JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

IMPACT OF THE GLOBAL REGULATOR SARA ON TOXINS PRODUCTION IN *STAPHYLOCOCCUS AUREUS*

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https://doi.org/10.15414/jmbfs.4155

ARTICLE INFO

Received 2. 1. 2021
Revised 24. 4. 2021
Accepted 7. 5. 2021
Published 1. 10. 2021

Regular article

OPEN ACCESS

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that is responsible for many infectious diseases in human, some of which may be fatal. The regulation of toxins production and virulence factors in this pathogen is controlled by many global regulators including the staphylococcal accessory regulator, SarA. In this study, we outlined the influence of SarA on toxins production in *S. aureus*. The expression of genes encoding toxins was measured using real-time PCR and toxins production was quantitatively assayed. Gene expression and quantitative toxins production were compared in three staphylococcal strains; UAMS-1 wild type clinical strain, its *sarA* mutant strain and a revertant strain. Genes encoding for proteases (including *sspA, aur, scpA* and *sspB*), genes encoding for hyaluronidases (including *hysA*1 *and hysA*2) and gene encoding for staphylokinase (*sak*) were upregulated in response to the *sarA* mutation. On contrary, genes encoding for lipases (including *lip1* and *lip2*) were downregulated. Moreover, the quantitative production of protease, hyaluronidase and staphylokinase was significantly increased due to the *sarA* mutation. On the other hand, the quantitative production of lipase was significantly altered. Overall, our findings indicate that SarA is an effective global regulator of toxins production that can suggest new prospective therapeutics for the treatment of *S. aureus* infections.

Keywords: Gene expression; Global regulator; SarA; *Staphylococcus aureus*; Toxins production

INTRODUCTION

In the clinical context, *Staphylococcus aureus* (*S. aureus*) is considered the most pathogenic organism in the Gram-positive Staphylococcus genus **(Bitrus *et al.*, 2018)**. It is a commensal bacterium, which colonizes the majority of the human population **(Tong *et al.*, 2015)**. *S. aureus* becomes opportunistic when it gains access to open wounds, or when it colonizes populations suffering from lowered immunity including immunocompromised patients, diabetics, elderly people and children **(Tong *et al.*, 2015; Bitrus *et al.*, 2018)**. This opportunistic pathogen can lead to high morbidity and mortality. *S. aureus* can cause a vast variety of diseases ranging from mild and moderate skin infections to more severe and life-threatening infections such as endocarditis, food poisoning, osteomyelitis and toxic shock syndrome **(Becker, 2018; Hamzah *et al.*, 2019)**.
Disease severity and the multiple pathogenic implications of *S. aureus* are attributed to the diversity of virulence factors that act in a complicated harmonious manner such as the production of biofilm, surface proteins, exfoliative toxins, exoenzymes and exotoxins **(Connolly *et al.*, 2017)**. Staphylococcal ability to produce these virulence factors enables its attachment to the host tissues and evading the host immune system to subsequently cause its cellular and systemic toxic effect **(Ionescu *et al.*, 2015)**. Indeed, *S. aureus* produces many potent exotoxins, co-factors and exoenzymes, among which, proteases, lipases, staphylokinase and hyaluronidases are especially important **(Lacey *et al.*, 2016)**. The regulation of virulence determinant production in *S. aureus* involves several global regulatory loci; of these, SarA, a regulatory nucleic acid binding protein, is particularly important **(Arvidson & Tegmark, 2001; Jones *et al.*, 2008; Mauro *et al.*, 2016; Jenul & Horswill, 2019)**.
S. aureus secretes many types of proteases that play a crucial role in the staphylococcal proteolytic effects, breaking down host peptide bonds. Protease production is mediated by different operons encoding seven serine proteases (*sspA*) and serine protease–like proteins (*spls*), two cysteine proteases (staphopain A, *scpA* and staphopain B, *sspB*) and a metalloprotease (aureolysin, *aur*) **(Singh & Phukan, 2019)**. These proteases are able to lyse many host proteins such as fibrinogen and fibronectin **(Pietrocola *et al.*, 2017)**. Besides protease production, *S. aureus* is known as a potential lipase producer. Lipase production by this pathogen enables immune evasion by interfering with the phagocytosis by human granulocytes **(Chen & Alonzo, 2019)**. Lipases can inactivate antimicrobial lipids, and also facilitate host cell lysis by helping degradation of cell membrane lipids. Lipase effect on host lipids results in release of free fatty acids from lipid stores **(Cadieux *et al.*, 2014; Chen & Alonzo, 2019)**. There are two types of lipases; Sal1 and glycerol ester hydrolase (Geh = Sal2) encoded by *gehA* and *gehB*, respectively **(Nguyen *et al.*, 2018; Chen & Alonzo, 2019)**. Furthermore, staphylokinase is a 136 amino acid long co-factor found associated with the cell surface and in the environment of cell culture. It can bind to host antibacterial peptides including α defensins and revoke their antibacterial action. It does not have proteolytic ability on its own, but its activity arises after the formation of 1:1 complex with plasmin to form Sak–plasmin active complex that converts plasminogen (PLG) into plasmin with broad proteolytic range **(Pietrocola *et al.*, 2017; Tam & Torres, 2019)**. Besides, the ability of *S. aureus* to go through tissues rich in hyaluronic acid (HA) is based on its secreted hyaluronidase enzyme or hyaluronate lyase that was previously known as the spreading factor. Hyaluronidase is encoded by *hysA1*, however, in some *S. aureus* strains, another type of hyaluronidase enzyme was found that is about 75.9% identical to hyaluronate lyase 1 and is encoded by *hysA2* **(Tam & Torres, 2019)**.
In the current study we depicted effect of the global regulator SarA on protease, lipase, staphylokinase and hyaluronidase production in *S. aureus*. This was fulfilled by comparing the gene expression as well as quantitative production of these toxins between wild type, *sarA* mutant and revertant *S. aureus* strains.

MATERIALS AND METHODS

Bacterial strains and culture conditions

S. aureus strains utilized in this study are described in Table 1 **(Beenken *et al.*, 2003; Morrison *et al.*, 2012)**. Tryptic soy broth (TSB) was used for routine cultivation of *S. aureus* strains. Moreover, extraction of toxins and their quantitative assay were carried out in TSB.

Table 1 Bacterial strains used in this study

Bacterial strain	Characters	Reference
UAMS-1	Clinical standard isolate; virulent; methicillin sensitive; isolated from bone infection	**(Beenken *et al*., 2003; Morrison *et al*., 2012)**
UAMS-1 *sarA* mutant	UAMS-1, *sarA* mutant	**(Beenken *et al*., 2003; Morrison *et al*., 2012)**
UAMS-1 *sarA* revertant	Revertant strain of UAMS-1 *sarA* mutant strain	**(Beenken *et al*., 2003; Morrison *et al*., 2012)**

Measurement of bacterial growth

S. aureus strains were inoculated in TSB and incubated at *37*°C overnight on a shaker incubator adjusted at a speed of 150 rpm. Thereafter, an aliquot from each previously grown cultures was subcultured into fresh TSB and subsequently incubated with shaking at *37*°C. The bacterial cell density and growth rate were monitored by determining the optical density of the cultures at 600 nm (OD_{600}) every 60 minutes to establish the growth curve and to determine the bacterial growth phases.

Real-time PCR (rt-PCR)

S. aureus strains were inoculated in TSB and incubated at *37*°C with shaking, and RNA isolation was established during the log phase of bacterial growth. Cell pellets were harvested by centrifugation at *10000 rpm for 5 minutes at 4*°C. *Then, the resultant cell pellets were mixed with* 300 µL of triazole reagent (TRI reagent®, Sigma-Aldrich, USA) and *150 mg* glass beads (212-300 µm, 50- 70 U.S. sieve, unwashed, Sigma-Aldrich, USA). The reaction mixtures were heated in a heating block adjusted at *60*°C for 20 minutes with vortexing every 5 minutes, and then were vortexed for 30 minutes. Afterwards, the cells were incubated in a *-80*°C freezer for 3 minutes, then incubated at *37*°C for another 3 minutes. This cycle of freezing and thawing was repeated 3 times to disrupt the cell integrity and was followed by centrifugation at 6000 rpm for 5 minutes at *4*°C. Following the TRI reagent® manufacturer's protocol, RNA was precipitated, air dried for 10 minutes and resuspended in 20 µL of RNase free water. RNA samples were treated with DNase set (Qiagen, Hilden, Germany), according to the manufacturer's protocol, and then were kept at *-80*°C. The quality of isolated RNA was checked and its concentration was measured using NanoDrop (Thermo Fisher Scientific inc., Massachusetts, USA). Using SensiFAST™ cDNA synthesis kit (Bioline, Bloomberg, USA), synthesis of cDNA was performed according to the manufacturer's guidelines from the purified RNA. Real-time PCR (rt-PCR) was conducted using 5x HOT FIREPol® EvaGreen HRM Mix (Solis BioDyne, Tartu, Estonia) according to the manufacturer's guidelines and the Rotor-Gene Q (Qiagen, Hilden, Germany). Each reaction mixture consisted of 4 µL of 5x HOT FIREPol® EvaGreen HRM Mix, 2 µL of template cDNA, 12 µL of sterile water (PCR grade), 1 µL of forward primer (250 nM) and 1 µL of reverse primer (250 nM) (Table 2). In every PCR run, negative control reaction mixtures were included and were prepared by excluding the reverse transcriptase enzyme or cDNA template from the reaction mixture. In each run, a pool (8 µL) was created from the cDNA of tested strains, and 4 dilution points from this pool were included in each run to establish a standard curve. PCR was carried out according to the conditions; primary activation for 15 minutes at 95°C. Then, 45 cycles of denaturation for 12 seconds at 95°C, annealing for 20 seconds at the appropriate temperature and extension for 20 seconds at 72°C. Amplification specificity was determined from the amplicon melting curves. *16S rRNA* was used as a house-keeping gene to normalize the expression of tested genes. The RNA concentration of each sample was calculated through comparing C_t of each gene sample to the established standard curve. In these experiments, the house-keeping gene was used as an endogenous reference gene, where the level of expression of each gene in different samples was calculated compared to the house-keeping gene by the comparative method ($\Delta\Delta C_t$) using UAMS-1 as a calibrator sample **(Schmittgen & Livak, 2008)**.

Table 2 Oligonucleotide primers used in this study

Gene	Accession number		Nucleotide sequence 5'~ 3'	Position	Size (bp)
16S rRNA	JTJK01000002	F	**AACTAAGCTTAAGGGTTGCG**	417--601	185
		R	**GGAGCATGTGGTTTAATTCG**		
sspB	JTJK01000001	F	**CATTAGCAACAACTGCTAGG**	141--382	242
		R	**GCGTACATTATACCCTGAAG**		
sspA	JTJK01000001	F	**TGAGTTACCACCAGTTGTAC**	364--601	238
		R	**CGAAGGTGATTTAGCAATCG**		
hysA1	JTJK01000001	F	**CTAATGGATGATATGCTCAC**	601--843	243
		R	**GGCAGAATCTTGAACATAAG**		
hysA2	JTJK01000002	F	**ACATTGATCATCAAGACGTC**	881--1100	220
		R	**TCATTTTCACGACTGATAGC**		
lip2	JTJK01000001	F	**CATGATAAAGCAGCACCAAC**	541--786	246
		R	**TTTATCTGTCGGTTTCTCCG**		
lip1	JTJK01000002	F	**GTCTGCTTTTTGTCTATCGC**	322--572	251
		R	**ATTCAAGAGTAGACTTCGGG**		
scpA	JTJK01000002	F	**AAGTTAATGTCGAGGACAAG**	119--363	245
		R	**ATCCTTAGTGACTTTTGGTG**		
sak	JTJK01000002	F	**TGTTATAGGGAAAGACTTCG**	91--339	249
		R	**TTGATGGTAAATGTGACTGG**		
aur	JTJK01000002	F	**CATGCTTCGTAAAGCATCTC**	301--570	270
		R	**AATAACGCTGCATGGATTGG**		

Assessment of the protease activity

S. aureus strains were inoculated in TSB and incubated at *37*°C with shaking at 150 rpm for an appropriate time. Thereafter, cultures were centrifuged at 10000 rpm for 10 minutes at *4*°C. The obtained supernatants were filtered through 0.2 µM Millipore filter and subsequently used in the assay of protease activity. Protease quantitative assay was carried out on bacterial supernatants according to **(Elgaml *et al*., 2013; Elgaml *et al*., 2014; Abdel-Sattar *et al*., 2016)**. Azocasein (1.0 mg) (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 50 mM Tris HCl (pH 8.0) in volume of 0.6 mL. From this solution, 0.2 mL was mixed with 0.4 mL of the supernatants. The reaction mixtures were incubated for an appropriate period of time at 30°C. Thereafter, 1.4 mL of 5% trichloroacetic acid (TCA) was added to cease the reactions. The reaction mixtures were centrifuged at 5000 rpm for 5 minutes at *4*℃ and the obtained supernatants were added to equal volumes of 0.5 M NaOH. Finally, the absorbance was determined at 440 nm. In these experiments, TSB was used as a negative control.

Assessment of the lipase activity

Reaction mixtures were prepared by mixing 20 µL of the prepared bacterial supernatants (as described above in protease assay), 75 µL of 20 mM p-nitro phenyl palmitate (P-NPP) and 0.1 M tris HCl buffer (pH 8.5) to a final volume of 3 mL. The reactions were incubated for 10 minutes at *37*°C, and then were frozen at -20°C for another 10 minutes to cease the lipolytic action. The yellow color of the reaction product was spectrophotometrically measured at 410 nm **(Sarkar *et al*., 2012; El-baz *et al*., 2017)**. In these experiments, TSB was used as a negative control.

Assessment of the staphylokinase activity

Staphylokinase extraction was carried out according to **(Shagufta Naseer *et al*., 2014)**. In a 10 mL of TSB, colonies of *S. aureus* stains were inoculated and incubated on a shaker incubator at 150 rpm overnight at *37*°C. Then, OD_{600} of the cultures was adjusted to 0.2-0.275. In a 10 mL of TSB, 200 µL of the adjusted bacterial suspensions were subcultured and incubated for 24 hours at *37*°C on a shaker incubator at 200 rpm. Then, the cultures were centrifuged for 15 minutes at a speed of 3000 rpm at *4*°C. To the separated supernatants, 40% (v/v) cold absolute alcohol was added and they were frozen at -20°C for two days to completely precipitate the staphylokinase enzyme. Staphylokinase enzyme was collected as pellets after centrifugation of the enzyme precipitate at 10000 rpm for 20 minutes at *4*°C. The pellets were subsequently dissolved in 0.1 M phosphate buffer solution (pH 7.2) (one-tenth of the original supernatant volume) and stockpiled at -20°C for later use. Thereafter, staphylokinase enzyme quantitative assay was established. Initial weights of Eppendorf tubes were measured (X_1) and 500 µL of freshly collected human blood was transferred to the Eppendorf tubes, and incubated for 60 minutes at 37°C to enable blood clotting. After complete clotting of blood samples, the produced serum was aspirated and the Eppendorf tubes were reweighed (X_2). The clot weight was determined by calculating the difference between the second and the first weights ($X_2 - X_1$). To each Eppendorf tube, 250 µL of the previously prepared enzyme extract was added, and then the tubes were incubated at 37°C for 2 hours to evaluate clot lysis. After lysis, every Eppendorf tube was weighed subsequent to fluid removal. For each sample, the difference in clot weights (before and after lysis) was calculated. Afterwards the clot lysis percentage, which represents the staphylokinase plasmolytic action was calculated. In these experiments, TSB was used as a negative control.

$$\% \text{ lysis of clot} = \frac{Difference\ in\ clot\ weight\ before\ and\ after\ lysis}{Clot\ weight\ before\ lysis} \times 100$$

Assessment of the hyaluronidase activity

Hyaluronidase quantitative assay was executed on previously separated bacterial supernatants (as described above in protease assay) using 1% agarose plates containing 0.4 mg/mL of hyaluronic acid (Sigma, H-1504) in 0.3 M sodium phosphate buffer (pH 5.3) and 1% bovine serum albumin (BSA). Under aseptic conditions, wells were cut in agarose to embody 100 µL of the supernatant of tested strains. After 24 hours of incubation at 37°C, 2 M acetic acid was added to flood the plates. The undigested hyaluronic acid was detected as opaque precipitation in conjugation with BSA behind the clear zones of the hydrolyzed hyaluronic acid. The clear zone diameters were measured in millimeters (mm) **(Kumar *et al*., 2012; Abdelkader *et al*., 2018)**. In these experiments, TSB was used as a negative control.

Statistical analysis

Significance of difference was assessed in the results by repeating the experiments three independent times and comparing the data using student's t-test. P values lower than 0.05 were regarded significantly different.

RESULTS

Effect of *sarA* disruption on protease activity

The UAMS-1 wild type strain and its *sarA* mutant strain as well as the revertant strain were compared for protease toxins gene expression and toxin production. Expression of *sspA, aur, scpA* and *sspB* was significantly increased in the *sarA* mutant strain as compared to the wild type and revertant strains (Figure 1A). The relative gene expression of the mutant strain versus the wild type strain was 32.10, 81.30, 5.85 and 9.81 for *sspA, aur, scpA* and *sspB*, respectively. Moreover, protease production was significantly higher in the *sarA* mutant strain than the wild type and revertant strains (Figure 1B). The protease activity of the mutant strain was 2.87 of that of the wild type strain.

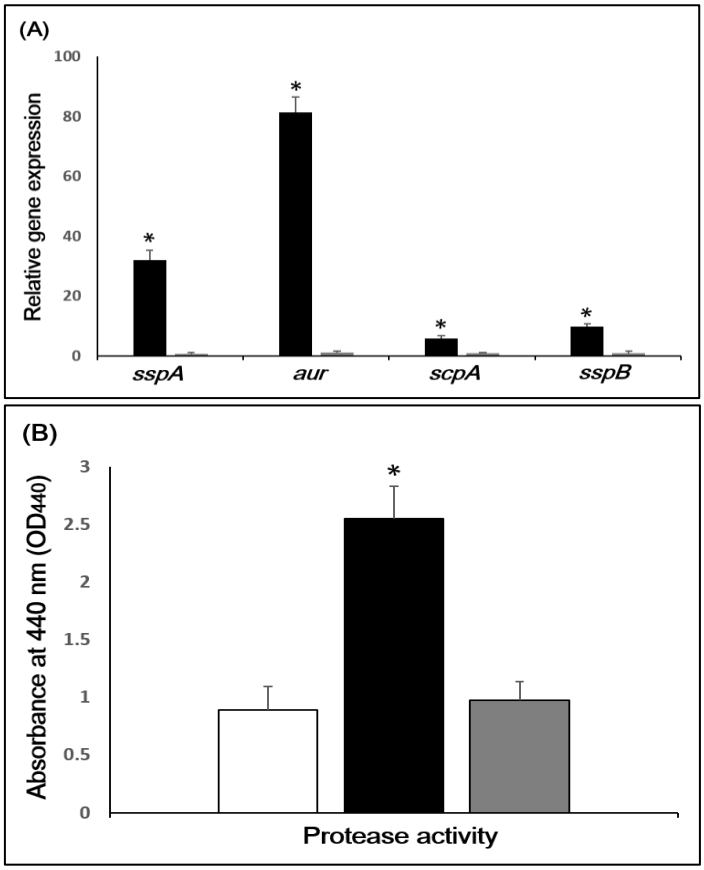
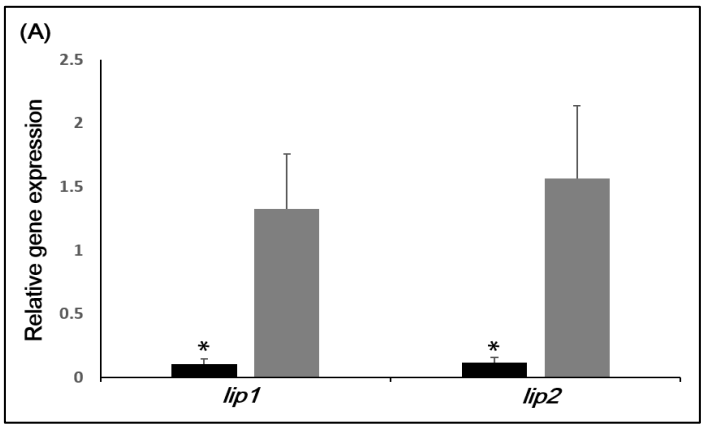


Figure 1 Effect of *sarA* disruption on *sspA, aur, scpA* and *sspB* gene expression levels (A) and on protease activity (B). Data was represented as mean ± S.D. The asterisk (*) indicates significant difference ($P < 0.05$) between *sarA* mutant strain (black bar) and both UAMS-1 wild type strain (white bar) and revertant strain (grey bar).

Effect of *sarA* disruption on lipase activity

Mutation of *sarA* was noticed to downregulate *lip1* and *lip2* expression (Figure 2A). The relative gene expression of the mutant strain versus the wild type strain was 0.11 and 0.12 for *lip1* and *lip2*, respectively. This result was confirmed by the quantitative assay of lipase, where, lipase production was significantly altered in the *sarA* mutant strain compared to both the wild type and revertant strains (Figure 2B). The lipase activity of the mutant strain was 0.11 of that of the wild type strain.



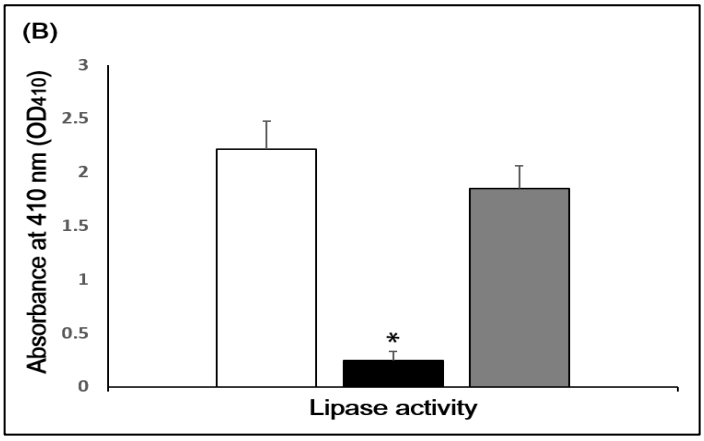


Figure 2 Effect of *sarA* disruption on *lip1* and *lip2* gene expression levels (A) and on lipase activity (B). Data was represented as mean ± S.D. The asterisk (*) indicates significant difference (P < 0.05) between *sarA* mutant strain (black bar) and both UAMS-1 wild type strain (white bar) and revertant strain (grey bar).

Effect of *sarA* disruption on staphylokinase activity

Expression of *sak* was significantly upregulated in the *sarA* mutant strain in contrast to the wild type and revertant strains (Figure 3A). The relative gene expression of the mutant strain versus the wild type strain was 3.35 for *sak*. In addition, staphylokinase production level increased noticeably in response to the *sarA* mutation compared to the wild type and revertant strains (Figure 3B). The staphylokinase activity of the mutant strain was 1.55 of that of the wild type strain.

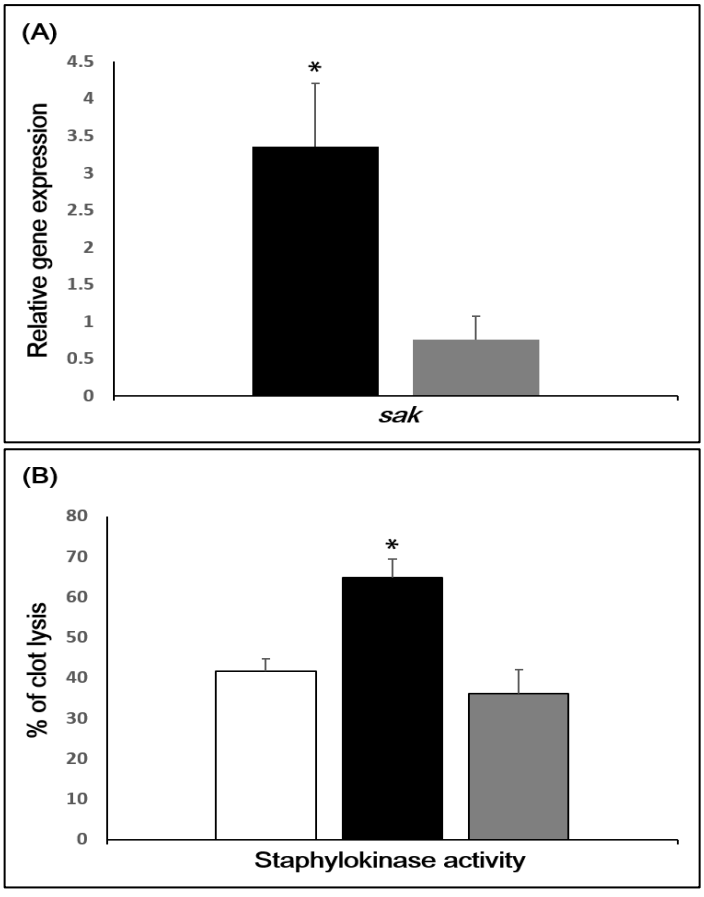


Figure 3 Effect of *sarA* disruption on *sak* gene expression level (A) and on staphylokinase activity (B). Data was represented as mean ± S.D. The asterisk (*) indicates significant difference (P < 0.05) between *sarA* mutant strain (black bar) and both UAMS-1 wild type strain (white bar) and revertant strain (grey bar).

Effect of *sarA* disruption on hyaluronidase activity

Expression of *hysA1* and *hysA2* was upregulated due to the *sarA* mutation in comparison to the wild type and revertant strains (Figure 4A). The relative gene expression of the mutant strain versus the wild type strain was 9.89 and 230.89 for *hysA1* and *hysA2*, respectively. Furthermore, quantitative assay of hyaluronidase activity revealed lower production levels in the wild type and revertant strains, and a higher level in the *sarA* mutant strain (Figure 4B). The hyaluronidase activity of the mutant strain was 2.06 of that of the wild type strain.

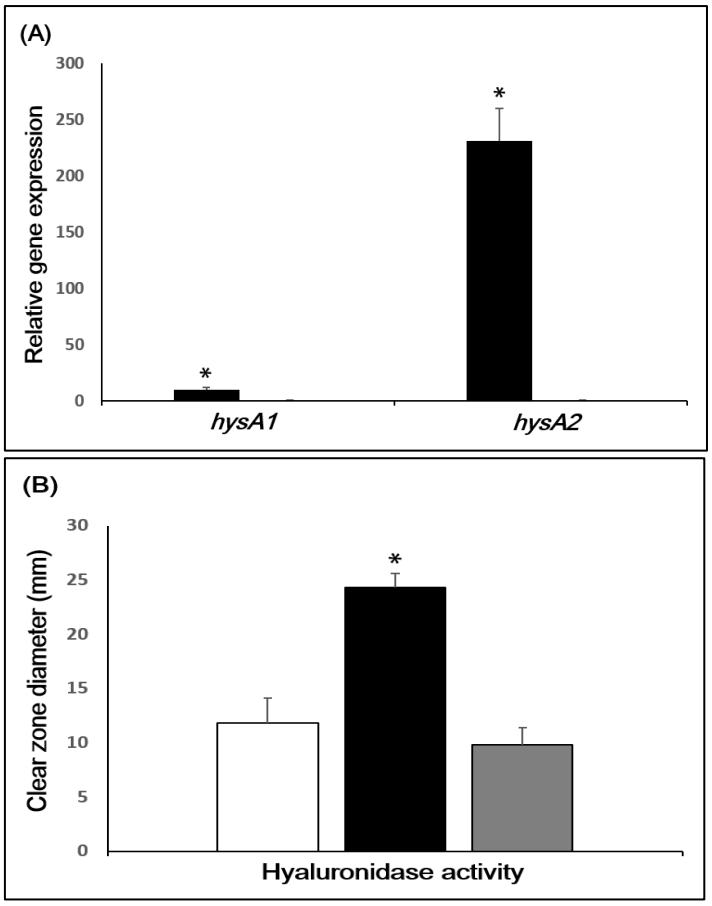


Figure 4 Effect of *sarA* disruption on *hysA1 and hysA*2 gene expression levels (A) and on hyaluronidase activity (B). Data was represented as mean ± S.D. The asterisk (*) indicates significant difference (P < 0.05) between *sarA* mutant strain (black bar) and both UAMS-1 wild type strain (white bar) and revertant strain (grey bar).

DISCUSSION

S. aureus is an important opportunistic Gram-positive pathogen that is responsible for a number of clinical infections **(Chuang-Smith & Schlievert, 2021)**. Previous studies showed that *S. aureus* is implicated in many clinical cohorts **(Mehraj *et al*., 2016)**. It is a major cause of skin and soft tissue infections worldwide as it is the causative agent of more than 80% of the skin and soft tissue infections. It is the most commonly isolated pathogen from infections in surgical sites, purulent cellulitis, wounds and chronic ulcers **(Mehraj *et al*., 2016)**. It can systemically infect most tissues in the host such as eyes, brain, bones, joints, kidneys, lungs, hearts and muscles **(Kuehl *et al*., 2020)**.

S. aureus is armed with various types of virulence factors that support its pathogenicity and allow its evasion from immune system **(Haddad *et al*., 2018)**. Expression of virulence factors genes in *S. aureus* is controlled by global regulators such as Agr, SarA, Rot and MgrA **(Jenul & Horswill, 2019)**. SarA is a 124 amino acid long winged helix protein encoded by *sarA* locus, which is composed of three overlapped transcripts (*sarA* P_1, *sarA* P_3 and *sarA* P_2). It is encoded by a 372 bp *sarA* open reading frame (ORF) that forms the final SarA protein form **(Liu *et al*., 2006; Cheung *et al*., 2008)**. SarA is known to regulate the expression of about 120 genes, of which 76 genes are upregulated, while 44 genes are downregulated either by direct DNA binding or by indirect pathways **(Liu *et al*., 2006; Jones *et al*., 2008)**. Murine models infected with *sarA* mutant *S. aureus* strain showed a suppressed virulence pattern, and reduced susceptibility to septic arthritis and osteomyelitis. Moreover, these models revealed that *sarA* mutant strain was less likely to produce biofilm, and therefore was more susceptible to antibiotics **(Gordon *et al*., 2013)**.

Our findings in this study demonstrated that proteases levels [staphylococcal serine protease (SspA), metalloprotease (aureolysin; Aur), staphopain (ScpA) and cysteine protease (SspB)] were increased in the *sarA* mutant strain when compared to the wild type and revertant strains. This was found to be accordant

to **Zielinska *et al*., (2012), Atwood *et al*., (2015), Kong *et al*., (2016)** and **Rom *et al*., (2017)**. Our results also were found to be accordant with the findings of **Karlsson & Arvidson, (2002), Pragman & Schlievert, (2004)** and **Arya & Princy, (2013)**, who found that increased *sarA* expression leaded to a decrease in the proteases levels.

In contrast to proteases, the genes encoding for lipases namely, *lip1* and *lip2*, were found in our study to be downregulated upon the *sarA* mutation. These findings are similar to those that were found by **Blevins *et al*., (2002)** and **Pragman & Schlievert, (2004)**.

Furthermore, the staphylokinase cofactor encoded by *sak* was noticed to be upregulated in response to the *sarA* mutation. This result is found to be in agreement with **Ziebandt *et al*., (2001)** and **Pragman & Schlievert, (2004)**, who found that SarA downregulated staphylokinase production.

Moreover, hyaluronidase was found in higher levels in the staphylococcal *sarA* mutant strain in comparison to the wild type and revertant strains. Our finding was similar to **Hart *et al*., (2013)** and **Factor, (2014)**, who found that hyaluronidase expression increased in the *sarA* mutant strain as compared to the wild type strain.

CONCLUSION

To sum up, SarA increased the lipase production. On the other hand, staphylococcal serine protease, metalloprotease, staphopain protease, cysteine protease, staphylokinase and hyaluronate lyases were found to be downregulated by SarA. Therefore, it can be concluded that SarA is an important global regulator that controls the expression and production of many toxins in *S. aureus*. Our data support previous literature that SarA may act either as a positive regulator (may be through increasing the stability of mRNA of the genes) or a negative regulator (may be through decreasing the transcription of target genes) or may act through other regulatory mechanisms in *S. aureus* (Figure 5). This can open the door to set novel therapeutic approaches, targeting SarA, for the treatment of certain bacterial infections and help in tackling the overwhelming problem of antibiotic resistance. This can be achieved through using either new key inhibitors or modulators of SarA to control the production of virulence factors that are responsible for the clinical characteristics of each infection.

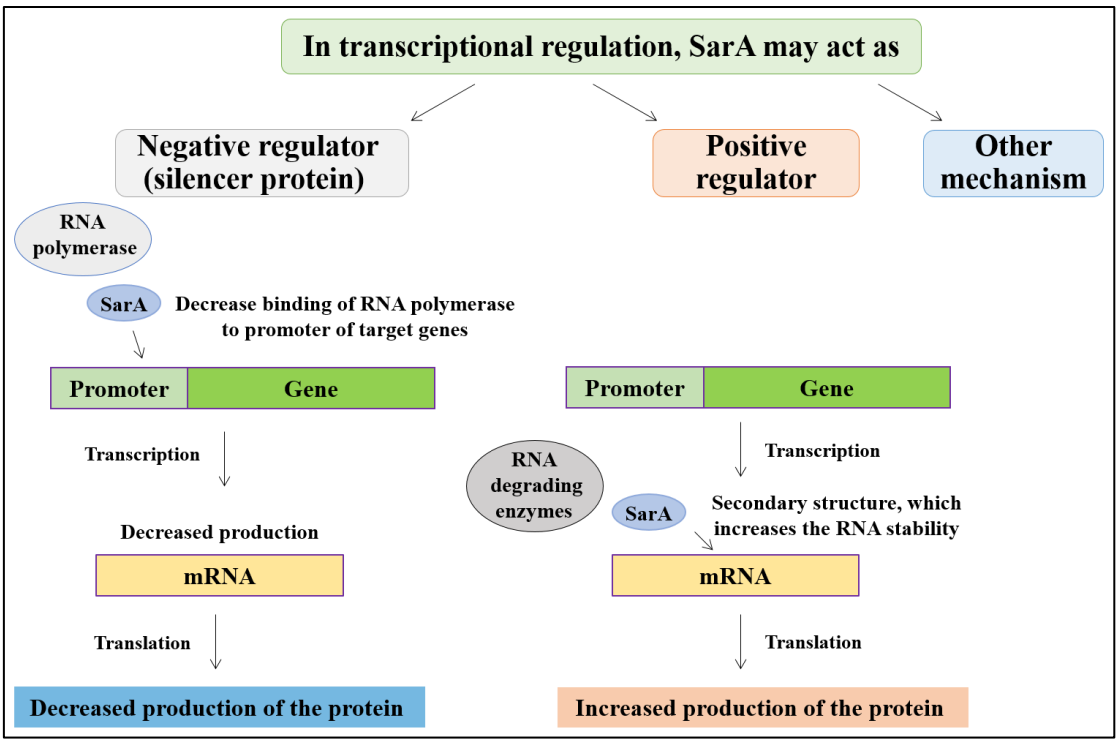


Figure 5 Proposed action of SarA as a global regulator in *S. aureus*. SarA may act either as a positive regulator (may be through increasing the stability of mRNA of the genes) or a negative regulator (may be through decreasing the transcription of target genes) or may act through other regulatory mechanisms.

Acknowledgment: All thanks and appreciation to Prof. Mark S. Smeltzer, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, USA, for providing the *Staphylococcus aureus* UAMS-1 standard strain, its *sarA* mutant strain and revertant strain. This study was performed at Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Conflict of interest: The authors declare that they have no conflicts of interest.

REFERENCES

Abdel-Sattar, E. S., Miyoshi, S., & Elgaml, A. (2016). Regulation of *Vibrio mimicus* metalloprotease (VMP) production by the quorum-sensing master regulatory protein, LuxR. *Journal of Basic Microbiology, 56*(10), 1051-1058. https://doi.org/10.1002/jobm.201600002

Abdelkader, S. A., El-Mowafy, M., Abdelmegeed, E., & Hassan, R. (2018). Analysis of hyaluronidase expression by qPCR in Egyptian clinical isolates of *Staphylococcus aureus* and its correlation with phenotypic plate assay. *Advances in Microbiology, 8*(7), 614-624. https://doi.org/10.4236/aim.2018.87041

Arvidson, S., & Tegmark, K. (2001). Regulation of virulence determinants in *Staphylococcus aureus*. *International Journal of Medical Microbiology, 291*(2), 159-170. https://doi.org/10.1078/1438-4221-00112

Arya, R., & Princy, S. A. (2013). An insight into pleiotropic regulators Agr and Sar: molecular probes paving the new way for antivirulent therapy. *Future Microbiology, 8*(10), 1339-1353. https://doi.org/10.2217/fmb.13.92

Atwood, D. N., Loughran, A. J., Courtney, A. P., Anthony, A. C., Meeker, D. G., Spencer, H. J., . . . Smeltzer, M. S. (2015). Comparative impact of diverse regulatory loci on *Staphylococcus aureus* biofilm formation. *MicrobiologyOpen, 4*(3), 436-451. https://doi.org/10.1002/mbo3.250

Becker, K. (2018). Pathogenesis of *Staphylococcus aureus*. In *Staphylococcus aureus* (pp. 13-38): Elsevier. doi: https://doi.org/10.1016/C2015-0-04757-8

Beenken, K. E., Blevins, J. S., & Smeltzer, M. S. (2003). Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. *Infection and Immunity, 71*(7), 4206-4211. https://doi.org/10.1128/iai.71.7.4206-4211.2003

Bitrus, A., Peter, O., Abbas, M., & Goni, M. (2018). *Staphylococcus aureus*: a review of antimicrobial resistance mechanisms. *Veterinary Sciences: Research and Reviews, 4*(2), 43-54. https://doi.org/10.17582/journal.vsrr/2018/4.2.43.54

Blevins, J. S., Beenken, K. E., Elasri, M. O., Hurlburt, B. K., & Smeltzer, M. S. (2002). Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus*. *Infection and Immunity, 70*(2), 470-480. https://doi.org/10.1128/iai.70.2.470-480.2002

Cadieux, B., Vijayakumaran, V., Bernards, M. A., McGavin, M. J., & Heinrichs, D. E. (2014). Role of lipase from community-associated methicillin-resistant *Staphylococcus aureus* strain USA300 in hydrolyzing triglycerides into growth-inhibitory free fatty acids. *Journal of Bacteriology, 196*(23), 4044-4056. https://doi.org/10.1128/JB.02044-14

Chen, X., & Alonzo, F. (2019). Bacterial lipolysis of immune-activating ligands promotes evasion of innate defenses. *Proceedings of the National Academy of Sciences, 116*(9), 3764-3773. https://doi.org/10.1073/pnas.1817248116

Cheung, A. L., Nishina, K. A., Trotonda, M. P., & Tamber, S. (2008). The SarA protein family of *Staphylococcus aureus*. *The international Journal of Biochemistry & Cell Biology, 40*(3), 355-361. https://doi.org/10.1016/j.biocel.2007.10.032

Chuang-Smith, O. N., & Schlievert, P. M. (2021). Staphylococcal enterotoxin C subtypes are differentially associated with human infections and immunobiological activities. *mSphere*, *6*(1), e01153-20. https://doi.org/10.1128/mSphere.01153-20

Connolly, J., Boldock, E., Prince, L. R., Renshaw, S. A., Whyte, M. K., & Foster, S. J. (2017). Identification of *Staphylococcus aureus* factors required for pathogenicity and growth in human blood. *Infection and Immunity*, *85*(11), e00337-00317. https://doi.org/10.1128/IAI.00337-17

El-baz, R., Rizk, D. E., Barwa, R., & Hassan, R. (2017). Virulence characteristics and molecular relatedness of methicillin resistant *Staphylococcus aureus* harboring different staphylococcal cassette chromosome *mec*. *Microbial Pathogenesis*, *113*, 385-395. https://doi.org/10.1016/j.micpath.2017.11.021

Elgaml, A., Higaki, K., & Miyoshi, S.-i. (2013). Regulation system of serine protease production in *Vibrio vulnificus* strain NCIMB 2137, a metalloprotease-gene negative strain isolated from a diseased eel. *Aquaculture*, *416-417*, 315-321. doi: https://doi.org/10.1016/j.aquaculture.2013.09.041

Elgaml, A., Higaki, K., & Miyoshi, S.-i. (2014). Effects of temperature, growth phase and *luxO*-disruption on regulation systems of toxin production in *Vibrio vulnificus* strain L-180, a human clinical isolate. *World Journal of Microbiology and Biotechnology*, *30*(2), 681-691. https://doi.org/10.1007/s11274-013-1501-3

Factor, C.-R. V. (2014). *Staphylococcus aureus* hyaluronidase is Cod-Y regualted virulence factor. *Infection and Immunity*, *82*(10), 4253-4264. https://doi.org/10.1128/IAI.01710-14

Gordon, C. P., Williams, P., & Chan, W. C. (2013). Attenuating *Staphylococcus aureus* virulence gene regulation: a medicinal chemistry perspective. *Journal of Medicinal Chemistry*, *56*(4), 1389-1404. https://doi.org/10.1021/jm3014635

Haddad, O., Merghni, A., Elargoubi, A., Rhim, H., Kadri, Y., & Mastouri, M. (2018). Comparative study of virulence factors among methicillin resistant *Staphylococcus aureus* clinical isolates. *BMC Infectious Diseases*, *18*(1), 560. https://doi.org/10.1186/s12879-018-3457-2

Hamzah, C., Mardziah, A., Yeo, C. C., Puah, S. M., Chua, K. H., & Chew, C. H. (2019). *Staphylococcus aureus* infections in Malaysia: A review of antimicrobial resistance and characteristics of the clinical isolates, 1990–2017. *Antibiotics*, *8*(3), 128. https://doi.org/10.3390/antibiotics8030128

Hart, M. E., Tsang, L. H., Deck, J., Daily, S. T., Jones, R. C., Liu, H., . . . Smeltzer, M. S. (2013). Hyaluronidase expression and biofilm involvement in *Staphylococcus aureus* UAMS-1 and its *sarA*, *agr* and *sarA agr* regulatory mutants. *Microbiology*, *159*(Pt 4), 782-791. https://doi.org/10.1099/mic.0.065367-0

Ionescu, B., Ionescu, D., Gheorghe, I., Curuțiu, C., Banu, O., Bleotu, C., . . . Bezirtzoglou, E. (2015). Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania. *Romanian Biotechnological Letters*, *20*(3), 10536-10546. https://www.rombio.eu/rbl3vol20/19.pdf

Jenul, C., & Horswill, A. R. (2019). Regulation of *Staphylococcus aureus* virulence. *Microbiology Spectrum*, *7*(2), 10.1128/microbiolspec.GPP3-0031-2018. https://doi.org/10.1128/microbiolspec.GPP3-0031-2018

Jones, R. C., Deck, J., Edmondson, R. D., & Hart, M. E. (2008). Relative quantitative comparisons of the extracellular protein profiles of *Staphylococcus aureus* UAMS-1 and its *sarA*, *agr*, and *sarA agr* regulatory mutants using one-dimensional polyacrylamide gel electrophoresis and nanocapillary liquid chromatography coupled with tandem mass spectrometry. *Journal of Bacteriology*, *190*(15), 5265-5278. https://doi.org/10.1128/JB.00383-08

Karlsson, A., & Arvidson, S. (2002). Variation in extracellular protease production among clinical isolates of *Staphylococcus aureus* due to different levels of expression of the protease repressor *sarA*. *Infection and Immunity*, *70*(8), 4239-4246. https://doi.org/10.1128/iai.70.8.4239-4246.2002

Kong, C., Neoh, H.-m., & Nathan, S. (2016). Targeting *Staphylococcus aureus* toxins: a potential form of anti-virulence therapy. *Toxins*, *8*(3), 72. https://doi.org/10.3390/toxins8030072

Kuehl, R., Morata, L., Meylan, S., Mensa, J., & Soriano, A. (2020). When antibiotics fail: a clinical and microbiological perspective on antibiotic tolerance and persistence of *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, *75*(5), 1071-1086. https://doi.org/10.1093/jac/dkz559

Kumar, D. M., Rejitha, R., Devika, S., Balakumaran, M., Rebecca, A., & Kalaichelvan, P. (2012). Production, optimization and purification of lipase from Bacillus sp. MPTK 912 isolated from oil mill effluent. *Advances in Applied Science Research*, *3*(2), 930-938. https://www.cabdirect.org/cabdirect/abstract/20123359848

Lacey, K. A., Geoghegan, J. A., & McLoughlin, R. M. (2016). The role of *Staphylococcus aureus* virulence factors in skin infection and their potential as vaccine antigens. *Pathogens*, *5*(1), 22. https://doi.org/10.3390/pathogens5010022

Liu, Y., Manna, A. C., Pan, C.-H., Kriksunov, I. A., Thiel, D. J., Cheung, A. L., & Zhang, G. (2006). Structural and function analyses of the global regulatory protein SarA from *Staphylococcus aureus*. *Proceedings of the National Academy of Sciences*, *103*(7), 2392-2397. https://doi.org/10.1073/pnas.0510439103

Mauro, T., Rouillon, A., & Felden, B. (2016). Insights into the regulation of small RNA expression: SarA represses the expression of two sRNAs in *Staphylococcus aureus*. *Nucleic Acids Research*, *44*(21), 10186-10200. https://doi.org/10.1093/nar/gkw777

Mehraj, J., Witte, W., Akmatov, M. K., Layer, F., Werner, G., & Krause, G. (2016). Epidemiology of *Staphylococcus aureus* nasal carriage patterns in the community. *Current Topics in Microbiology and Immunology*, *398*, 55-87. https://doi.org/10.1007/82_2016_497

Morrison, J. M., Anderson, K. L., Beenken, K. E., Smeltzer, M. S., & Dunman, P. M. (2012). The staphylococcal accessory regulator, SarA, is an RNA-binding protein that modulates the mRNA turnover properties of late-exponential and stationary phase *Staphylococcus aureus* cells. *Frontiers in Cellular and Infection Microbiology*, *2*, 26. https://doi.org/10.3389/fcimb.2012.00026

Nguyen, M.-T., Luqman, A., Bitschar, K., Hertlein, T., Dick, J., Ohlsen, K., . . . Götz, F. (2018). Staphylococcal (phospho) lipases promote biofilm formation and host cell invasion. *International Journal of Medical Microbiology*, *308*(6), 653-663. https://doi.org/10.1016/j.ijmm.2017.11.013

Pietrocola, G., Nobile, G., Rindi, S., & Speziale, P. (2017). *Staphylococcus aureus* manipulates innate immunity through own and host-expressed proteases. *Frontiers in Cellular and Infection Microbiology*, *7*, 166. https://doi.org/10.3389/fcimb.2017.00166

Pragman, A. A., & Schlievert, P. M. (2004). Virulence regulation in *Staphylococcus aureus*: the need for *in vivo* analysis of virulence factor regulation. *FEMS Immunology & Medical Microbiology*, *42*(2), 147-154. https://doi.org/10.1016/j.femsim.2004.05.005

Rom, J. S., Atwood, D. N., Beenken, K. E., Meeker, D. G., Loughran, A. J., Spencer, H. J., . . . Smeltzer, M. S. (2017). Impact of *Staphylococcus aureus* regulatory mutations that modulate biofilm formation in the USA300 strain LAC on virulence in a murine bacteremia model. *Virulence*, *8*(8), 1776-1790. https://doi.org/10.1080/21505594.2017.1373926

Sarkar, P., Yamasaki, S., Basak, S., Bera, A., & Bag, P. K. (2012). Purification and characterization of a new alkali-thermostable lipase from *Staphylococcus aureus* isolated from Arachis hypogaea rhizosphere. *Process Biochemistry*, *47*(5), 858-866. https://doi.org/10.1016/j.procbio.2012.02.023

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C T method. *Nature Protocols*, *3*(6), 1101-1108. https://doi.org/10.1038/nprot.2008.73

Shagufta Naseer, B., Ravi, M., Gaddad, S. M., & Jayaraj, Y. M. (2014). Screening of staphylokinase producing *Staphylococcus aureus* from clinical samples. *4*(2), 46-48. https://www.isroset.org/journal/IJSRBS/index.php

Singh, V., & Phukan, U. J. (2019). Interaction of host and *Staphylococcus aureus* protease-system regulates virulence and pathogenicity. *Medical Microbiology and Immunology*, *208*(5), 585-607. https://doi.org/10.1007/s00430-018-0573-y

Tam, K., & Torres, V. J. (2019). *Staphylococcus aureus* secreted toxins and extracellular enzymes. *Microbiology Spectrum*, *7*(2), 10.1128/microbiolspec.GPP3-0039-2018. https://doi.org/10.1128/microbiolspec.GPP3-0039-2018

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, *28*(3), 603-661. https://doi.org/10.1128/CMR.00134-14

Ziebandt, A. K., Weber, H., Rudolph, J., Schmid, R., Höper, D., Engelmann, S., & Hecker, M. (2001). Extracellular proteins of *Staphylococcus aureus* and the role of SarA and σB. *Proteomics*, *1*(4), 480-493. https://doi.org/10.1002/1615-9861(200104)1:4<480::AID-PROT480>3.0.CO;2-O

Zielinska, A. K., Beenken, K. E., Mrak, L. N., Spencer, H. J., Post, G. R., Skinner, R. A., . . . Smeltzer, M. S. (2012). SarA-mediated repression of protease production plays a key role in the pathogenesis of *Staphylococcus aureus* USA 300 isolates. *Molecular Microbiology*, *86*(5), 1183-1196. https://doi.org/10.1111/mmi.12048