# Detection of Virulence Factors *hla* and *hlb* of *Staphylococcus aureus using PCR Technique*

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## Abstract

Thirty isolates were collected from patients sputum and throat swabs who admitted at Teaching General Hospital in Hilla city during a period of two months lasting from (Febraury to March 2014). The morphological characterization and biochemical reactions showed 18 isolates diagnosed as *Staphylococcus aureus*, of which obtain only six isolates, two from sputum samples (25%) and four from throat swabs (0.4%) have *hlb* gene and only four isolates, two from sputum samples(25%) and two from throat swabs (0.2%) have *hla* gene using PCR techniques.

**Keywords:** S. aureus , virulence factors , PCR , Antibiotic .

**الخلاصة:**

تم جمع ثلاثون عينة من عينات القشع ومسحات البلعوم في المستشفى التعليمي في مدينة الحلة خلال فترة شهرين من (كانون الثاني الى شباط 2014). واظهرت النتائج الشكلية والبايوكيمائية ان ثماني عشر عينة مشخصة كانت لبكتريا العنقوديات الذهبيه , والتي تشمل منها ست عينات فقط تحوي (*hlb*) جين,منها اثنان من عينات القشع(٢٥%) واربع من مسحات البلعوم (٠.٤%) , واربع عينات فقط تحوي (*hla*) جين, منها اثنان من عينات القشع (٢٥%) واثنان من مسحات البلعوم (٠.٢%) باستخدام تقنية تفاعل البلمرة المتسلسل PCR.

مفتاح البحث : البكتريا العنقودية , عوامل الضراوة , تفاعل البلمرة المتسلسل , المضادات الحياتية.

## Introduction

*Staphylococcus aureus* is a Gram-positive, facultative anaerobic and non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus and characterized by individual cocci which divide in more than one plane to form grape-like clusters [1].

*Staphylococcus aureus* known as golden staph, when viewed through a microscope appear as large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates [2].

*Staphylococcus aureus* subspecies distinguished from *Staphylococcus aureus* by lack of pigment and clumping factor and by the inability to carry out anaerobic fermentation of mannitol, to grow at 45°C, to produce acetoin from glucose to reduce nitrate, to produce b-glucosidase, and to produce acid from galactose, lactose, mannose, mannitol, ribose, and trehalose [3].

*Staphylococcus aureus* is a dangerous human pathogen in both community-acquired and nosocomial infections. *Staphylococcus aureus* which can be found as part of the normal skin flora and in anterior nares of the nasal passages and it is the most common species of *Staphylococcus* to cause Staph infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies [4].

A fundamental biological property of this bacterium is its ability to asymptomatically colonize healthy individuals [5].

*Staphylococcus* produce many potential virulence factors such as toxins like alpha, beta and gamma toxin

leukocidin, toxic shock syndrome toxin and different types of enterotoxins. Alpha and beta toxins are exotoxins hemolysins in nature cause lysis of erythrocytes by pore formation. A wide range of cell types is affected by alpha toxins including erythrocytes, monocytes, lymphocytes, macrophages and epithelial cells [6].

*Staphylococcus aureus* a-toxin a polypeptide encoded by *hla* is a pore-forming cytotoxin that is produced by the majority of *S. aureus* strains and targets a broad range of host cell types.Like most staphylococcal extracellular proteins, a-toxin is not expressed constitutively but is tightly regulated by an array of extracellular and intracellular signals [7].

However *α*-toxin is secreted as a water-soluble monomer that undergoes a series of conformational changes to generate a heptameric, *β*-barrel structure in host membranes [8].
On the other hand β-toxin is a neutral sphingomyelinase secreted by certain strains of *Staphylococcus aureus*. This virulence factor lyses erythrocytes in order to evade the host immune system as well as scavenge nutrients [9].

*β-Toxin* (*hlb*) among *S. aureus* toxins, the function of *β*-toxin in pneumonia and lung injury. This *β*-toxin is a Mg2+-dependent neutral sphingomyelinase that hydrolyzes sphingomyelin of the host cell plasma membrane to generate phosphocholine and the bioactive secondary messenger [10].

*β*-toxin does not lyse most types of host cells but leaves them susceptible to a number of other lytic agents, such as *α*-toxin and Panton-Valentine leukocidin ,the cytotoxic effect of *β*-toxin is cell type-specific and species-specific, suggesting that its primary virulence activity is to modulate host processes that affect pathogenesis, rather than to directly kill host cells [11].

# Materials and Methods

## Collection of specimens:

The study was conducted at Teaching General Hilla Hospital in Babylon Governorate. 30 samples collected from patients sputum and throat swabs during the period from (Febraury to March 2014). Only18 isolates of *Staphylococcus aurues* were obtained from patients with tonsillitis, pharyngitis, sinusitis and otitis by standard bacteriological methods.

## Bacterial identification:

The samples were processed on blood agar, macCkonkey agar and selective media (Salt milk agar) were incubated at 37°C performed by standard biochemical methods (catalase test, oxidase test, coagulase test, urea hydrolysis test, hemolysin , produce of lipase produce of phosphatase, reduce of nitrate to nitrite , ferments mannitol and gelatin liquefaction) according to Bergy's Manual for Determinative Bacteriology [12].

## DNA extraction for gram positive bacteria:

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Promega, USA).

## Detection of some virulence gene markers by PCR:

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in table (1). The primers includes *hla* and *hlb* genes, Each 25µl of PCR reaction contained 2.5µl of each upstream and downstream primer, 2.5µl of free nuclease water, 5µl of DNA extraction and 12.5µl of master mix. The PCR amplification product were visualized by electrophoresis on 1% agarose gels for 45min at 70v. The size of the amplicons were determined by comparison to the 100 bp allelic ladder (Promega, USA).

**Table (1):** Primers sequences and PCR condition

| Genes | Primer sequence (5′-3′) | Size of product bp | PCR condition | Reference |
|---|---|---|---|---|
| ***hlbF*** <br> ***hlb*R** | GCC AAA GCC GAA TCT AAG <br> GCG ATA TAC ATC CCA TGG C | **833** | 94ºC 2min 1x <br> 94ºC 1min <br> 54ºC 1min 30x <br> 72ºC 1min <br> 72ºC 10min 1x | [13] |
| ***hla F*** <br> ***hla* R** | TTGGCTGGGGAGTTGAAGCACA <br> CGCCTGCCCAGTAGAAGCCATT | **306** | 94ºC 2min 1x <br> 94ºC 1min <br> 55ºC 1min 30x <br> 72ºC 1min <br> 72ºC 10min 1x | [14] |

## Results

*Staphylococcus aureus* isolates were subjected to produce major cytotoxic agent ($\alpha$-toxin and $\beta$-toxin). Molecular detection of Staphylococcal $\alpha$-toxin (*hla*) and $\beta$-toxin (*hlb*) was done by using specific PCR primer. The results showed that only six of investigated isolates contained the (*hlb)* gene, two from sputum isolates ( 25%) and four from throat swabs (0.4%) as shown in figure (1-1).

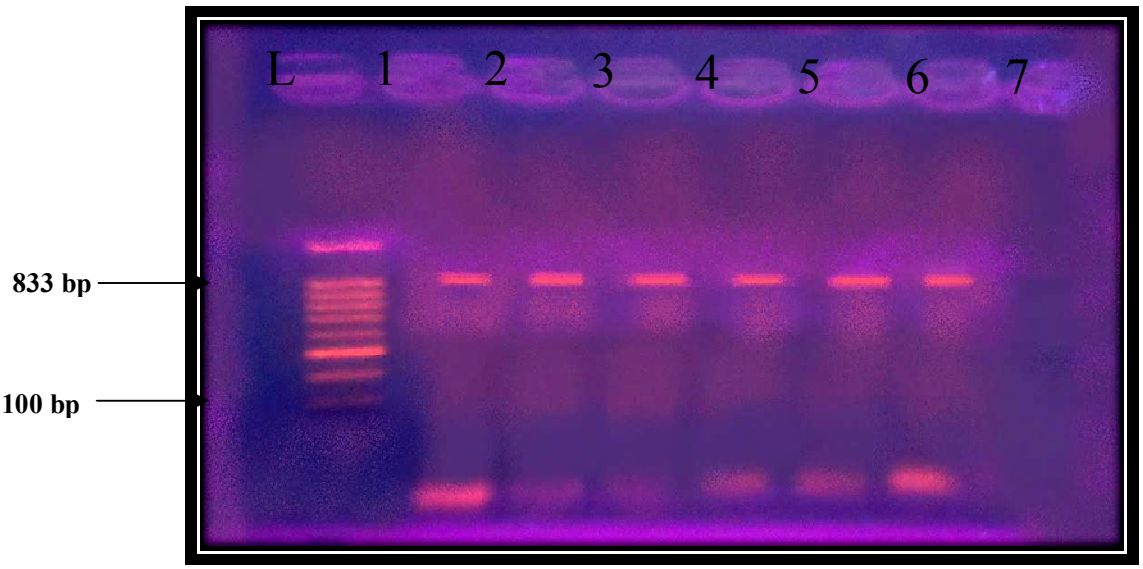


**Figure (1-1) :** Gel electrophoresis of PCR product of *hlb*
(1,2,3,4,5,6)isolates with positive result for *hlb* (1,2) isolates from sputum (3,4,5,6) isolates from throat swabs. L= ladder(1500-100). The electric current was allowed at 70 volt for 30 min.

Also the results revealed that only four isolate gave positive amplification for *(hla*),two from sputum samples (25%) and two from throat swabs ( 0.2%) as shown in figure (1-2).

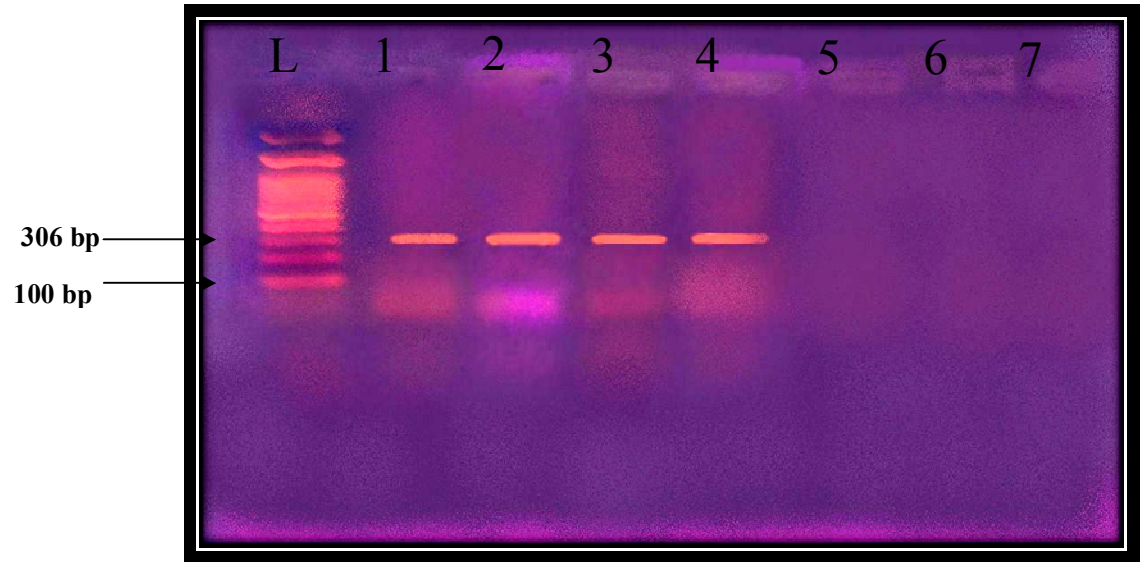


**Figure (1-2) : Gel electrophoresis of PCR product of *hla***
(1,2,3,4) isolates with positive result for *hla* (1,4) isolates from sputum (5,6)isolates from throat swabs. L= ladder(1500-100). The electric current was allowed at 70 volt for 30 min.

## Discussion

The majority of initial inflammatory responses to inhaled bacteria is signaled by mucosal cells lining the respiratory tract. *Staphylococcus aureus* has a potential to activate the host inflammatory response in several different ways through the adherence of intact bacteria to the host epithelial cells, by internalization of the bacteria and by direct interaction of

bacterial adhesins and toxins with the mucosal epithelium [15]. Many factors of Staphylococci are known but the most frequently mentioned and detected in this study by using PCR with primers specific genes includes: *hla* and *hlb*.

It was found that (*hlb*) is present in only six isolates. This result is correlated with the results done by [16] who investigated the presence of this gene among most *S. aureus* isolates.

Also the results revealed that only four isolate gave positive amplification for (*hla*). This result is agreement with the results done by [17] who investigated the presence of this gene among most *S. aureus* isolates. This gives the important role of this gene in pathogenesis of *S. aureus* among hospitalized patients.

Other study investigate by [15] indicated that 96% of *hla and hlb* give positive isolates obtained from nasal carriage .

Harris *et al*., [18] and Cheung *et al* .,[19] reported that *Hla* is positively controlled by *agr, sarA,* and *sae*. It appears that *agr* activates *hla* at both the transcriptional and translational levels, whereas *sarA* exerts a complex positive impact on *hla* expression by both *agr*-dependent and *agr*-independent pathways.

In addition, *sae* appears to positively activate *hla* via an *agr* dependent pathway *in vitro* [20].

Suchart, and Jorgen [2] and Ryan and Ray [21] reported that $\alpha$-toxin possesses additional biological functions such as binding to a putative glycoprotein receptor on host cells, activation of intracellular signaling, and modulation of several processes.

It was recently described, that $\alpha$-toxin facilitates the secretion of newly synthesized chemokines into the airway and exaggerates neutrophil-mediated inflammatory lung injury [22].

Study of [23] and [24] uncovered a previously unknown *in vivo* function of $\beta$-toxin in pneumonia. $\beta$-toxin has been shown to maximize lung injury not through its cytotoxic activity, but rather through its capacity to enhance PMN infiltration in a syn-decan- 1-dependent manner.

The study show has been done by [25] the beta toxin is the hot-cold toxin because of its unique activity on sheep blood agar plates. At 37°C, beta toxin interacts with sheep red blood cells but does not lyse them. If the red cells are then placed at 4°C the cells lyse, this is observed as a lack of hemolysis on blood agar plates at 37°C and then complete hemolysis at 4°C.

A survey by [26] found that beta toxin was produced in 72% of bovine mastitis isolates, in 11% of healthy human nasal isolates, and in 13% of human septicemia isolates. Due to the likelihood of contamination from one or more cytolysins and the differential and species dependent susceptibility to beta toxin.

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