Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.11.009

Expression of hemolysin specific for S. aureus in different bacterial species isolated from variant clinical sources

Zeena Hashim Abd Al- Wahid [1], Munaff Jawdat Abd Al- Abbas[2*]

[1,2]Department of Biology, College of Science, University of Basrah, Basrah, Iraq

***Corresponding author:** Munaff Jawdat Abd Al- Abbas, Department of Biology, College of Science, University of Basrah, Basrah, Iraq, Email: munaff.abdalabbas@uobasrah.edu.iq

Submitted: 24 March 2023; Accepted: 17 April 2023; Published: 09 May 2023

ABSTRACT

Staphylococcus aureus is the main source of the virulence factors, particularly hemolysins causing serious and fatal infections. The hemolysins were overexpressed in several bacterial species from various clinical sources indicating the actual problem of gene transfer. The highest expression level was detected in the hld gene comparison to hla, hlb and hlg. Furthermore, they all showed variable expression levels depending on the bacterial species and isolation sources. Finally, the presence of the hemolysin substrate such as blood in the bacterial culture media increased the expression level.

Keywords: *Hemolysins, Bacterial, Gene*

INTRODUCTION

Staphylococcus aureus is a human pathogens that causes disease ranging from chronic to life threating infections including bacteremia, skin and soft tissue infections, endocarditis, arthritis, osteomyelitis, pulmonary infections, gastroenteritis, meningitis, urinary tract infections and toxic shock syndrome (Bartlett, 2008; Monecke et al., 2014; Mahdi et al., 2021). It is possible for S. aureus to successfully colonize in a wide range of environments, inanimate hosts or matrices due to their various virulence mechanisms through a global regulatory system that includes the accessory regulatory gene (Agr) and the sigma factor (B), S. aureus has the capacity to regulate the expression of virulence factors in accordance with the environmental conditions in which it is present, since the ability of S. aureus to adapt to various microenvironments with various environmental, nutritional and stress conditions may result in the acquisition of genes coding for virulence factors that allow its survival in addition to gene regulation (Kong et al., 2016). As a result to horizontal gene transfer, S. aureus becomes a source for their specific hemolysin in other bacterial species (Abd Alwahid and Abd Al abbas, 2023). S. aureus has a many virulence factors that cause invasion of host including hemolysin encoded by hla, hlb, hld, hlgA, hlgB, and hlgC having the main role for colonization and pathogenicity (Dekker et al.,2016). Alpha hemolysin is a pore-forming toxin that is heptamerized and has the ability to lyse many types of mammalian cells since it has hemolytic, dermonecrotic and neurotoxic activity, the toxin is coded by the *hla* gene located on the chromosome (Dings *et al*., 2000). Beta hemolysin is a sphingomyelinase in *S. aureus* that helps to form biofilm by host skin attachment and is encoded by the *hlb* gene (Gordon and Lowy, 2008). Gamma hemolysin is a pore-forming toxin that is lyse the white and red blood cells. Furthermore, γ-toxin caused toxic effects against phagocytic cells at the site of infection to evade

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

the immune system, the toxin consists of two subunits including the F and S unit (Blake *et al*., 2018; Tarenzi *et al*., 2022). Delta hemolysin, also called delta lysin, is a peptide toxin consisting of 26 amino acids produced by some strains of *Staphylococcus*. It is encoded by the *hld* gene a part of an accessory gene regulator related to phenol-soluble modulins (PSMs), which is the family of peptide toxin (Recsei *et al.*, 1986; Zhou *et al*.,2021). The toxin has several functions, including the lysis of erythrocytes, the toxic effect on other bacterial cells by the targeting of protoplasts and spheroplasts, disrupting the mast cells leading to the development of atopic dermatitis and inducing biofilm formation (Nakamura et al.,2013; Otto, 2014).

The study aimed to examine if there is a difference in production level of hemolysin according to the different bacterial species and\ or different bacterial sources.

MATERIALS AND METHODS

The present study is focused on the bacterial isolates (n=48) from variant clinical sources (Abd Al-Wahid and Abd Al-Abbas, 2023).

Bacterial RNA extraction

RNA extraction was begin after a single colony of bacterial isolate (n=48) activated in Brain heart infusion broth (TM, India) and incubated at 37°C for 18 h according to the procedure of the GENEzol™ TriRNA Pure kit (Geneaid, Taiwan). All steps of extraction was performed on -4°C. The concentration and quality of RNA was measured by Nanodrop spectrophotometer (Avans Biotechnology Inc., Taiwan) and the A 260/ A 280 ratio was calculated after standard the blank with RNase free water.

Stimulation of hemolysin production in bacterial isolates (New experiment)

Bacterial isolates of S. aureus were stimulated by culturing in semisolid blood agar (3.5gm of agar / L) and incubated at 37◦ C for 18 h. Bacterial growth were isolated by centrifuging for 30 min, RNA extracted as mentioned above.

cDNA synthesis by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total RNA (400 ng) from each sample was transformed to a cDNA using Accupower® Rocket script™ RT PreMix kit (Bioneer, Korea). Total RNA and nuclease free water (Bioneer, Korea) were added to the cDNA master mix tubes and mixed well by mini vortex then placed in thermal cycler (Bioneer, Korea) for 10 min at 37◦C, 1 hr. at 60◦C and 5 min at 95◦C.

Estimation of genes (hla, hlb, hlg and hld) expression levels

Real time-PCR was performed to estimate the expression of hla (alpha), hlb (beta), hlg (gamma) and hld (delta) genes using the primers listed in the Table (1). The 16s rRNA gene was used as a housekeeping gene. All genes were amplified by SYPR green dye as Zhang et al.,2016; Cafiso et al., (2012). Each sample had three technical repeats and contains reagents as following: 10 µl of Go Taq qPCR master mix (Promega, USA), 1 µl from each forward and reverse primer (Alpha, USA), 1 µl of cDNA and 7 µl of Nuclease free water (Bioneer, Korea) that is mixed well and subjected to Accurate x96 thermal cycler (D-lab, USA). The program used to amplify hemolysins were included one cycle at 94◦C for 3 min. 45 cycles at 94◦C for 30 sec, 52◦C for 30 sec and 72◦C for 40 sec. The obtained qPCR data were analyzed using the ΔCT method as the CT values for each gene (hla, hlb, hlgC and hld) as well as for the house keeping gene (16S rRNA) as Livak and Schmittgen,(2001).

TABLE 1: Primers for amplifying hemolysins genes and their length

No.	primer	Primers Sequence	Primer Length (b)
1	F - 16	5-TGAGATGTTGGGTTAAGTCCCGCA-3	24
	R - 16	5-CGGTTTCGCTGCCCTTTGTATTGT-3	24
2	F-hla	5-ATGGTGAATCAAAATTGGGG-3	20
	R-hla	5-GTTGTTTGGATGCTTTTC-3	18

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

3	F -hlb	5-GCCAAAGCCGAATCTAAG-3	18
	R -hlb	5-CGAGTACAGGTGTTTGGT-3	18
4	F- hlg C	5-CTCTTGCCAATCCGTTATTA-3	20
	R - hlg C	5-GCTTTAACATGATTAGTTTT-3	20
5	F- hld	5-CTGAGTCCAAGGAAACTAACTCTAC-3	25
	R - hld	5-TGATTTCAATGGCACAAGAT-3	20

RESULTS

Expression level for hemolysins genes

The amplification curves for the hemolysins hla, hlb, hlgC, hld and 16S rRNA were shown in Figure(1).

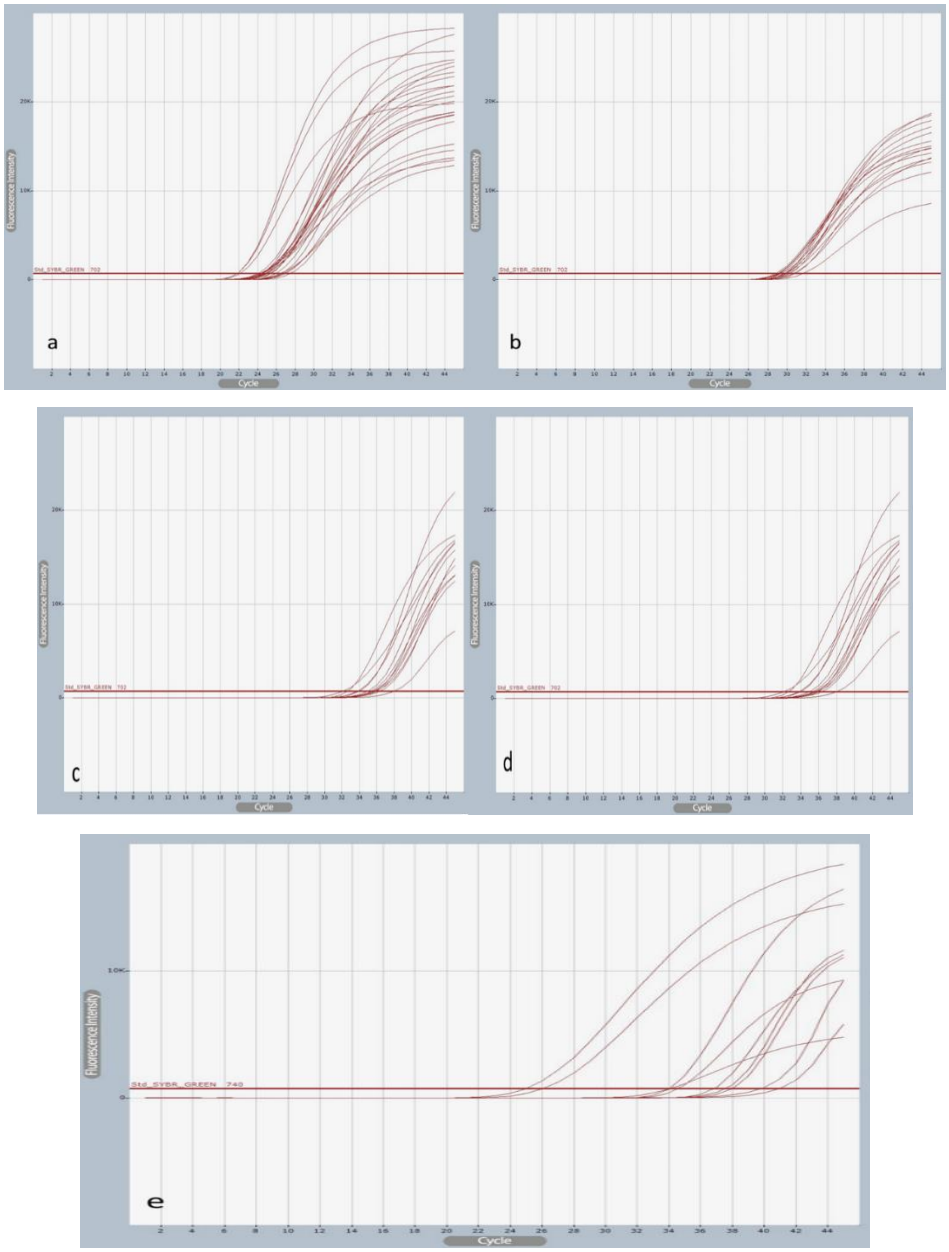

FIGURE 1: Amplification curves of hemolysins using SYBR Green chemistry. A: Amplification curve of 16S rRNA gene, B: Amplification curves of hlb gene, C: Amplification curves of hla gene, D: Amplification curves of hlgC gene and E: Amplification curves of hld gene.

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

hlb expression levels from different isolation sources

The expression of the hlb gene was 6.09362, 9.65, 9.417 and 115.7357 fold more than control for P. aeruginosa, S. aureus, S. epidermidis and gram negative bacteria including E. coli and K. pneumonia respectively (Figure 2).

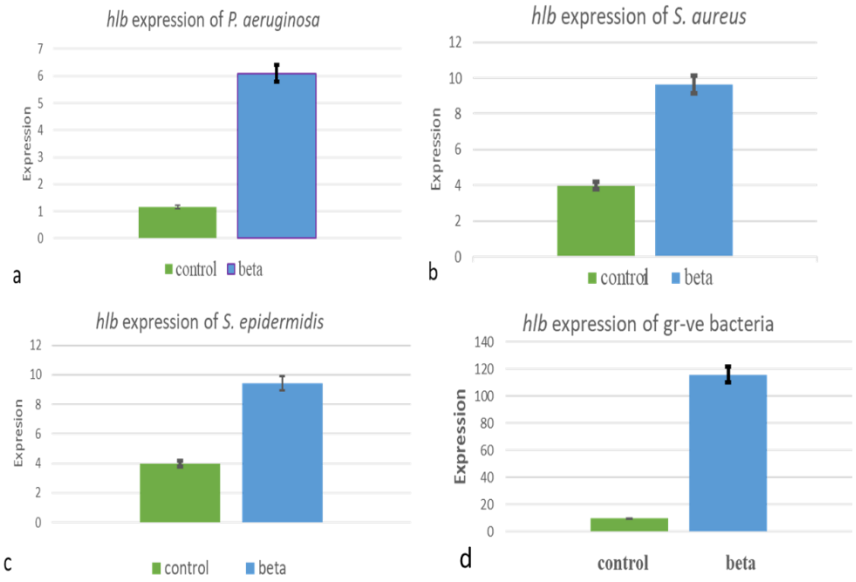

FIGURE 2: Expression levels of hlb in comparison with control. A: hlb of P. aeruginosa, B: hlb of S. aureus, C: hlb of S. epidermidis and D: hlb of E. coli and K. pneumoniae.

The expression level of hlb for P. aeruginosa was differed depending on the source. Wound swab showed the higher level, followed by burn swab, stool and blood with no significant differences at $P \leq 0.05$ as Table (2).

TABLE 2: hlb expression of P. aeruginosa according to their sources

Bacterial species	No. of isolates	hlb expression	Source of isolates
P. aeruginosa	23	0.070316	Stool
	63	0.105843	Wound swab
	76	0.087171	Burn swab
	95	0.06983	Blood
hlb expression = 6.09362			

$p \leq 0.05$

The *hlb* levels of *S. aureus* from burn patients were 7.780 which significantly differed from other sources including ear infections(0.004158) and body fluid (0.000367). *S. epidermidis* showed a variant levels of expression with the variance of the isolation sources, burn patients (0.03516) followed by blood infections (0.02241), nasal swab (0.00205) and (0.00028) without significant difference (Table 3).

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

TABLE 3: hlb expression level of S. aureus and S. epidermidis according to their sources

Bacterial species	No. of isolates	hlb expression	Source of isolates
S . aureus	1	0.000367	Aspirate
	11	0.004158	Ear swab
	102	7.780	Burn swab*
hlb expression = 9.65			
S. epidermidis	28	0.00028	Nasal swab
	49	0.00205	
	67	0.02241	Blood
	70	0.03516	Burn swab
hlb expression = 9.417			

p≤0.05

The estimated level of *hlb* hemolysin for *E. coli* isolated from urine (5.21103, 1.16444, 0.000182 and 0.000113) was significantly higher than stool (0.000125). On the other hand, *K. pneumonia* showed a close expression level for wound swab (0.00023) and stool (0.00011) with no significant difference at P ≤ 0.05 (Table 4).

TABLE 4: hlb expression for E. coli and K. pneumonia according to their sources

Bacterial species	No. of isolates	hlb expression	Source of isolates
K. pneumonia	3	0.00023	Wound swab
	21	0.00011	Stool
E. coli	9	5.21103*	Urine
	15	1.16444*	
	5	0.000182	
	57	0.000113	
	33	0.000125	Stool
hlb expression= 115.7357			

p≤0.05

hlb expression from burn clinical isolates

The expression of the *hlb* gene in different bacterial species isolated from burn infections was estimated to be two-fold higher than control (Figure 3). There were no significant differences between the bacterial species expression of E. hormaechei (0.00826), S. hominis (0.01379), E. faecalis (0.03191), 2 of P. aeruginosa (0.04329 and 0.03039), K. pneumonia (0.05219), S. epidermidis (0.25349), A. baumannii (0.14063) and S. aureus (0.01097) as Table (5).

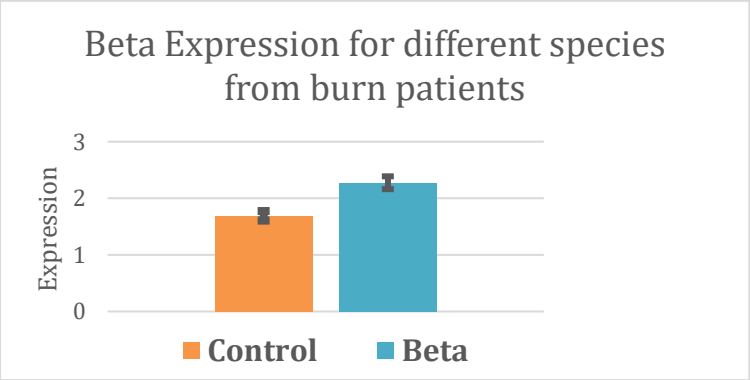

FIGURE 3: Expression levels of hlb for bacterial species from burn patients

J Popul Ther Clin Pharmacol Vol 30(11):e69–e80; 09 May 2023.
This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

TABLE 5: hlb expression for different bacterial species from burn patients

Source of isolates	No. of isolates	Bacterial species	hlb expression
Burn swab	74	E. hormaechei	0.00826
	75	S. hominis	0.01379
	78	E .faecalis	0.03191
	81	P. aeruginosa	0.04329
	84	K. pneumonia	0.05219
	86	S. epidermis	0.25349
	88	P. aeruginosa	0.03039
	90	A. baumannii	0.14063
	101	S. aureus	0.01097
hlb expression = 2.274			

$p \leq 0.05$

hla expression

The expression level of hla for S. aureus was 7.03588 fold more than control (Figure 4). The level of hla differs with the difference of S. aureus isolation sources but with no significant differences including aspirate (0.05633), body fluid (0.08077), ear swab (0.00062), nasal swab (0.00338) and burn swab (0.01023) as Table (6).

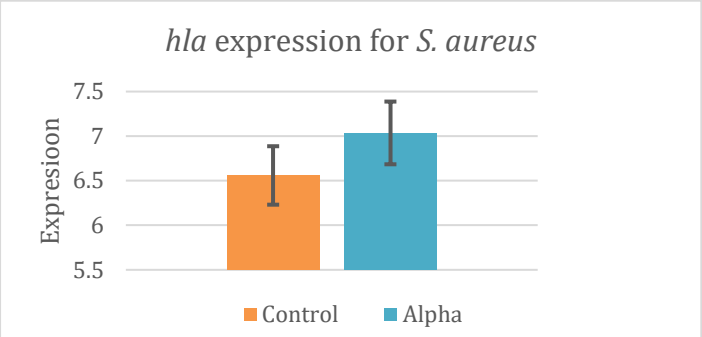

FIGURE 4: hla expression level for S. aureus.

TABLE 6: Expression level of hla for S. aureus according to the sources

Bacterial species	No. of isolates	hla expression	Source of isolates
S . aureus	1	0.05633	Aspirate
	4	0.08077	Body fluid
	11	0.00062	Ear swab
	20	0.00338	Nasal swab
	108	0.01023	Burn swab
hla expression =7.03588			

$p \leq 0.05$

The hla level was 5.883 fold higher than the normal form (Figure 5). It was varied for variant bacterial species in spite of all these bacterial species isolated from burn patients. Since, A. baumannii (8192) was the first followed by E. hormaechei (0.04481), P. aeruginosa (0.03467) , S. hominis (0.00808) S. aureus (0.00269) and S. haemolyticus (0.00116) with significant differences at $p \leq 0.05$(Table 7).

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

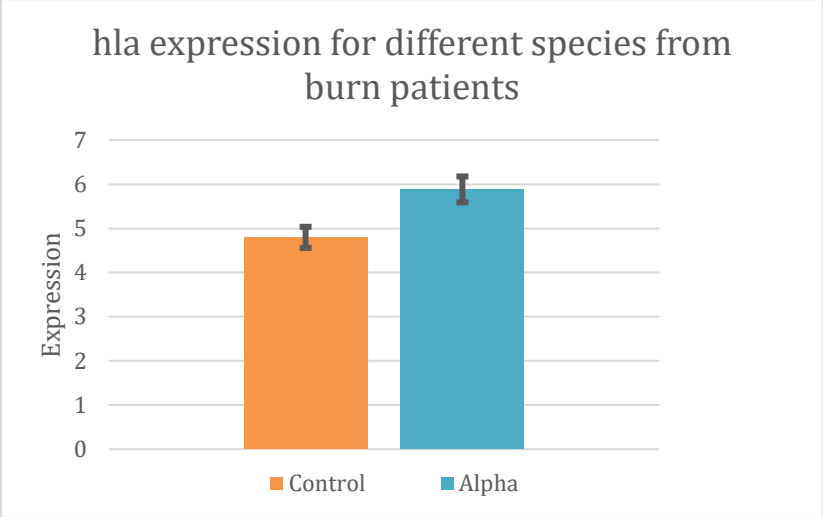

FIGURE 5: hla expression of bacterial isolates from burn patients.

TABLE 7: hla expression of different bacterial species from burn patients

Source of isolates	No. of isolates	Bacterial species	hla expression
Burn swab	73	S. hominis	0.00808
	79	S. haemolyticus	0.00116
	80	A. baumannii	8192*
	82	P. aeruginosa	0.03467
	105	S. aureus	0.00269
	109	E. hormaechei	0.04481
hla expression = 5.883			

$p \leq 0.05$

hlgC expression level

The hlg expression of E. faecalis was 8.9499, followed by S. aureus (4.451) and P. aeruginosa (1.376) when they were compared with control (Figure 6).

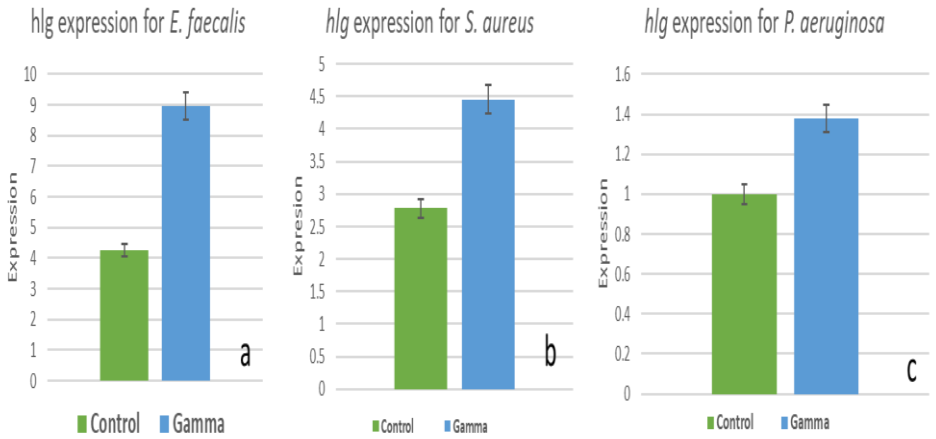

FIGURE 6: Gene expression level of hlgC and controls. (a) expression of hla for E. faecalis (b) S. aureus (c) P. aeruginosa ($p \leq 0.05$).

J Popul Ther Clin Pharmacol Vol 30(11):e69–e80; 09 May 2023.
This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

The expression of hlgC was differed depending on the isolation source (Table 8).In detail, E. faecalis showed higher expression in sore throat (7.41) followed by burn swab (2.13), wound swab of isolate No. 61 (0.00724), urine (0.00044) and wound swab of isolate No. 10. In spite of that, *S. aureus* showed closer expression in aspirate and burn swab without significant differences ($p \leq 0.05$).

TABLE 8: hlg expression of different bacterial species according to their sources

Bacterial species	No. of isolates	hlgC expression	Source of isolates
E .faecalis	10	0.00033	Wound swab
	16	0.00044	Urine
	19	7.41	Sore throat*
	61	0.00724	Wound swab
	78	2.13	Burn swab*
hlg expression = 8.9499			
S. aureus	1	0.06381	Aspirate
	102	0.00501	Burn swab
hlg expression = 4.451			

$p \leq 0.05$

Similarly, P. aeruginosa produced hlgC at a variant level despite being isolated from the same sources without significant differences (Table 9).

TABLE 9: hlg level of P. aeruginosa

Bacterial species	No. of isolates	hlgC expression	Source of isolates
P. aeruginosa	6	0.00329	Ear swab
	23	0.00105	Stool
	63	0.03125	Wound swab
	76	0.04639	Burn swab
	88	0.00067	
	96	0.11111	
	94	0.00089	Blood
	98	0.00113	
hlg expression = 1.376			

$p \leq 0.05$

hld expression

The hld expression level of twenty bacterial isolates was equal to 8604.077 in comparison with a control of 7.964 (Figure 7). P. aeruginosa showed there was significant differences in hld expression levels when it was isolated from wound swab (1.3944), burn swab (0.00043 and 9.0729) and blood (3.1594 and 3.1594) as Table (10).

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

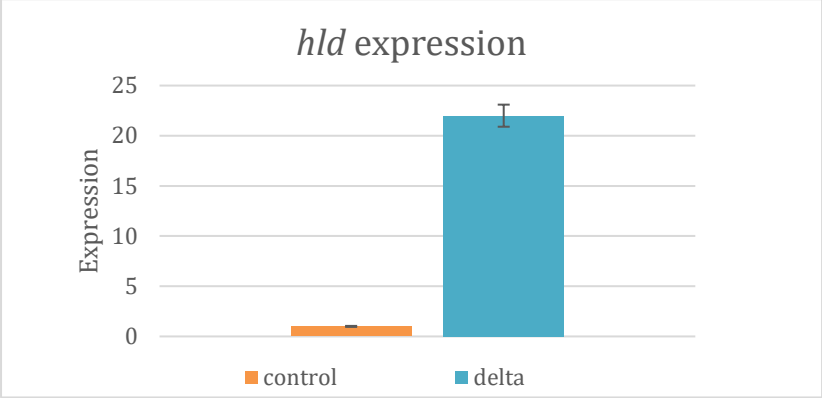

FIGURE 7: hld expression level of in comparison with control .

TABLE 10: hld expression of P. aeruginosa according to their sources

Bacterial species	No. of isolates	hld expression	Source of isolates
P. aeruginosa	63	1.3944	Wound swab
	76	0.00043	Burn swab
	96	9.0729*	
	94	3.3907	Blood
	98	3.1594	

p≤0.05

At the same time, the hld level of other bacterial species showed differences when the isolation sources was different as Table (11). E.coli showed the higher expression followed by the other bacterial species including E. hormaechei, K. pneumonia, A. baumannii and Staphylococcus spp.

TABLE 11: hld level for different bacterial species according to their sources

Bacterial species	No. of isolates	hld expression	Source of isolates
K. pneumonia	3	1.65823*	Wound swab
	21	1.1326*	Stool
	77	0.00024	Burn swab
E. hormaechei	74	0.00055	Burn swab
	109	2.01341*	
A. baumannii	90	0.02936	Burn swab
E.coli	110	6.4964*	Urine
S. epidermidis	67	0.00151	Blood
	86	0.00064	Burn swab
	93	0.00167	
S. hominis	73	0.00729	
	75	0.03125	
S. haemolyticus	79	0.00443	
S. aureus	105	0.0625	
	108	0.00112	

p≤0.05

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

Enhancing the expression of hemolysins by bacteria culturing in blood media

The expression level of hemolysin genes including hla , hlb and hlg showed higher expression after extraction the RNA of bacteria growing in blood cultures but with no significant differences at $P \leq 0.05$ (Table 12). hla expression of S. aureus for two isolates (1 and 102) were equal to 0.00059 and 0.01184, respectively, while these isolates showed expression equal to 0.04299 and 0.05517 after isolation from blood culture respectively. Also, hlb level was estimated at 0.01323 and 0.01709 before using the blood while it was estimated 0.11111 and 0.06886 after the use of blood respectively. Finally, the expression level of hlgC before the experiment was 0.06381 and 0.00502 while it was equal to 1.6582 and 0.02179 after the experiment respectively.

TABLE 12: Expression level of hemolysins gene before and after using blood

Culturing on blood							
Bacterial species	No.	hla		hlb		hlg	
		before	after	before	after	before	after
S. aureus	1	0.00059	0.04299	0.01323	0.11111	0.06381	1.6582
	102	0.01184	0.05517	0.01709	0.06886	0.00502	0.02179

$p \leq 0.05$

DISCUSSION

In spite of the fact S. aureus was the source of beta hemolysin, hemolysins showed higher expression in E. coli and K. pneumoniae in comparison to S. aureus, S. epidermidis and P. aeruginosa indicating that the genetic transfer of the virulence factor did not effect on their activity. Moreover, the expression level was differ with the different isolation sources. Furthermore, the variance in isolation sources leads to variant expression for each strain of the same species (Tables 2, 3 and 4). hlb codes for an sphingomyelinase responsible for the breakdown of sphingomyelin which is the content of the plasma membrane explaining the expression of the toxin in different isolation sources not just in blood cells and may be the content of sphingomyelin in each cell determines the level of expression. The bacterial species' physiology and the surrounding growth conditions may be an essential cause for the difference in expression when the different bacterial species are isolated from the same source (Table 5). The hlb level was two-fold higher than the control in burn infections leading to aggregative infections via colonizing infection sites which is an enrichment environment for different bacterial species.

Even if the alleles are the same, the *hla* of *S. aureus* strains from different isolation sources recorded variant expression as a result of polymorphism in the promoter region and a complex regulatory system called a two-component systems (TCS) including the accessory gene regulator (AGR) controlled by other factors concerning external influence and the cells' signals including SaeRS, ArlRS and SrrAB, alternative sigma factors (sB) and transcription factors (Tavares et al., 2014). The expression level was varied even if the bacterial species source was the same or different (Tables 6 and 7). Perhaps refers to the physiology of the strain itself. Since, in chronic infections, the immune responses of the host and the use of antibacterial drugs such as ciprofloxacin leads to the overexpression of the *hla* gene as a way to colonize the mucosa and evade the host especially in the respiratory tract (Huseby et al., 2010).

Also, as with beta and alpha hemolysins, gamma and delta hemolysins showed a higher expression in the bacteria species, despite the fact that *S. aureus* is the primary source of hemolysins (Tables 8, 9, 10 and 11). In addition, the expression showed a difference in each strain which may refers to the same causes of alpha

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

toxin. hlg overexpression caused the bacteria to over replicate, which increased cytokine production at the infection site and raised mortality, the varied of expression depending on many factors including the promoter activity variance as a result of the polymorphism in this region containing two SNPs or one insertion, the ability of translation, mRNA stability and the SNP in a coding region of hlg affected the mRNA stability and protein level. Moreover, the proteins translated from an operon gave different levels, since hlg is an operon consisting from three subunits hlgA, hlgB and hlgC (Pivard et al., 2022). The four hemolysins were overexpressed leading to lysis the cells immediately and the activity of each gene is independent on each other to increase the ability of bacteria to colonize the host (Zhang et al., 2016).

The detection of hemolysins, their genetic variation and variant expression among different bacterial strains when isolated from different sources may express evolution through the ability to adapt to different environments (Adame-Gómez *et al.*, 2020).

Enhancing the expression of hemolysins

This experiment was inspired from the theory of induced enzyme and the substrate, the expression of three hemolysins including hla, hlb and hlg in S. aureus after culturing with blood were higher than the expression of them when culturing without blood that is, of course, the logical result of the main target for hemolysins is the blood reveling express the real dangerous of these hemolysins by overexpressing In vitro to extend to the expression of hemolysin In vivo.

CONCLUSIONS

The hemolysins **(***hla, hlb, hlgC & hld***)** specific for *S. aureus* are active in other bacterial species. Generally, there expression is not influenced by the differences of the bacterial species or the isolation sources. Furthermore, the expression level of hemolysins induced by blood utilizing.

REFERENCES

1. Abd Al-Wahid, Z. H., & Abd Al-Abbas, M. J. (2023). Distribution of hla, hlb, hlgC, hld & cylA hemolysin genes and their alleles in different bacterial species isolated from the variant clinical sources in Basrah. Journal of Population Therapeutics and Clinical Pharmacology, 30(3), 180-198.
2. Adame-Gómez, R., Castro-Alarcón, N., Vences-Velázquez, A., Toribio-Jiménez, J., Pérez-Valdespino, A., Leyva-Vázquez, M. A., & Ramírez-Peralta, A. (2020). Genetic diversity and virulence factors of S. aureus isolated from food, humans, and animals. International journal of microbiology, 2020.
3. Bartlett, J. G. (2008). Methicillin-resistant Staphylococcus aureus infections. Top HIV Med, 16(5), 151-155.
4. Blake, K. J., Baral, P., Voisin, T., Lubkin, A., Pinho-Ribeiro, F. A., Adams, K. L., ... & Chiu, I. M. (2018). Staphylococcus aureus produces pain through pore-forming toxins and neuronal TRPV1 that is silenced by QX-314. Nature communications, 9(1), 1-15.
5. Cafiso, V., Bertuccio, T., Spina, D., Purrello, S., Campanile, F., Di Pietro, C., ... & Stefani, S. (2012). Modulating activity of vancomycin and daptomycin on the expression of autolysis cell-wall turnover and membrane charge genes in hVISA and VISA strains. PLoS One, 7(1), e29573.
6. Dekker, D., Wolters, M., Mertens, E., Boahen, K. G., Krumkamp, R., Eibach, D., ... & May, J. (2016). Antibiotic resistance and clonal diversity of invasive Staphylococcus aureus in the rural Ashanti Region, Ghana. BMC Infectious Diseases, 16(1), 1-6.
7. Dings, M. M., Orwin, P. M., & Schlievert, P. M. (2000). Exotoxins of Staphylococcus aureus. Clin. Microbiol. Rev, 13, 16-34.
8. Gordon, R. J., & Lowy, F. D. (2008). Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clinical infectious diseases, 46(Supplement_5), S350-S359.
9. Huseby, M. J., Kruse, A. C., Digre, J., Kohler, P. L., Vocke, J. A., Mann, E. E., ... & Earhart, C. A. (2010). Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. Proceedings of the National Academy of Sciences, 107(32), 14407-14412
10. Kong, C., Neoh, H. M., & Nathan, S. (2016). Targeting Staphylococcus aureus toxins: a potential form of anti-virulence therapy. Toxins, 8(3), 72.
11. Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), pp.402-408.

J Popul Ther Clin Pharmacol Vol 30(11):e69–e80; 09 May 2023.
This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.

12. Mahdi, M. A., Abd Al-Abbas, M. J., & Alsamak, A. M. (2021). Distribution of OatA alleles detected by a new designed primer in bacteria isolated from eye infections in Basrah governorate/Iraq. Annals of the Romanian Society for Cell Biology, 8258-8277.
13. Monecke, S., Müller, E., Büchler, J., Stieber, B., & Ehricht, R. (2014). Staphylococcus aureus in vitro secretion of alpha toxin (hla) correlates with the affiliation to clonal complexes. PloS one, 9(6), e100427.
14. Nakamura, Y., Oscherwitz, J., Cease, K. B., Chan, S. M., Muñoz-Planillo, R., Hasegawa, M., ... & Núñez, G. (2013). Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. Nature, 503(7476), 397-401.
15. Otto, M. (2014). Phenol-soluble modulins. International Journal of Medical Microbiology, 304(2), 164-169.
16. Pivard, M., Caldelari, I., Brun, V., Croisier, D., Jaquinod, M., Anzala, N., & Vandenesch, F. (2022). Complex regulation of gamma-hemolysin expression impacts S. aureus virulence. bioRxiv, 2022-10.
17. Recsei, P., Kreiswirth, B., O'reilly, M., Schlievert, P. M., Gruss, A., & Novick, R. P. (1986). Regulation of exoprotein gene expression in Staphylococcus aureus by agr. Molecular and General Genetics MGG, 202(1), 58-61.
18. Tarenzi, T., Lattanzi, G., & Potestio, R. (2022). Membrane binding of pore-forming γ-hemolysin components studied at different lipid compositions. Biochimica et Biophysica Acta (BBA)-Biomembranes, 183970.
19. Tavares, A., Nielsen, J. B., Boye, K., Rohde, S., Paulo, A. C., Westh, H., ... & Miragaia, M. (2014). Insights into alpha-hemolysin (Hla) evolution and expression among Staphylococcus aureus clones with hospital and community origin. PLoS One, 9(7), e98634.
20. Zhang, H., Zheng, Y., Gao, H., Xu, P., Wang, M., Li, A., ... & Du, H. (2016). Identification and characterization of Staphylococcus aureus strains with an incomplete hemolytic phenotype. Frontiers in cellular and infection microbiology, 6, 146.
21. Zhou, X., Zheng, Y., Lv, Q., Kong, D., Ji, B., Han, X., ... & Jiang, Y. (2021). Staphylococcus aureus N-terminus formylated δ-toxin tends to form amyloid fibrils, while the deformylated δ-toxin tends to form functional oligomer complexes. Virulence, 12(1), 1418-1437.

This article is distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License. ©2021 Muslim OT et al.