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RESEARCH ARTICLE

Staphylococcus haemolyticus Profiles that Carrier of IE Gene as Nosocomial Infection in a Hospital Environment

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ABSTRACT

In the presented study, 40 clinical *Staphylococcus haemolyticus* isolates were isolated from 200 patients admitted to Marjan Teaching Hospital in Babylon Governorate. Those (40) isolates were isolated from 15 males and 25 females from blood, urine and skin. The isolates were diagnosed as *S. haemolyticus* using the biochemical test, *API* Staph System, Vitek2 compact system and culture assays, and identified by polymerase chain reaction PCR. The biofilm-forming capacity was examined by the microtiter plate method. *S. haemolyticus* showed That the total number of isolates that formed biofilm is 33 distributed among 28 (70%) isolates that formed strong biofilm and 5 (12.5%) moderate, while 7 (17.5%) don't form biofilm. The virulence *atlE* gene was detected by polymerase chain reaction. *atlE* gene. PCR analysis of the virulence agent *atlE* gene in *S. haemolyticus* isolates revealed that 10 (25%) of bacteria isolates were positive for this gene, indicating a link between biofilm and the *atlE* gene.

INTRODUCTION

S. haemolyticus is the second most common CoNS isolate and it is considered one of the pathogens in hospitals, where hospital infections include sepsis, urinary tract infection, wounds, bones, arthritis, and others.[1, 2] The injury is usually associated with the insertion of foreign bodies, usually as some operations such as prosthetic limbs, intravascular catheters, artificial valves, and dialysis catheters.[3, 4] It is also characterized by a high ability to resist antibiotics and the formation of biofilms.[5]

S. haemolyticus is a Gram-positive, coagulase-negative bacterium inhabiting the skin of humans and mucous as a commensal M.O. It occurs mainly associated with BIs and medical device-associated infection.[6]

Staphylococcus haemolyticus can cause septicity by infecting the M.O. Part of the urinary tract, and women, especially pregnant women, are more susceptible to infection[7] because the urethra is shorter in them, allowing bacteria to enter the bladder more quickly.[8]

AtlE gene of genes encode the adhesive characteristics and bind to the host factors vitronectin through various cell wall proteins such *as AtlE and Fbe* as well as intercellular adhesion and fibrinogen.[9, 10]

Isolation of bacteria

150 samples were collected and distributed as follows (50 blood, 30 urine and 70 skin lesions). and cultured on blood aga for 24 hrs. at 37°C. The morphology of the colony, size, shape, color, biochemical tests, API staph and Vitek2 compact system was used to identify the isolated bacteria.

Biochemical tests

A group of biochemical tests were carried out to investigate the bacteria, and these tests are an examination of oxidase,[11]

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catalase,[12] coagulase test[13] and hemolysis test.[14]

- Oxidase Test: A single colony of bacteria were mixed with a few drops of the oxidase reagent on filter paper using a sterilized wooden stick. A positive reaction was then shown by the appearance of a purple color within 10 - 30 seconds.[11]
- Catalase Test: one or two drops of catalase reagent (H2O2) were put on a single colony of bacteria. (O2 + H_2O = bubbles) was a positive outcome.[12]
- Coagulase test: This test is used to identify the different forms of Staphylococcus spp. An enzyme-like protein called coagulase is able to clot plasma.[13]
- Hemolysis Test: The bacterial colony was observed and cultured on the blood agar plates for 24 to 48 hours at 37°C. As a result of the full lysis of RBCs, ß-hemolysis results in a clear circular zone surrounding the bacterial colony, whereas α-hemolysis appears to result in a partly clear zone and frequently green coloration surrounding the bacterial colony.[14]

Identification of bacteria by API Staph System

This technique is employed to identify *S. hemolyticus* members. Twenty biochemical tests were contained in the strip's tiny tubes, microtubes equivalent to 0.5 McFarland were produced and filled with bacterial suspension. After that, they were incubated at 37°C for 18-24 hours. Color in a microtube would alter during incubation due to physical, and metabolic processes or the addition of chemicals. The test's outcome is then read aloud. Following both positive and negative test findings, a seven-digit profile number was established by looking up the profile number in the API Staph Index. The microbe was then identified using these numbers.[11, 14]

Identifying biofilm

Utilizing a microtiter plate reader, this method is used to estimate biofilm generation. According to an author[15] Classification of biofilm formation as strongly positive (OD570 ≥ 0.24), weakly positive (0.12 ≤ OD570 < 0.12), or negative (OD570 < 0.12)

Molecular study

The DNA from *S. haemolyticus* isolates was extracted using the Presto TMMini DNA bacterium Kit Quick methodology (Geneaid, Canada) as shown in Table 1, primers for polymerase chain reactions (PCR) were designed using the online NCBI Genbank sequence database design program and manufactured by Alpha DNA, Canada (1).

Table 1: The particular primers and their order

Primer Name	*Sequences 5'-3'*	*Size (bp)*
16S rRNA	TCTTGCCATCAGATGTGCCC	250
	TAACCACAACACCTTCCTCCCC	
Atl	ACGCTGATTATGCTGCAAC	230
	CCAAGGTGCTACTTGCTTC	

RESULTS AND DISCUSSION

Distribution of patients according to age

Table 2 shows the distribution of patients infected with *S. haemolyticus* according to age, where the mean of age appeared at (40.47), the maximum age was at 62 years and the minimum was at 23 year.

In the present study, we found the mean of age at (40.47 year) in patients infected with *S. haemolyticus*, these results was consistent with Denkinger *et al.* who reported that the patients in this age and older are one of the hospital's main reservoirs for multidrug-resistant infections such as *S. haemolyticus*,[16] in addition Garcia *et al.*[17] reported that as people age, the prevalence of isolates resistant to antibiotics that target DNA synthesis increases. As a preventative step to lower the incidence of resistant infections in each susceptible population, they emphasize the significance of patient age in the selection of antibiotics.

Distribution samples with the site of infection

A total of 200 swab samples have been selected from blood, urine and skin. Of those 200 samples, only 40 (20%) samples were found to be *S. haemolyticus*. The urine and skin were the most frequent where the samples were collected, which represented 25 (62.5% skin) and 12 (30% from urine). Only 3 isolates were collected from a blood sample as shown in Table 2.

The results of the current study revealed a high rate of *S. haemolyticus*. The increased prevalence of *S. haemolyticus* as the causative agent of hospital infections may increase the possibility of antibiotic resistance.[18] On the other hand, it was mentioned that based on data, these pathogens were responsible for a 10-fold greater prevalence of infections in hospitalized patients.[19]

The 40 strains were isolated from 15 males and 25 females, as shown in Figure 1. We note that females are more infected with bacteria, and this is likely due to the nature of their continuous dealing with contaminated places such as floors and walls and when visiting hospitals during pregnancy or health care centers when following up on the growth and vaccinations of children.[20]

Biofilm production detection

A total of 40 isolates were tested for biofilm formation in this study. *S. haemolyticus* formed a strong biofilm in 28 (70%) of the

Table 2: Distribution of patients infected with *S. haemolyticus* according to the age

The Age of	N	Minimum	Maximum	Mean		Std.
				Statistic	Std. Error	Deviation
patients	15	23	62	40.47	3.159	12.235

Table 3: Frequency distribution of the total *S. haemolyticus* isolated from the whole samples

Type of sample	*No. of S. haemolyticus*	*%*	*No.% from 200 samples*
skin	25	62.5	12.5
urine	12	30	6
blood	3	7.5	1.5
total		100	20

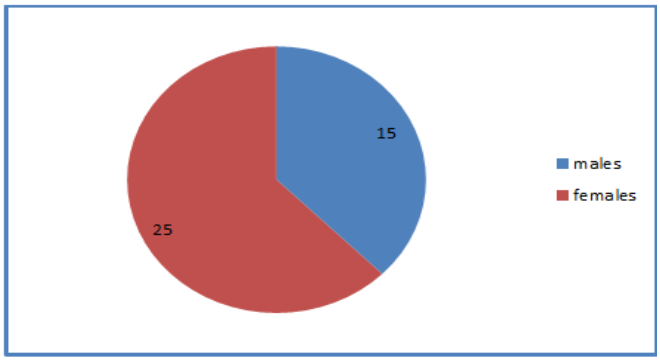

Figure 1: distribution of the total S. haemolyticus isolated according to gender.

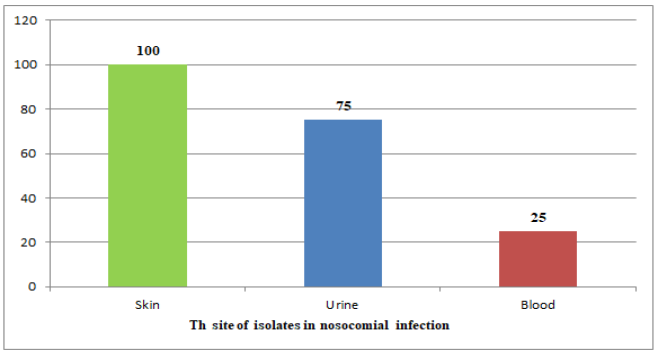

Figure 3: Distribution of the *S. haemolyticus* isolated according to the site of infection

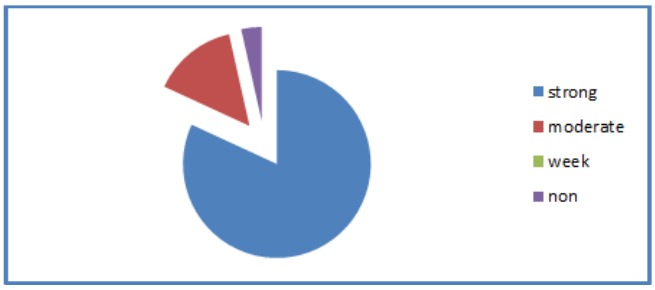

Figure 2: Biofilm results of the isolated bacteria.

cases and a moderate biofilm in 5 (12.5%). Figure 2 shows that 17.5% of seven *S. haemolyticus* isolates never formed a biofilm.

Numerous bacterial pathogens, including *S. haemolyticus, A. baumannii,* and *E. cloacae*, can grow as scattered (planktonic) cells or as matrix-enclosed colonies known as biofilms.[21] The findings of the current investigation were consistent with,[22] who showed that the proclivity of coagulase-negative staphylococci to form biofilm causes CoNS to form most frequently after medical equipment installation. *S. haemolyticus* is the second most common CoNS found in people with hospital-acquired illnesses. When the biofilm-forming abilities of 72 clinical *S. haemolyticus* isolates were investigated, 53 (74%) of the isolates formed; nevertheless, it was proven that all isolates of *S. haemolyticus* have the potential to create biofilms. The findings were consistent with previous reports that clinically significant and contaminated isolates of *S. haemolyticus* were obtained.[23]

Distribution of the *S. haemolyticus* isolated according to the site of infection

Figure 3 shows the distribution of the *S. haemolyticus* isolated according to the site of infection, where the highly percentage was in skin at 100%, followed the urine at 75%, then 25% for blood sample.

Our result shows that most patients infected with *S. haemolyticus* isolated from skin at 100 samples. These results reflect that most of the isolates of this bacteria were isolated from the skin because the skin is more in contact with the hospital environment, contaminated surfaces and tools contaminated with this bacteria. The researcher indicates Krzymińska *et al*.,[24] who reported that the infection by *S. haemolyticus* strains is clearly increasing in the skin and also in the urine, while its access to the blood is less due to the presence of phagocytes and other immune cells clearly. Frequency distribution of the total *S. haemolyticus* isolated from the whole samples (Table 3).

The correlation between the age and the site of infection

Figure 4-A,4-B and 4-C shows the correlation between the age and the site of infection in patients infected with *S. haemolyticus*, where the high percentage of infection in the skin showed at age (35 years), while the lowest infection showed at age (62 years). In addition, the highest percentage of infection in urine showed at age (35 years), while the lowest infection showed at age (32 years). Finally, the high percentage of infection in blood showed at age (35 & 37 years), while the lowest infection showed at age (62 years).

Prevalence of hospital-*Staphylococcus* associated infection, particularly coagulase-negative *S. haemolyticus*, is increasing. In this present study, *S. haemolyticus* isolates were predominantly found (more than 200 samples) in different clinical samples, the most aged infection with the *S. haemolyticus* (35–45 years), Figure 4a,b. The researcher mentioned Ghaed'a *et al.*,[25] who explained that the highest infection rate was at the age of 30 to 39 years, at a rate of 64% for bacteria samples isolated from different parts of the body. In addition, the characteristics of the opportunistic pathogens which were previously considered as normal flora, commensally found in human skin, urine, particularly in the axilla[26] in addition the patients infected with *S. epidermidis* and *S. haemolyticus* is recently considered as the three most frequently etiologic agents of *Staphylococcus*-associated infections.[27] Also, *S. haemolyticus* strains have the ability to develop multiple resistance to a wide range of antibiotics, and thus its areas of spread within the body vary and increase in areas that are in direct contact with areas contaminated with bacteria, which leads to an increase in its clinical importance that exceeds the spread of other types of *Staphylococcus*.[28]

The correlation coefficient between the sites of infection and age

Table 4 shows the correlation coefficient between the sites of

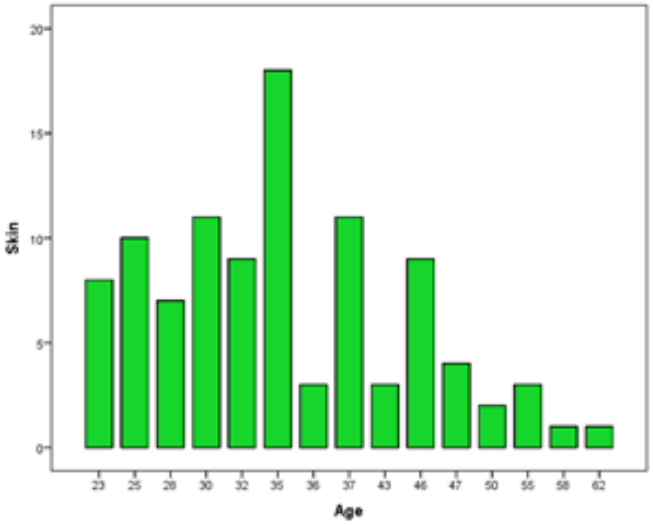

Figure 4-A: Correlation between the age and skin in patients infected with *S. haemolyticus*

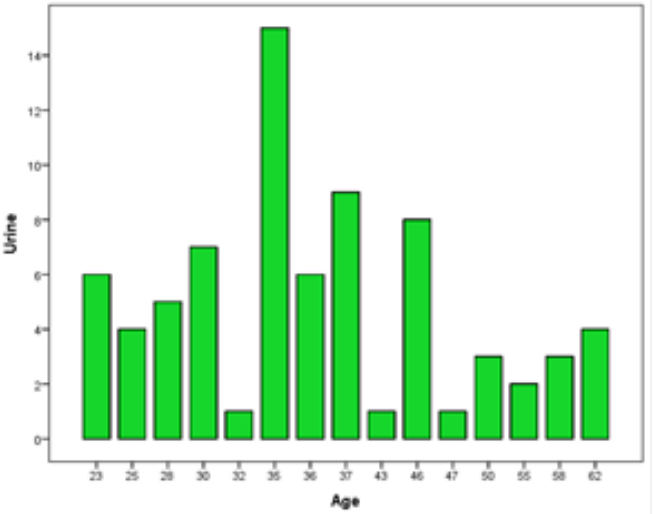

Figure 4-B: Correlation between the age and urine in patients infected with *S. haemolyticus*

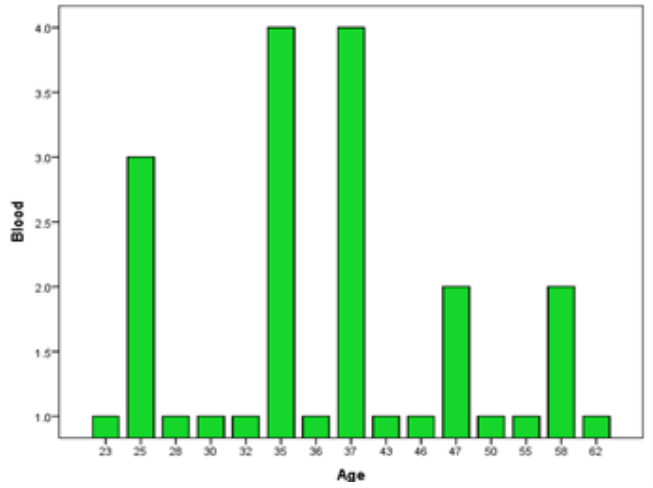

Figure 4-C: Correlation between the age and blood in patients infected with *S. haemolyticus*

infection, where there are significant differences between age and skin at (0.653), between skin and urine at (0.767), skin and blood at (0.606), a correlation between urine and age at (0.767), between urine and blood at (0.580), the statistical analysis makes at probability level ≤0.01 and ≤0.05.

S. haemolyticus is noted to be found colonizing not only the skin, but also the urethra and periurethra of both males and females.[29] Table 4 shows the correlation coefficient between the *S. haemolyticus* and the site of infection, which was found to highly correlate in urine at 0.767, where some reports indicated the diagnosis of *Staphylococcus* bacteria with many patients who were suffering from epididymitis, and the culture of the urine sample on nutritious bacterial media showed its presence in large numbers, especially in urine samples and bladder of patients with marbled cystitis.[30,31] Also, *S. haemolyticus* has been identified as a frequent cause of bacteremia, cited in one study as the most common. A bloodstream pathogen in probable or probable bloodstream infections.[32]

Molecular study

Molecular detection of 16S rRNA

S. haemolyticus was identified by PCR, as indicated in Figure 3.

AtlE gene detection in S. haemolyticus

As indicated in Figure 4, agar gel electrophoresis was performed as a result of PCR analysis of the virulence agent *atlE* gene in *S. haemolyticus* isolates, revealing that 10 (25%) of bacterium isolates were positive for this gene.

The outcomes were *S. haemolyticus* isolates were positive for the *atlE* gene in 10% (25%) of cases. This information was

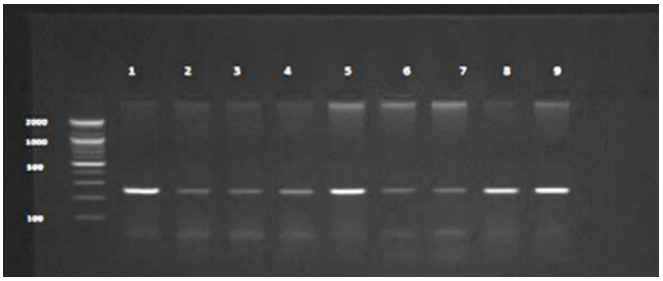

Figure 5: Images from agarose-gel electrophoresis revealed the PCR result. *S. haemolyticus* 16S rRNA gene investigation Lane (M): marker ladder (100–2000 bp), positive 16S rRNA gene at 250bp. Size of the PCR result.

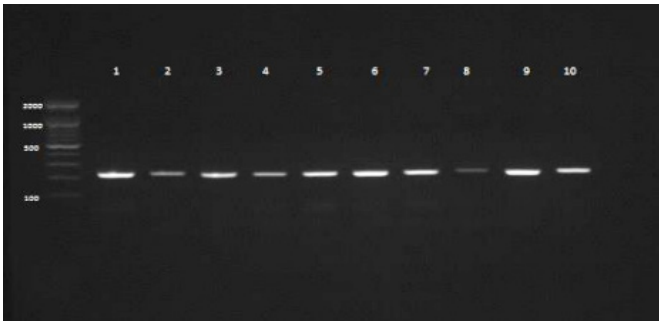

Figure 6: Agarose gel electrophoresis revealed a result of PCR investigation of the virulence factor AtlE gene in *S. haemolyticus* isolates. Lane (M) (100–2000 bp), lane (1- 10): revealed positive AtlE gene at 230 bp PCR product size.

Table 4: The correlation coefficient between the sites of infection and age

Correlations					
		Age	*Skin*	*Urine*	*Blood*
Age	Pearson Correlation	1	-.653-**	-.312-	-.177-
	Sig. (2-tailed)		.008	.258	.529
	N	15	15	15	15
Skin	Pearson Correlation	-.653-**	1	.767**	.606*
	Sig. (2-tailed)	.008		.001	.017
	N	15	15	15	15
Urine	Pearson Correlation	-.312-	.767**	1	.580*
	Sig. (2-tailed)	.258	.001		.023
	N	15	15	15	15
Blood	Pearson Correlation	-.177-	.606*	.580*	1
	Sig. (2-tailed)	.529	.017	.023	
	N	15	15	15	15

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 5: Correlation between the ability of bacteria to form a biofilm and to have an atlE gene

No. of isolates from biofilm	*No. of isolates have atlE gene*	*No %*
28 strings	8	20%
5moderate	2	5%
7 non	0	0
total	10	25%

consistent with,[33] *S. haemolyticus* was discovered to carry *fbe* and *atlE* genes (Figures 5 and 6).

Furthermore,[34] observed that 16.7% of CoNS contained the *embp* gene, which is similar to the current study results and attained by an author[35, 36], who discovered that the ratio of total biofilm genes *atlE*, *aae*, *embp*, and *fbe* ranged from 74 to 100%.

Correlation between the ability of bacteria to form a biofilm and to have an atlE gene

The total number of isolates that formed biofilm is 33 distributed among 28 isolates that formed strong biofilm and 5 media, and when investigating the gene, it was found that 10 isolates possess this gene distributed among 8 from the group of strong biofilms and 2 media as shown in Table 3.

Biofilm development begins with proteins expressed on the bacterial cell wall, such as autolysin *atlE* and fibrinogen binding protein *fbp*.[37] The current study, on the other hand, contradicted the findings of,[38] who found a low frequency of *fbe* and *atlE* 30% genes (Table 5).

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