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ABSTRACT

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DETECTION OF SMALL NON-CODING RNA, RSAE, REGULATES THE *ICA* OPERON EXPRESSION IN *STAPHYLOCOCCUS AUREUS* IN HOLLY KARBALA CITY

Introduction. One of the pathogenic mechanisms by which *Staphylococcus aureus* is able to adhere to and persist on many types of medical equipment is its ability to produce biofilms. S. aureus conserved small noncoding RNA, RsaE, is known to regulate several metabolic processes.

Aim: This study aimed to investigate the correlation between small non-coding RNA RsaE and biofilm formation ica operon of S. aureus.

Material and Methods. In this study, different clinical samples (n=250) were collected from patients who attended to the hospitals at Holly Karbala City, Iraq. Based on cultural and biochemical approaches, the bacteria isolated from clinical samples were identified as S. aureus (n=60), which was further confirmed by Vitek2 compact. To determine the potential of S. aureus for biofilm formation, a microtiter plate as a quantitative method was used. Thereafter, the biofilm genes icaADBC and regulatory gene small non-coding RNA, RsaE, were detected using a polymerase chain reaction. Finally, the expression of biofilm operon icaADBC and regulatory gene rsaE was estimated in S.aureus isolates using a real-time quantitative PCR method.

Results. S. aureus (n=60) was isolated from different clinical samples (n=250) and identified according to the morphological and biochemical characteristics. Depending on the quantity of biofilm formation, the isolates were divided into strong (25%), moderate (67%), and weak (8.3%). All isolates (100%) had biofilm genes icaADBC, and regulatory small non-coding RNA gene, rsaE. Notable, S.aureus isolates with strong and moderate biofilm formation revealed high expression levels in icaA, icaD, and rsaE genes while *S.aureus* isolates with weak biofilm formation appeared with low expression levels of icaA, icaD, and rsaE genes.

Conclusion. This study proved that rsaE was responsible for the regulation of biofilm formation in the S.aureus clinical isolates through positively controlling the biofilm genes icaADBC.

Keywords: Staphylococcus aureus, biofilm formation, icaADBC, PIA/ica, rsaE gene.

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ABBREVIATIONS

sRNAs	small noncoding RNA
TCA cycle	Krebs cycle
QS	quorum sensing
PIA	polysaccharide intercellular adhesion
eDNA	extracellular DNA
Ica	intracellular adhesion
cDNA	complement DNA

INTRODUCTION / ВСТУП

S. aureus is a Gram-positive bacteria that can be found on the skin, anterior nares, and mucous membranes [1, 2]. These bacteria are opportunistic pathogens, capable of inducing a variety of illnesses in both animals and humans [3]. *S. aureus* is present in the nasal microbiome of approximately 30% of humans and can also transform into a highly dangerous and potentially lethal disease [4]. Skin infections, abscesses, impetigo, necrotizing pneumonia, septicemia, catheter-induced endocarditis, atherosclerosis, and osteomyelitis are just a few of the illnesses that Staphylococcus aureus may cause. [5, 6]. Particularly, the nosocomial infections in hospitals are exceedingly grave. Because *S. aureus* can form biofilms and is a facultative intracellular parasite, it may withstand the effects of antibiotics and host immune responses. This presents serious therapeutic challenges for the medical community worldwide [7]. Several factors contribute to the formation of a biofilm, which provides a favorable environment for evading the effects of antimicrobial agents and the immune system. Biofilm development is a process that involves quorum sensing (QS) [8]. As the bacteria grow, they will release tiny signals known as autoinducers (AIs), which will build up in the extracellular medium. Once a sufficient number of bacteria is attained, the artificial intelligences will facilitate the ability of a solitary bacterium to detect and interact with other bacteria in its vicinity, perhaps leading to the formation of biofilms [9].

Furthermore, the presence of biofilms enhances the ability of *S. aureus* to survive within cells, resulting in the development of persistent infections [10, 11]. Most medicines are widely recognized for their limited capacity to pass through biofilms and their reduced effectiveness against biofilm-forming *S. aureus* compared to planktonic *S. aureus* [12, 13]. Presently, *S. aureus* biofilms pose a significant enigma, with no viable therapy options currently accessible. The expression of genes that contribute to the severity of an infection is influenced by the availability of energy resources. Global regulators closely control the utilization of carbon sources. This adaptability is facilitated by a multitude of trans-acting regulators, which encompass small non-coding RNA (sRNAs) among other factors [14, 15]. Bacteria possess a substantial quantity of sRNAs. A significant number of them regulate mRNA translation and stability by forming base pairs with one or more specific RNAs. They exert influence on nearly all cellular functions, hence contributing to the process of adaptation and the maintenance of bacterial homeostasis [16]. The sRNA RsaE, consisting of 102 nucleotides, has garnered interest among researchers due to its significant conservation within the Bacillales order [17]. In *S. aureus*, RsaE modifies the expression of several genes linked to the TCA cycle, folate metabolism, and oligopeptide transport [18, 19].

RsaE enhances the creation of the biofilm matrix mediated by polysaccharide intercellular adhesion (PIA), mostly by activating the expression of the *icaADBC* operon through the regulation of small non-coding RNA regulatory gene *icaR*. Additionally, it inhibits the TCA cycle and redirects the central carbon metabolism of staphylococci towards the synthesis of PIA precursors. In addition, Extracellular DNA (eDNA), an essential portion of the biofilm matrix that aids in stabilizing the biofilm structure, is released more easily when RsaE is present, which promotes the growth of biofilms. Given the varied expression of RsaE within biofilms, so this

small RNA acts as a catalyst for phenotypic diversity and promotes the division of labour in *S. aureus* biofilm communities [20].

MATERIALS AND METHODS

Study Design

Different clinical samples (n=250) were collected from patients who attended the hospitals, Al-Husain Medical City and Al-Hassan al Mujtaba Teaching Hospital, at Holly Karbala City, Iraq from December 2021 to June 2022. The samples included nares swabs (n=55), abscesses (n=60), ulcers (n=5), blood (n=30), midstream urine (n=40), and wound swabs (n=60).

Ethical approval

Ethical approval for this study was obtained from the Ethical Approval Committee of the University Of Baghdad, Iraq. The study was conducted from December 2021 to June 2022.

Bacterial culture conditions

The bacterial isolates from clinical samples were identified as *Staphylococcus aureus* (n=60) according to the cultural method using mannitol salt agar [21] then the growing colonies were submitted to biochemical assays (production of catalase and coagulase). Furthermore, a VITEK 2 compact (bio- Mérieux, America) was employed for conclusive verification [22, 23].

Quantifying Biofilm Production Detection

An assay for microtiter plates was used to assess the biofilm development of *S. aureus* isolates, following prior instructions [24]. In summary, every *S. aureus* isolate was cultivated for 24 hours at 37°C on Trypticase Soy Agar (TSA, Merck, Co., Germany). The grown colonies were then suspended in sterile physiological saline, with the turbidity adjusted to 0.5 Mc- Farland. 180µl of Trypticase Soy Broth (TSB, Merck, Co., Germany) supplemented with 1% glucose and 20µl of bacterial suspension added to each well made up the 96 well microdilution plates (Cell and Tissue Culture plates, flat well bottom, Guangzhou Jet Bio-Filtration Products Co., Ltd. Guangdong, China). Once the plates had been incubated for 24 hours at 37 degrees Celsius, the broth was carefully removed, and three gentle washes with sterile phosphate-buffered saline (PBS) were performed. 200µl of a 2% crystal violet dye solution in water was applied to each well for the purpose of quantifying biofilm, and the plates were left to remain at room temperature for 40 minutes. After that, sterile PBS was used three times to rinse the wells in order to remove any remaining crystal violet. Using 200 millilitres of 95% ethanol, crystal violet attached to the biofilm was removed, and the extracted safranin's absorbance was quantified at 490 nm using an ELISA reader (BioTek, USA). As a negative control, background optical density (OD) was measured using TSB+1% glucose media. The cut-off OD (ODc) for biofilm formation was determined as the average OD of negative control +3× standard deviation (SD) of negative control [25]. Every microtiter plate's OD value was computed separately. In this investigation, the mean optical density (OD) of the negative control was found to be 0.092. The calculated standard deviation of the optical density (OD) value was found to be 0.00173. Therefore, the cut-off value was calculated to be 0.0971. The biofilm gradation in clinical isolates was then assessed based on the criteria outlined in (Table 1) [26].

Table 1 – Classification of biofilm generated by Staphylococcus aureus strains [24]

Optical density values OD	Criteria outlined
OD ≤ 0.097	Non biofilm formation
0.0971 < OD ≤ 0.194	Weak biofilm formation
0.194 < OD ≤ 0.388	Moderate biofilm formation
0.388 < OD	Strong biofilm formation

Extraction of bacterial Genomic DNA

DNA from *S. aureus* isolates was extracted following the manufacturer's instructions for a Gram-positive DNA extraction kit (Norgen Canada). The isolated genomic DNA was analyzed for both quality and quantity with the use of 2% agarose gel electrophoresis and a QubitTM dsDNA HS assay kit (ThermoFisher® USA). The DNA samples were ultimately placed in a 1 µL microtube. then stored at a temperature of -20ºC for subsequent investigations [27].

PCR detection of ica operon and rsaE gene

The extraction of genomic DNA was performed using the previously outlined method [28]. The PCR reactions were conducted using a Gradient thermal cycler (SimpliAmp, Germany). The PCR reaction mixture had a final volume of 20µl and consisted of several components. These components included 2 µl of a 10X PCR buffer, 1.5ml of a 25mM $MgCl_2$ solution, 2 µl of a 10mM dNTPs solution, 1µl of each primer, 2.5µl of the DNA sample, and 1.25U of Taq DNA polymerase from NEB® (England). The final volume was then adjusted to 20µl using 10.5µl of nuclease-free water. The PCR

products were subjected to amplification and then separated by electrophoresis on a 1.5% agarose gel in TBE 0.5× buffer (containing 5.4g Tris base, 2.75g Boric acid, and 2ml 0.5M EDTA per liter) at a voltage of 100V for 90min. The products were identified through the application of Green Viewer Dye for staining, followed by photography. The primers were designed based on the *Staphylococcus aureus* genome information accessible on the National Centre for Biotechnology Information (NCBI) and were supplied in a lyophilized form by Macrogen Company (Korea). The size of the genes being examined and their matching genetic sequences were precisely ascertained for the objectives of this investigation [29]. The primers and PCR programmes are provided in Table 2.

Table 2 – Primer pairs and PCR conditions were used to detect genes in this study

Gene target	Primer/sequence (5'-3')	PCR condition	Amplicon size (bp)
icaA	F: CGACGTTGGCTACTGGATAC R: ACACATGGAAGCGGTTCATA	30 sec. 94ºC, 45 sec 56º C, 45 sec 72º C	106
icaB	F: CACAGGTCATGTTGGGGAAGAA R: TGCAAATCGTGGGTATGTGTITC	1 min 94º C, 45 sec 53º C, 45 sec 72º C	118
icaC	F: GCGTTAGCAAATGGAGACTATTGG R: TGCGTGCAAATACCCAAGATAACA	30 sec.94º C, 45 sec 53º C, 45 sec 72º C	101
icaD	F:TCGCTATATCGTGTGTCTTTTGGA R: CGCGAAAATGCCCATAGTTTCA	30 sec.94º C, 45 sec 53º C, 45 sec 72º C	164
rsaE	F: AGGTTGAAATGGAAGAGATAGCGT R: CACGACGCTAAACAAAGGGGA	30 sec.94º C, 45 sec 60º C,45 sec 72º C,	116
16S rRNA	F: CTCAACCGTGGAGGGTCATTG R: CCTGTTTGACCCCACGCTTT	30 sec.94º C, 45 sec 57º C, 45 sec 72º C	169

Molecular Detection of reference gene 16S rRNA

The procedure involved adding 12.5 µl of OneTaq (NEB®) mastermix, 3 µl of DNA sample, 1 µl of 10 pmol/µl primer for each primer, and 7.5 µl of free-nuclease water into a PCR tube. The experiment was conducted using the appropriate polymerase chain reaction (PCR) settings specified in Table 2.

RNA extraction from S. aureus isolates

The RNA from *S. aureus* isolates was extracted using the TRIzolTM (Thermo Fisher®, USA) according to the manufacturer's protocol. The isolates were cultured in Tryptic Soy broth (TSB) supplemented with 1% glucose for a duration of 18 hours, after which they were transferred to microtiter plates to generate biofilm cells. The plates were thoroughly cleaned with distilled water to remove any traces of methanol, so removing any cells that were not adhered to the wells. After the biofilm on the glass surface became invisible, the biofilm cells were gathered by suspending them in cold, sterile normal saline using a pipette. During the entire procedure, the cells were kept on ice to prevent the degradation of RNA. Afterwards, the bacterial cells were relocated to micro-centrifuge tubes having a 1.5 ml capacity. The tubes were subsequently centrifuged at a force of 14000g for a duration of two minutes. After centrifugation, the liquid part above the settled cells, referred to as the supernatant, was completely extracted. In order to determine the suitability of the samples for future usage, the concentration of extracted RNA was measured using a Quantus fluorimeter .The assay used to quantify RNA concentrations exhibits a high degree of selectivity for RNA and provides reliable measurements for initial sample concentrations ranging from 10 picograms per microliter (pg/µL) to 100 nanograms per microliter (ng/µL). The test is conducted at ambient conditions, specifically at the temperature of the room, and the signal remains constant for a duration of 3 hours. The assay can effectively accommodate common impurities such as salts, free nucleotides, solvents, detergents, or proteins without any adverse effects.

Synthesis of reverse transcription

The NEB®-UK protoscript cDNA synthesis kit was employed to perform reverse transcription of mRNA extracted from total RNA and the primers for the studied genes (*icaA, icaD, icaR,* and *rsaE*) as well as reference gene *16S rRNA* transcripts were designed (Table 2). The experiment was carried out following the manufacturer's protocol (New England Biolabs) in a reaction volume of 20 µl. Then, cDNA had been stored at -80° C until use.

Real-time Quantitative PCR (RT-qPCR) assay

The qRT-PCR was performed using the QUBIT® Real-time PCR System (Thermofisher®, USA) and qPCRsoft software. The Real-Time PCR programme was established using the specified thermocycling methodology outlined in Table 3.

Table 3 – Thermocycling protocol for RT-qPCR

Cycle No.	Stage	Temperature	Time
1	Initial Denaturation	95ºC	60 sec.
40-45	Denaturation	95ºC	15 sec.
	Extension	60ºC	30 sec.
1	Melt curve	60-95ºC	40 min.

Determination the expression of ica operon and rsaE gene by RT-qPCR

The data was collected via the FAM/SYBR channel, then transferred to an Excel spreadsheet and processed using the LinRegPCR programme version 2015. In order to ascertain the value of the expression, the initial concentration of each sample was standardised in relation to the concentration of the housekeeping gene 16S rRNA. The cDNA template derived from the S. aureus isolates exhibiting low biofilm formation was employed as a control, and each trial was repeated a minimum of three times [30]. The $2^{-\Delta\Delta CT}$ method is commonly employed as a methodology for relative quantification in the analysis of data obtained from real-time quantitative polymerase chain reaction (RT-qPCR). This method offers an easy way of assessing the comparative levels of gene expression across different samples. The computation relies on the threshold cycles (CTs) provided by the RT-qPCR apparatus [31]. The CT value of the target gene was normalised to match the CT value of the internal control gene. The results were assessed with the CT and Livac formulas as follows:

ΔCT = CT(a target gene)−CT(a reference gene)

ΔCT for the target sample= CT(a target gene A) – CT(a reference gene B)

ΔCT for the reference sample= CT(a target gene B) – CT(a reference gene B)

ΔΔCT = ΔCT (a target sample) −ΔCT(a reference sample)

Normalized Ct expression Formula= $2^{-\Delta\Delta Ct}$

From its arduous form to its computerized one, polymerase chain reaction (PCR) has become an essential instrument in biology and medicine. Several PCR-based infectious illness treatments exist. These include monitoring antibiotic resistance, developing diseases, biological threats, and specific or widespread infections. Prenatal diagnosis and newborn genetic disorders screening are made easier by PCR. Cancer research uses PCR to find tumor-related genes. PCR detected genetic and microbiological issues in several medical settings [32–41].

RESULTS

Isolation and identification of S. aureus isolates

A total of 250 clinical samples, including wound, nasal cavity, blood, urine, abscess, and ulcer samples, were obtained from patients who visited the hospitals in Holly Karbala City for the purpose of isolating *S. aureus*. A total of sixty bacterial isolates were classified as *S. aureus* based on their morphological and biochemical characteristics [42, 43]. These isolates exhibited round, smooth, elevated, mucoid, and glistening colonies. The microscopic analysis revealed that all of these isolates were gram-positive cocci. In addition, all isolates exhibited a negative oxidase result, a positive catalase and coagulase result, and the ability to ferment mannitol. A clear zone encircling *S. aureus* colonies on blood agar revealed hemolysis [44]. The prevalence of *S. aureus* isolates in the samples varied: wounds (n=21, 35%), nasal cavities (n=14, 23.33%), blood (n=5, 8.33%), urine (n=10, 16.66%), abscesses (n=9, 15%), and an ulcer (n=1, 66%) (Figure 1).

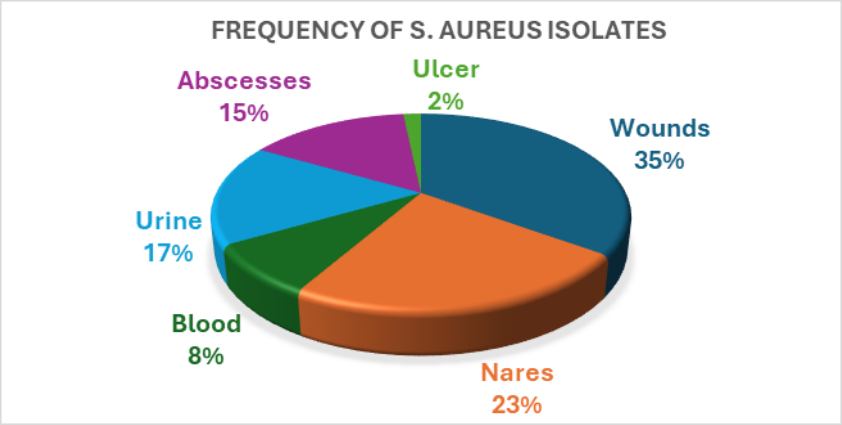

Figure 1 – Frequency of S. aureus isolates by sites of infection

The elevated prevalence of *S. aureus* in wounds may be attributed to their capacity for biofilm formation, which slows the healing process. We also demonstrated a link between wounds and the formation of biofilm. Within a fully developed biofilm, bacterial growth is slowed by limited nutrition availability, leading to the bacteria's resistance to antibiotics [45].

Formation of Biofilms by S. aureus Isolates

All *S.aureus* isolates demonstrated a potential for forming a biofilm with different profiles; 15 (25%) isolates had strong ability for biofilm production, the majority of *S. aureus* isolates (n=40, 67%) appeared with moderate biofilm formation, while 5 (8%) isolates revealed weak ability for biofilm formation (Figure 2). According to the data obtained, the *S. aureus* isolates shown a significant capacity for biofilm development. This suggests that wounds and ulcers are suitable environments for the generation of biofilms [46]. The pathogenicity of *S. aureus* is contingent upon its capacity to generate a diverse array of virulence factors that facilitate bacterial infiltration [47, 48].

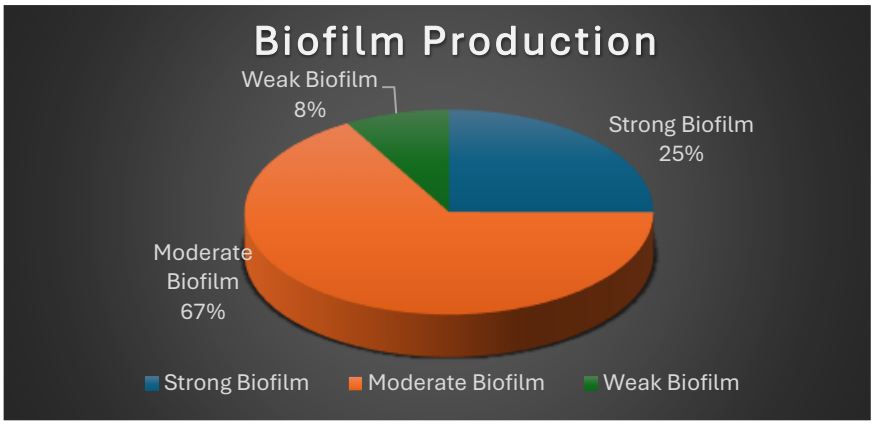

Figure 2 – Different biofilm production by S. aureus isolates

***ica* operon in *S. aureus* isolates**

All isolates showed the presence of the ica operon (Figure 3). The prevalence rates of the examined genes were as follows: *icaA* (100%), *icaB* (100%), *icaC* (100%), and *icaD* (100%) [49, 50]. The *ica* genes exert a significant influence on the generation of slime in *S. aureus*. So, bacteria that had the *icaADBC* cluster were identified as potential producers of biofilm [51]. The results of our study align with previous publications, as the ica genes were found in all isolates [52].

Molecular detection of small noncoding RNA, RsaE

An analysis was conducted on *S. aureus* isolates to detect the presence of a short noncoding RNA *rsaE* gene. The electrophoretic pattern of the *rsaE* gene can be seen in Figure 4. RsaE is expressed in a specific location and time within biofilms formed by *S. aureus* through PIA-mediated mechanisms. When RsaE is overexpressed, it induces the formation of PIA biofilms and the release of eDNA in a strain of *S. aureus* that produces a protein biofilm matrix. RsaE promotes the formation of biofilms by aiding in the release of extracellular DNA (eDNA), which acts as a stabilizing component of the biofilm structure [53]. Given the varied expression of RsaE within biofilms, our proposition suggests that this small RNA molecule (sRNA) has a function in enhancing the diversity of characteristics within *S. aureus* biofilm communities and aiding in the distribution of tasks among individuals. We hypothesize that the bacterial lysis facilitated by RsaE could be a type of bacterial altruism that aids in the organization of biofilms by supplying nutrients to nearby bacterial cells and releasing eDNA as the biofilm matrix's stabilizing element [54].

Expression of ica operon and RsaE in clinical S. aureus isolates

The expression of biofilm-production *ica* operon and non-coding RNA (*rsaE* gene) in 15 biofilm-producing *S. aureus* isolates were measured using RT-qPCR. To compare the values of strong, moderate, and weak biofilm production, we used the average of weak biofilm values as a control. The results indicated the expression of the *icaADBC* and *rsaE* gene was upregulated in *S. aureus* isolates exhibited strong and moderate biofilm production. In contrast, the expression of *icaADBC* and *rsaE* gene was downregulated in isolates that had weak biofilm production, summarized (Tables 4, 5, and 6).

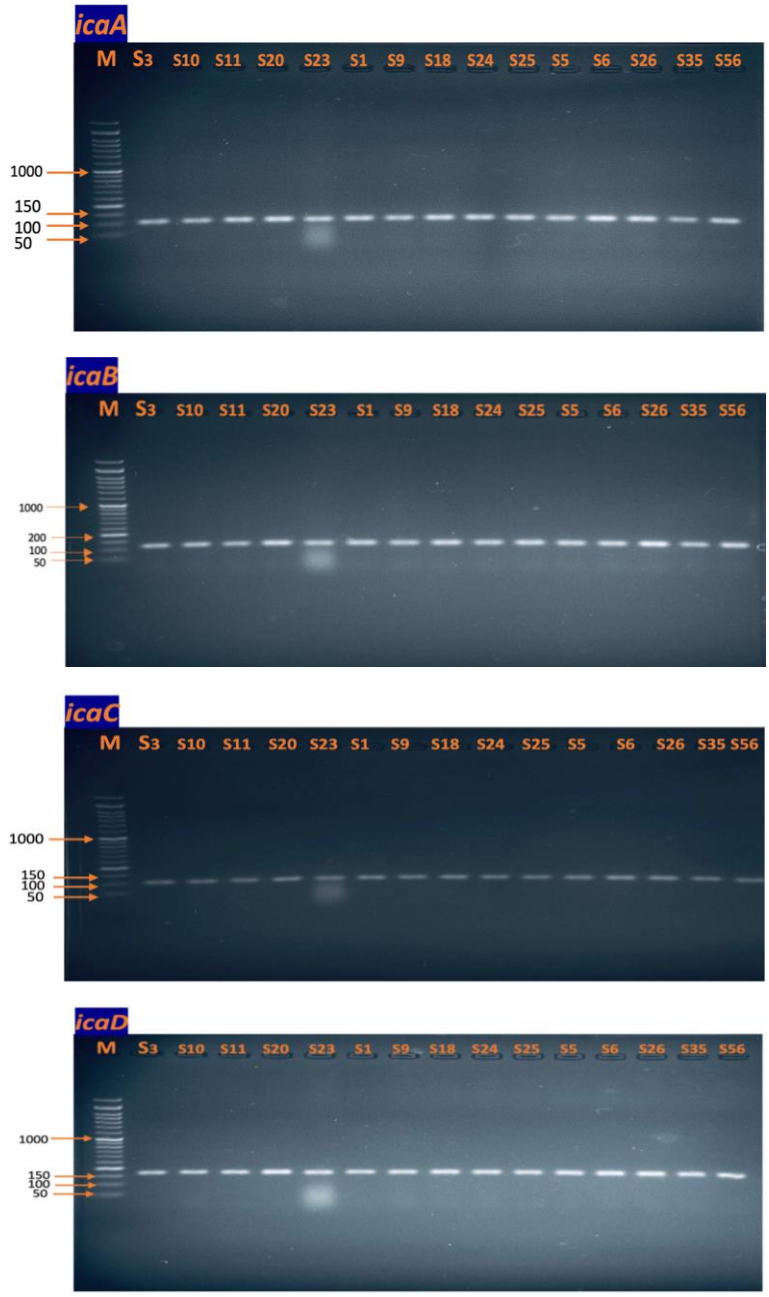

Figure 3 – Gel electrophoresis indicating the presence of ica operon in S.aureus isolates. M: molecular size marker

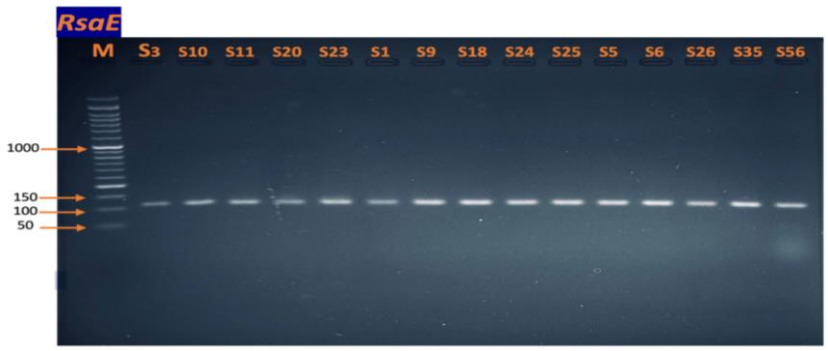

Figure 4 – Gel electrophoresis indicating the presence of small noncoding RNA, RsaE (141 bp) in S.aureus isolates. M: molecular size marker

Table 4 – Expression of *icaADBC* and noncoding RNA, *rsaE* gene in *S. aureus* isolates with strong biofilm production

Genes		Clinical isolates				
		S3	**S10**	**S11**	**S20**	**S23**
icaA	**Folding of expression**	0.233	2.549	4.184	2.027	**2.337**
	Induction	-	1.549	3.184	1.027	**1.33**
	Reduction	4.484	-	-	-	-
icaB	**Folding of expression**	0.137	1.972	4.027	1.717	**2.329**
	Induction	-	0.972	3.027	0.717	**1.329**
	Reduction	7.299	-	-	-	-
icaC	**Folding of expression**	0.18	2.12	6.43	1.753	**2.741**
	Induction	-	1.12	5.43	0.753	**1.741**
	Reduction	5.555	-	-	-	-
icaD	**Folding of expression**	0.083	1.84	3.105	1.717	**3.103**
	Induction	-	0.84	2.105	0.717	**1.103**
	Reduction	12.048	-	-	-	-
rsaE	**Folding of expression**	0.372	1.783	1.14	1.72	**1.866**
	Induction	-	0.783	0.14	0.72	**0.866**
	Reduction	2.688	-	-	-	-

Table 5 – Expression of *icaADBC* and noncoding RNA, *rsaE* gene in *S. aureus* isolates with moderate biofilm production

Genes		Clinical isolates				
		S1	**S9**	**S18**	**S24**	**S25**
icaA	**Folding of expression**	3.93	1.61	1.63	1.427	**0.278**
	Induction	2.93	0.63	0.63	0.427	-
	Reduction	-	-	-	-	**3.597**
icaB	**Folding of expression**	0.582	0.022	0.438	0.429	**0.203**
	Induction	-	-	-	-	-
	Reduction	1.718	45.454	2.283	2.331	**4.926**
icaC	**Folding of expression**	0.57	0.06	1.6	0.364	**0.222**
	Induction	-	-	0.6	-	-
	Reduction	1.754	16.66	-	2.747	**4.504**
icaD	**Folding of expression**	1.582	1.019	0.372	0.294	**0.215**
	Induction	0.582	0.019	-	-	-
	Reduction	-	-	2.688	3.401	**3.401**
rsaE	**Folding of expression**	2.58	1.114	2.14	2.53	**0.23**
	Induction	1.58	0.114	1.14	1.53	-
	Reduction	-	-	-	-	**4.347**

Table 6 – Expression of *icaADBC* and noncoding RNA, *rsaE* gene in *S. aureus* isolates with weak biofilm production

Genes		Clinical isolates				
		S5	**S6**	**S26**	**S35**	**S56**
icaA	**Folding of expression**	0.007	0.381	0.005	0.404	**1**
	Induction	-	-	-	-	-
	Reduction	3.597	142.85	200	0.227	-
icaB	**Folding of expression**	0.005	0.307	0.007	0.266	**1**
	Induction	-	-	-	-	-
	Reduction	200	3.257	142.85	3.759	-
icaC	**Folding of expression**	0.004	0.303	0.005	0.421	**1**
	Induction	-	-	-	-	-
	Reduction	250	3.30	200	2.375	-
icaD	**Folding of expression**	0.002	0.309	0.004	0.174	**1**
	Induction	-	-	-	-	-
	Reduction	500	3.236	250	5.747	-
rsaE	**Folding of expression**	0.0006	0.33	0.006	0.14	**1**
	Induction	-	-	-	-	-
	Reduction	1666.66	3.030	166.66	7.142	-

DISCUSSION

Staphylococcus aureus, a major bacterium commonly found in both hospital and community settings, plays a vital role in the development of wound infections. *Staphylococcus aureus* is the primary bacterium commonly associated with osteomyelitis, a disorder characterized by persistent and recurrent bone infections. The beginning and progression of *S. aureus* infection can be influenced by the virulence of the bacteria and the immunological components of the host. Pathogen-related factors refer to the complex bacterial structure and functional characteristics that provide them metabolic and adhesion abilities, allowing them to avoid the host's immune response. The virulence indicators and toxins of *S. aureus* demonstrate notable resemblances in several wound types. The outcomes of wounds are significantly influenced by the presence of various clonal lineages and virulence factors [55]. The production of biofilms has been documented to increase the pathogenicity of *S. aureus*, and strains that are capable of forming biofilms exhibit greater resistance to antibiotics, disinfectants, and other environmental variables [56]. Environmental factors, nutritional availability, specimen type, surface adhesion properties, genetic makeup, and place of origin are among the many factors that impact biofilm formation. The data we have collected indicates a significant occurrence of *S. aureus* in both wounds and nasal passages, which aligns with a recent study [57, 58]. This study demonstrates that *S. aureus* strains obtained from wound infections in hospitalized patients exhibit a significant ability to generate biofilms. When the human immune system or exogenous antimicrobial agents fail to remove free-floating bacteria, biofilms can form on any lesion [59].

The production of PIA/PNAG is strictly regulated and appears to predominantly occur at the transcriptional level, at least in vitro. Although the precise signals regulating PIA/PNAG production in living organisms remain unclear, various environmental factors influence production in laboratory settings. PIA/PNAG production can be induced by factors such as high temperature, anaerobiosis, high osmolarity, glucose, and ethanol. However, there is diversity across different strains in terms of which conditions lead to enhanced PIA/PNAG production [60]. Due to its ability to provide protection, biofilm development is considered a crucial survival strategy for numerous pathogenic organisms in unfavorable conditions [61]. Our examination of gene expression confirms that the *icaADBC* operon does indeed contribute to the development of biofilms by this bacteria. Therefore, our research conclusively demonstrates that all the genes analyzed in this operon are greatly increased and closely

aligned with the development and maturation of biofilms by *S. aureus.* So, our result shows a positive relationship between *icaADBC* operon and small noncoding RNA, RsaE enhances PIA-mediated biofilm matrix synthesis, possibly via stimulating the expression of the icaADBC operon, which is consistent with the findings of Schoenfelder et al. [62, 63]. Furthermore, Bohn C, et al documented that RsaE has a role in maintaining synchronized control of the central metabolism in situations when carbon supplies are limited, in order to facilitate the development of biofilms [64]. Given its varied expression, we see RsaE as a contributing factor that promotes population diversity. The results together provide insight into an unexpected function of sRNAs as contributors to the formation of *S. aureus* biofilms.

CONCLUSION

Biofilm formation is one of the major virulence factors for *Staphylococcus aureus*, which increases its pathogenicity and how to use antibiotics to treat it. It was found that wounds are the most places that contain the most highly virulent strains. It was also found that the formation of biofilm is affected by a number of environmental factors, as well as internal factors, such as non-coding RNA, which is considered a sensor for external conditions, such as non-coding DNA, RsaE, which is considered a regulator of the carbon source and thus increases the productivity of the biofilm.

AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

Zahraa Mohammed Wannas: sample collection, sample analysis, data collection, statistical analysis, and manuscript writing. ***Hayfa H. Hassani***: Conceptualization of the research, research design, and manuscript proofreading.

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The authors declare that they have no competing interests.

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