochemical testing (Ferens et al, 1998).

To Diagnose and identify *Staphylococcus* spp. All samples culturing in some media used in this study included: Nutrient Agar, the difference will be due to different factors such as Blood agar medium, Nutrient broth, and Mannitol salt agar medium. Use an MSA to distinguish between *S. aureus* and *S. epidermidis* Where bacteria *S. aureus* ferment mannitol sugar (Ryan and Ray, 2004) as shown in figure (1). Gram staining and the catalase test were performed on Staphylococcal colonies isolated from selective media. Purification was accomplished using several subcultures on the appropriate media. When cultivated on blood agar plates, staphylococcal has big, spherical colonies that frequently exhibit hemolysis and appear as staphylococci (grape-like clusters) when seen under a microscope as in Figure (2) *(*Ryan and Ray, 2004).

Investigate some virulence factors

The productivity of several virulence factors of the extracellular enzymes urease, hemolysin, coagulase, and catalase was investigated using a variety of laboratory culture conditions.

Investigate the prevalence of *coa & hIy* genes in bacterial isolates

Using customized primers (Table 1), that target the Co-specific genes (*coa, hIy*) by PCR to detect these genes. A total of 20 *S. aureus* isolates in total were chosen for the study (20 from samples of normal flora used as families, 10 from clinical samples, and 5 from isolates that tested negative for coagulase while the other 5 isolates tested positive for coagulase).

Table 1: Primer sequence used in this study

Primer	Sequence	Target size
Coa	F 5' ATAGAGATGCTGGTACAGG 3' R 5' GCTTCCGATTGTTCGATGC 3'	1- 2.5 Kbp
hIy	F 5' GGT TTA GCC TGG CCT T 3' R 5' CAT CAC GAA CTC GTT C 3'	100 bp -1 Kbp

3.RESULTS AND DISCUSSION

Sample collection

A total of 100 samples were collected to investigate the staphylococcal bacteria distributed as follows (30 samples from healthy people, 35 samples from wounds, and 35 from burns). Isolates were identified phenotypically and biochemically. It was found 40 isolates belonging to *S. aureus* were distributed as follows (20 from healthy people, 15 from wounds, and 5 from burns) as shown in Table (2).

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Table 2: *S. aureus* distribution according to leasion source

Source	No.	Percentage (%)
Healthy	20	50
Wound	15	37.5
Burns	5	12.5
Total	40	100

Figure 1: Bacterial growth on MAS medium

Figure 2: Staphylococcal bacteria on the blood agar

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The hemolysis is noted on the blood agar, and the comparison between the hemolysis in the clinical samples and the natural sample can note a small difference in the decomposition and this is due to the difference in the environment in which the bacteria are located (Schenck et al, 2016).

S. aureus can exist in humans as a component of the normal microbiota in the skin, gut mucosa, and upper respiratory tract (Wollina, 2017). *S. aureus* is classified as a pathobiont because, depending on the host and the environment, it can cause disease (Otto, 2010; Jassim and Al-Amery 2019; Al Alwany AA, 2022a; Abbas et.al. 2020)

Staphylococcus is catalase-positive, which indicates that it can manufacture the catalase enzyme. Hydrogen peroxide is changed by catalase into water and oxygen. Testing for catalase activity can help identify staphylococci from streptococci and enterococci. Previously, the coagulase test was used to distinguish S. aureus from other staphylococci. But not every strain of *S. aureus* is coagulase-positive. (Matthews, 1997; Varrone, 2014; Mahamda, and Al Alwany, 2022; Al Alwany 2022) and incorrect species identification can impact effective treatment and control measures.

Identification of staphylococci isolates:

Staphylococcus varieties. Using the coagulase test (slide test and tube test), Novobiocin sensitivity, and biochemical tests including the urea test and sugar fermentation, were used to identify them (Harrigan, 1998) and (Barrow and Gelthan, 1993)

Investigation of the production of staphylococci for a group of extracellular enzymes

S. aureus isolates from families as normal flora samples were 100% productive for hemolysin – ß, Urease, and Catalase but 70% (14 isolates) coagulase production, *S. aureus* isolates from clinical samples were 100% productive for catalase, Coagulase, and urease (Table 3).

Table 3: Some extracellular enzymes produced by *S. aureus*

Source	No.	Urease (%)	Coagulase (%)	Catalase (%)	Hemolysis (%)
Healthy	20	100%	70%	100%	100%
Wounds	15	100%	100%	100%	100%
Burns	5	100%	100%	100%	100%

Coagulase is used to differentiate between several *Staphylococcus* isolates (Becker *et al*., 2014). Instead, a coagulase-negative bacterium, such *S. epidermidis* or *S. saprophyticus*, would be detected by a negative test. It is now understood, nonetheless, that not all strains of *S. aureus* are coagulase-positive. Coagulase-negative Staphylococci are often not pathogenic, in contrast to coagulase-positive which is less frequent, Staphylococci are linked to opportunistic infections (Ryan & Ray, 2004; González-Martín et al 2020; Ortora *et al*, 2013).

The urease test detects organisms capable of hydrolyzing urea to yield ammonia and carbon dioxide. Christensen's urea agar can be used to identify a variety of bacteria' urease activity (Collee *et al* 1996).

Correlation between bacterial production of enzymes :

Only 30% of the isolates belonged to the normal flora, even though all pathogenic isolates tested positive for coagulase. Since the same people generated identical results, these germs serve as a fingerprint of those people (figure 3).

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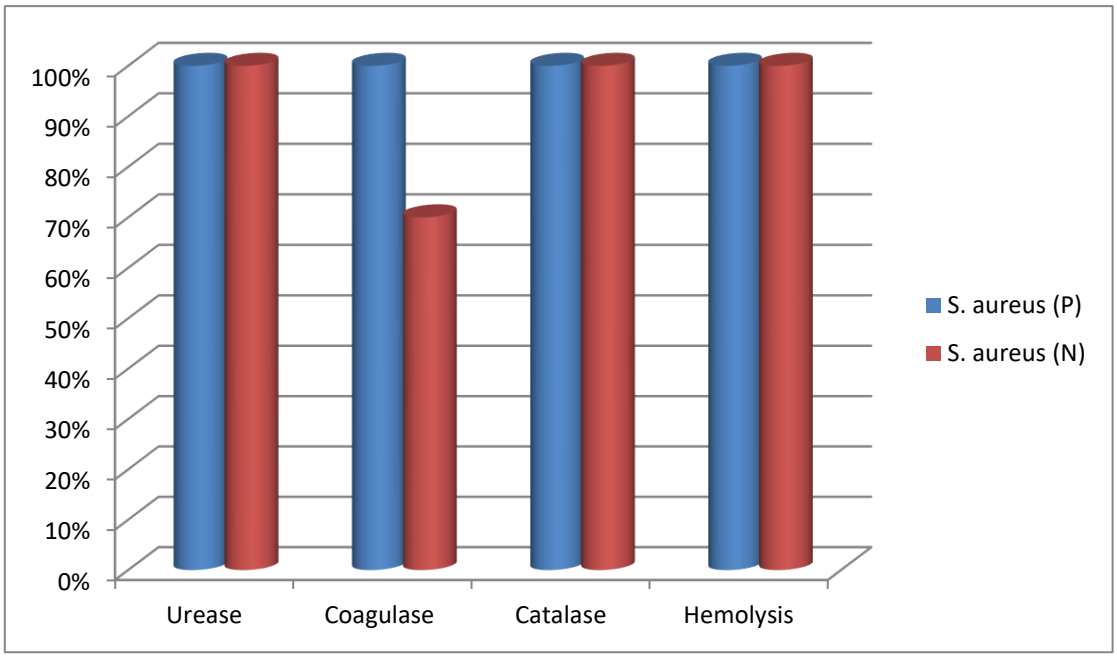

Figure 3: Tested *S. aureus* Enzymes

***P= Pathogenic. *N= Normal flora**

Molecular detection of *coa* and *hly*

A total of 20 coagulase and hemolysin producing isolates were chosen for PCR analysis (10 isolates from normal flora as families samples, 5 of which tested negative for coagulase while the other 10 isolates tested positive for coagulase, and 10 isolates from clinical samples). They revealed that the *hIy* was present in all examined isolates, whereas the *coa* was absent from six families out of the 10 clinical samples but present in all other isolates. (figures 4, 5, and 6).

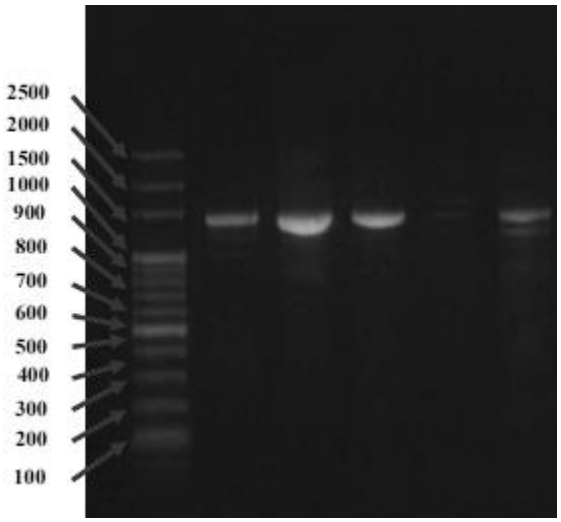

Figure 4: *S. aureus coa* PCR amplicons from healthy individuals on a 5.1% agarose

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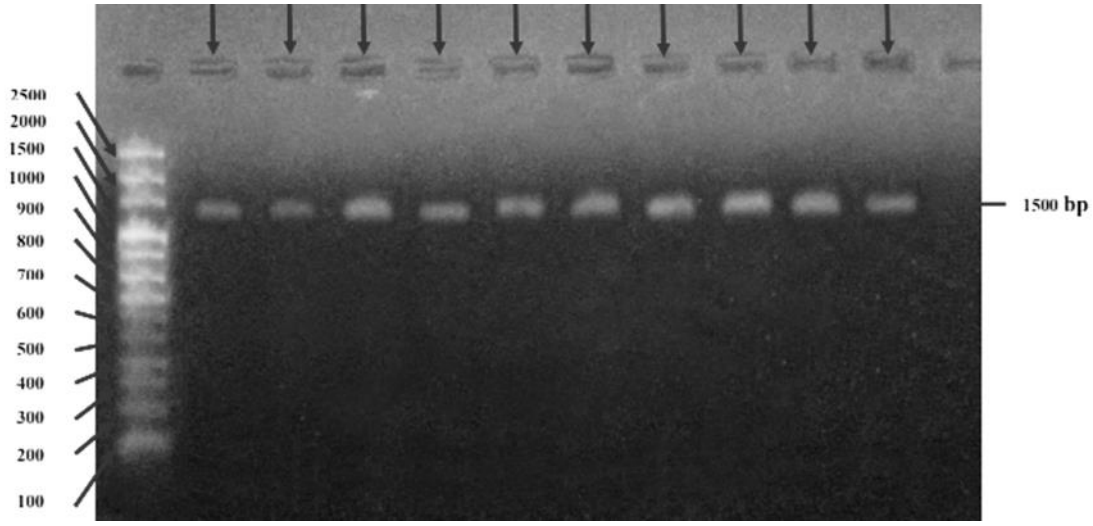

Figure 5: *coa S. aureus* PCR amplicons from burn infections and wounds were run on a 5.1%

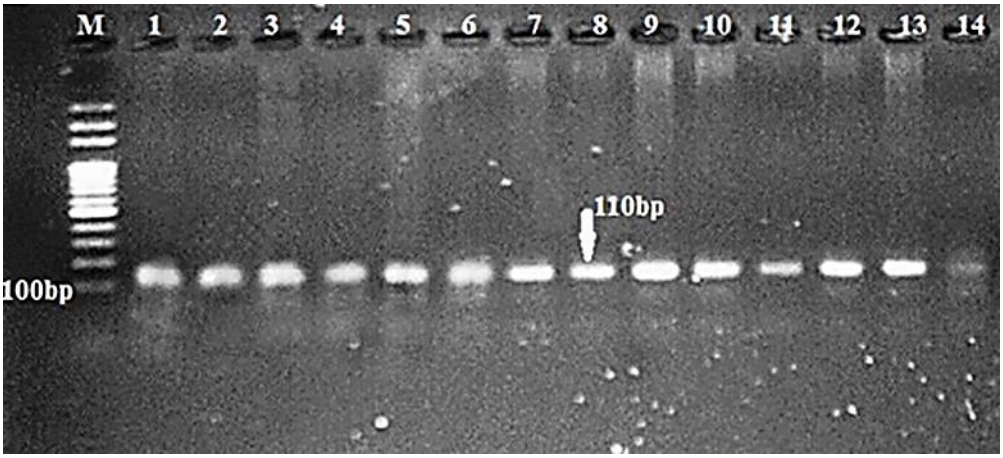

Figure 6: *S. aureus* hIy gene PCR amplicons from healthy individuals, wound, and burn infections were run on a 5.1% agarose gel

The results agree with Clark *et al* (1993). Most *S. aureus* isolates produce cos enzyme as an important factor in the pathogenicity of this bacterium with a rate of 39% (de Freitas *et al*, 2013) and the study's findings also agreed with what was indicated by Cuny *et al*, (2015) where methylene-resistant *Staphylococcus aureus* was utilized as a test subject, gene coagulase was used as a virulence factor. The relationship between gene distribution and the number of isolates and different sources.

The findings of the current investigation on the hIy gene agree with Ben *et al* (2008) Since it is an important virulence factor. The findings of the current investigation and any genes expressing hIb were in agreement with what was found by Budd et al (2015) who claimed that hemolysin- is more common than it is, at a rate of (4.43%)%, and the findings of the present study also agreed with their findings with what was found by Buzzola *et al* (2007).

Correlation between Bacterial enzymes production and gene analysis

It was discovered that some of the normal flora isolates, which tested negative for the coagulase assay, do not have cos gene. This information helps to establish a connection between the bacteria and the individuals isolated, which aids in the identification of the bacterial fingerprint for these families.

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The positive coagulase test clearly shows the existence of *coa*, whereas the isolates with the negative coagulase test were devoid of *coa* (table 3).

Table 3: Insidrnce of *coa* and *hly*

	Coagulase-positive			Coagulase-negative
	15			5
	P (10)	N (5)		N (5)
coa	+	+ (4)	- (1)	-
hly	+	+		+

4.CONCLUSION

Although not all isolates from healthy individuals could, the bacteria isolated from pathological conditions can produce several extracellular enzymes linked to virulence, including coagulase, catalase, hemolysin, and urease, but not all of the other enzymes, including catalase, hemolysin, and urease. It revealed that the hly and cos genes were present in every single clinical isolate that was chosen, but that only six isolates from the normal flora lacked the cos gene.

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