



## Molecular bacteriological study of the genes responsible for the production of hemolysin in *Staphylococcus spp* that cause urinary tract infections

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### Abstract

**Aims:** UTIs are a frequent health issue. females were more likely to be affected by human concerns than males. Rare *Staphylococcus spp* bacteria were identified and studied for virulence characteristics, such as the presence of hemolytic enzymes, by both phenotypic and genomic analysis.

**Methodology and results:** Urine was collected from 170 patients (100 females and 70 males) at Mosul General Hospital and Ibn Sina Teaching Hospital in Mosul city between (1/12/2021) and (30/3/2022), where phenotypic and partial detection of its virulence factors associated with the disease were conducted. Women were more likely to contract a urinary tract infection than men were; although men accounted for 29.4% of those infected, women accounted for 70.5%, and more than half of the bacteria isolated from female patients were Gram-negative. The bacterial isolates were primarily of Gram-positive bacteria, with *Escherichia coli* (30.36 percent), *Pseudomonas aeruginosa* (7.14 percent), *Kleipsella pneumoniae* (6.25%), *Proteus mirabilis* (2.6 percent), and *Cromobacterium violaceum* (2.6 percent) among the most common. The percentage of staphylococci where the coagulation test result was negative was (1.7%) and was due to 44.1% for staphylococcus aureus. *Staphylococcus haemolyticus* as a percentage. staphylococcus, epidermis (8.93), (9.82), and (2.68 xylosus) percent. Important bacteria like *E. faecalis*, *S. aureus*, *S. simulans*, *S. lugdunensis*, *Leuconostoc mesenteroides spp.*, and *S. hominis* were isolated from the rare bacteria that made up 0.89 percent of our study's total bacterial isolates

**Conclusion, significance and impact of study:** Hemolysin's damaging effect on kidney cells contributes significantly to urinary tract disease. The knowledge that it is produced by the *Staphylococcus* genus is crucial to understanding its etiology. There are three subtypes of hemolysin, each corresponding to a different gene: HLA for alpha hemolysin, HLB for beta hemolysin, and HLD for delta hemolysin. GeneKeywords: Genes, Molecular bacteriological, *Staphylococcus spp*, Urinary tract infections.

**Keywords:** molecular bacteriological, *Staphylococcus*, urine

### Introduction

Infectious diseases of the urinary tract impact 150 million individuals annually around the world (Flores-Mireles *et al.*, 2015) [7]. The bladder and the tissues around it get infected. UTIs are not just caused by bacterial infections. Viruses, fungi, and parasites can all cause UTIs. Although many cases of uncomplicated UTIs clear up on their own without treatment and the patient experiences no symptoms, many still choose for medical intervention. Frequent urination, suprapubic pain and discomfort, and dysuria are typical signs of this prevalent illness in women; treatment aims to stop the disease from spreading to the kidneys and developing to upper system disease and nephritis (Masajtis and Nowicki., 2017) [12]. Neglected instances of urinary tract infections (UTIs) are more likely to result in treatment failure or recurrence, and in the most extreme circumstances, death (Leslie and Sabih.,2021) [15]. The altered physiology of the elderly and those with incontinence makes them more susceptible to infection and environmental bacterial proliferation, which likely explains why these populations have a higher incidence of UTIs (Kim *et al.*, 2019) [10]. Urinary tract infections are a common cause of bacteremia, which requires systemic antibiotic treatment and can be fatal in frail elderly people due to urine

poisoning. According to research done by Fried *et al.* in 2001 [8], urinary tract infections are the second most common health problem among the elderly after lung infections. Age, gender, pregnancy status, geographic location, and water quality are only few of the factors that might affect the severity of an infection. However, the type of bacterium producing the infection and its virulence characteristics are the most important (Darouiche and Hull, 2012) [3].

### Materials and Methods

#### Isolation and diagnosis of bacterial isolates from UTI

From (1/12/2021) to (30/3/2022), 170 people in Mosul who sought medical advice at either Ibn Sina Teaching Hospital or Mosul General Hospital provided urine samples for analysis. Finally, after 24 hours incubation at 37°C, the samples were examined under the microscope and subsequently cultured on a preferred and diagnostic medium. Every colony's phenotypic characteristics were evaluated using the second each and every day, and several biochemical analyses were performed. and validated with a Vitic-2 instrument and an API assay kit. Comparable to Figure (1) and (2)

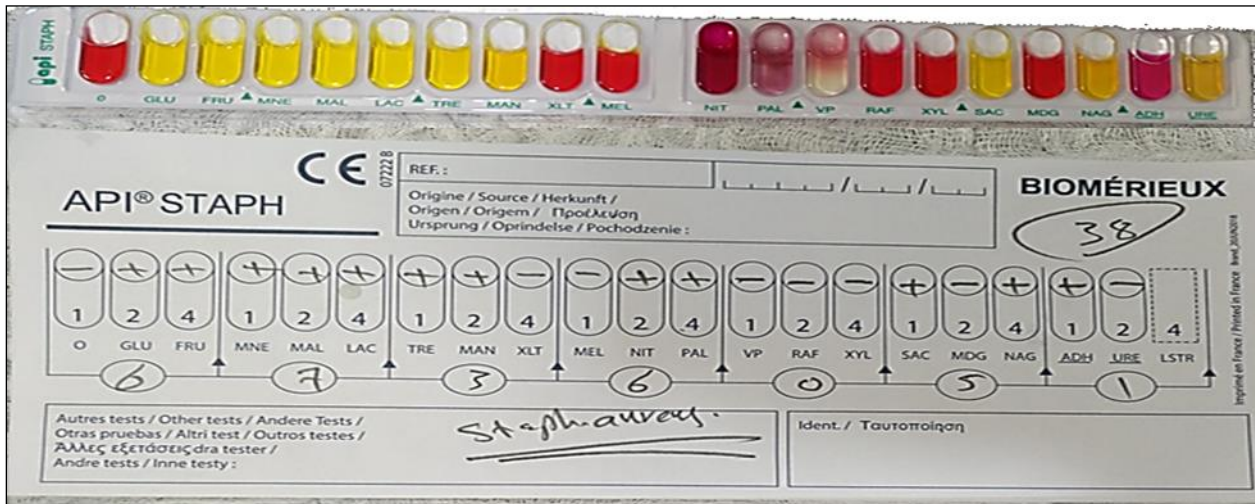


Fig 1: Diagnosis of Staph lugdunensis by vitek-2

Al-Mansur Laboratory  
Microbiology Chart Report

bioMérieux Customer: \_\_\_\_\_  
Patient Name: 71, 71  
Location: \_\_\_\_\_  
Lab ID: 382

Patient ID: 302  
Physician: \_\_\_\_\_  
Isolate Number: 1

Organism Quantity: \_\_\_\_\_  
Selected Organism : Staphylococcus lugdunensis

Source: \_\_\_\_\_ Collected: \_\_\_\_\_

Comments: \_\_\_\_\_

Identification Information	Analysis Time: 5.83 hours	Status: Final
Selected Organism	98% Probability Staphylococcus lugdunensis	
ID Analysis Messages	BiNumber: 010002006723231	

2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	+	14	CDEX	-	15	AspA	+	16	BGAR	+	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	+	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	+	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2a	-
64	OPTO	+															

Fig 2: Diagnosis of Staph lugdunensis by vitek-2

**DNA Extraction:** *Staphylococcus spp.* genomic DNA was extracted using a pre-made kit that was created by the (Junid) firm. The extraction method was carried out in accordance with the kit manufacturer's protocol.

**Electrophoresis and Polymerase Chain Reaction PCR Assay:** Electrophoresis was used to separate different sized

DNA molecules in accordance with Sambrook and Russell's methods for identifying virulence and resistance genes (2001) [16]. For your convenience, Scientific Researcher. Co. Ltd. has provided us with lyophilized powder (dry) versions of their primers in a range of picomole concentrations.

Table 1: Primer sequences that specifically target the genes in Staphelococcus spp species that produce hemolysin

gene		Primer sequence (5'-3')	Size Bp	References
HLA	F	CTGATTACTATCCAAGAAATTCGATTG	209	Jarraud <i>et al.</i> , 2002
	R	CTTCCAGCCTACTTTTTTATCAGT		

<b>HLB</b>	F	GTGCACTTACTGACAATAGTGC	309	Jarraud <i>et al.</i> , 2002
	R	GTTGATGAGTAGCTACCTTCAGT		
<b>HLD</b>	F	AAGAATTTTATCTTAATTAAGGAAGGAGTG	111	Jarraud <i>et al.</i> , 2002
	R	TTAGTGAATTTGTTCACTGTGTCGA		

The DNA sample and primer for each gene were combined with the Premix components in a 0.2 ml Eppendorf tube from the American firm promega, with the reaction volume set to 20 l, to create the master reaction mixes for each PCR reaction. The reaction tubes were then placed in a thermocycler with distilled water, and the transcription

reaction was carried out using specialized software. Then, we put Ladder DNA (provided by Biolaps) to one of the agarose gel wells containing 1% sample concentration. A device is used to analyze samples.

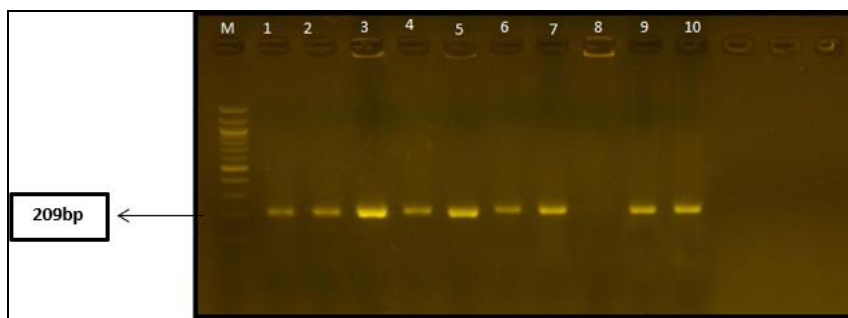
**Results and Discussion**

**Table 2:** shows the presence of genes responsible for the production of hemolysin enzyme in Staphylococcus spp.

virulence factor	Hemolytic genes	S. aureus (4)	S. Haemolyticus (2)	S.auriculari (1)	S. lugdunensis (1)	S. epidermidis (1)	S. simulans (1)
Haemolysin genes	HLA	100%	100%	100%	0 %	100%	100%
	HLB	50%	0%	0%	0%	0%	0%
	HLD	100%	100%	100%	100%	0%	100%

Ten different species of Staphylococcus were tested for the presence of the hla, hlb, and hld genes. isolates, plus two extra Staph. To the four S. isolated S. aureus strains (both mithicillin-resistant and -susceptible and a single strain of S. S. simulans and S. lugdunensis aureus. 90 percent of those infected with S. epidermidis and S. auricularis had the hla

gene and a 209 bp cross-product, as shown in table (2). (3). In contrast to the 50% of staphylococcus aureus bacteria that tested positive for the hlb gene and a reaction product of 309 (as shown in Figure), all of the isolates tested negative (4). As can be shown in Figure, all of the isolates were able to be positively identified as carrying the hld gene (5).

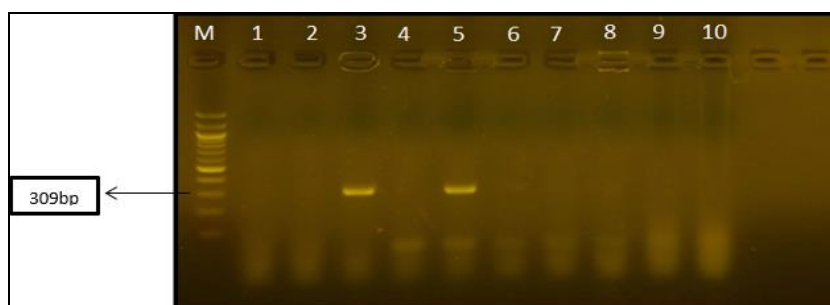


**Fig 3:** PCR reaction product electrophoresis to check for the presence of the hla gene

Our findings were consistent with those of (Ariyanti *et al.*, 2011) [1], who also found that 81.18 percent of staph aureus possessed the hla gene. While our findings coincided with those of the researcher (Fei *et al.*, 2011) [6] as the percentage of presence of the hlb gene was 42.60%, they did not coincide with those of the investigator (Ariyanti *et al.*, 2011) [1], whose study was conducted in Indonesia and found that the gene was present in only 18.18% of the population. Since d-haemolysis is only formed in the late exponential stages of growth, our findings on the hld gene corroborated those of the investigator (Divyakolu *et al.*, 2019) [5]. (Verdon *et al.*, 2009) [18].

It was found that the hld gene, which codes for the production of hemolysin, was present in both S.

haemolyticus and S. lugdunensis at identically high rates, while the hla gene was present in S. haemolyticus by 100% but not in S. lugdunensis, and neither species contained the hlb gene. Due to the fact that our findings matched those of the study done at Gleneagles Global Hospital in India/Hyderabad (Divyakolu *et al.*, 2019) [5]. According to our data, the hlb gene was not detected in staph haemolyticus, which is consistent with the findings of Brazilian researchers (Pinheiro *et al.*, 2015) [14], and our results for the hla gene were quite similar to theirs; the percentage of the presence of this gene was 91.7% in both species.

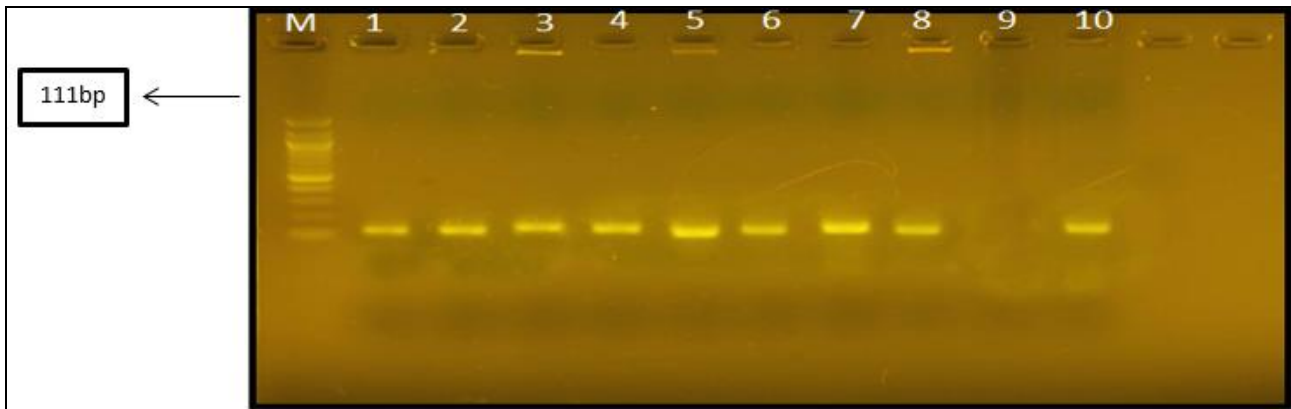


**Fig 4:** PCR reaction product electrophoresis to check for the presence of the hlb gene

*Staph. auricularis* bacteria were tested, and were found to have 100% *hla* and *hld* gene presence but 0% *hnb* gene presence. In contrast to the study by the investigator (Divyakolu *et al.*, 2019)<sup>[5]</sup>, our results were similar in terms of the existence of the *hld* gene, but different in terms of the *hla* gene, since it did not appear in the study.

*Staph. epidermidis* and *S. simulans* both had their *hla* genes identified, and the quality of their *hla* genes was also 100%, but neither strain had a *hnb* gene. Our findings agreed with the results of the investigator (Pinheiro *et al.*, 2015)<sup>[14]</sup> as the detection rate of *hla* and *hnb* genes in *Staph. epidermidis*

by 92.9%; however, the *hld* gene was only found in *Staph. simulans* and not in the *staph. epidermidis* bacteria. The hemolysin enzyme produced by coagulase-negative and coagulase-positive staphylococci is one of the most important virulence factors associated with pathogenesis; a study by researcher) Szemraj *et al.*, 2020<sup>[17]</sup> (in Poland found a 92.9 percent isolation rate of *hnb* and *hld* genes in *Staph. epidermidis*. Our results for *staph. simulans* differed from this, as no (Husmann *et al.*, 2009)<sup>[9]</sup>.



**Fig 5:** PCR reaction products electrophoresis to identify the presence of the *hld* gene

*Staph. auricularis* bacteria were tested, and were found to have 100% *hla* and *hld* gene presence but 0% *hnb* gene presence. As the alpha-enzyme secreted by bacteria isolated from urinary tract infections interacts with the surface receptors of the host cells to form small pores and works to release ions or influence the signals of host cells and stimulate cell death of various types of ce, our results were similar to the study of the investigator (Divyakolu *et al.*, 2019)<sup>[5]</sup> in terms of the presence of the *hld* gene, but it differed with it in terms of the absence of the *hla* gene (Liang *et al.*, 2009)<sup>[11]</sup>. As another example, the *hnb* gene's beta-encoded toxins have a potent analytic effect on RBCs (Dinges *et al.*, 2000)<sup>[4]</sup>. Since the *hld* gene's delta toxins are exchanged for their targets' membranes directly, even a low concentration of the toxin can cause a disruption in the membranes and eventually lead to cell lysis; the charge of peptides is a key factor in this process (Verdon *et al.*, 2009)<sup>[18]</sup>

### Conclusion

Since some species of non-coagulase-producing staphylococcus bacteria may have more than one gene responsible for hemolysis, this study provided a summary of their abilities to create hemolysin and hemolysis. It's not a bacteria, for sure. They are pollutants because they have virulence factors and can cause disease in those with compromised immune systems or diabetes.

### Acknowledgements

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