Iraqi Journal of Science

ISSN: 0067-2904

The Ability of *Staphylococcus aureus* to Establish Biofilm on Acrylic, Plastic, and Metallic Denture Materials

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Received: 26/3/2022 Accepted: 22/6/2022 Published: 28/2/2023

Abstract

Staphylococcus aureus is a common pathogenic agent due to its ability to cause various types of infections, ranging from mild skin infections to sever systemic diseases. One of the most virulence factors of this bacterium is its ability to from biofilms on solid surfaces by anchoring the planktonic cells and by producing a protective layer of extra polymeric substances. Biofilm formation is controlled through many genes. The most important ones are *icaA* and *icaD*. Dentures are prosthetic devices that are made of different materials to replace lost teeth. The aim of this study is to examine the ability of different types of denture materials to support the biofilm formation of *S. aureus* at phenotypic level by detecting bacterial growth on them using crystal violet and scanning electron microscope, as well as genotypic level through detection and estimation of gene *icaA* and *icaD* expression. Our findings showed that the denture materials do support biofilm formation and there is elevation in gene expression of *icaA* and *icaD*.

Keywords: *Staphylococcus aureus*, Biofilm, Denture, *icaA*, *icaD*

قدرة المكورات العنقودية الذهبية على تكوين الغشاء الحياتي على المواد الاساس لأطقم الاسنان الاكريليك والبلاستيكية والمعدنية

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الخلاصة

المكورات العنقودية الذهبية هي عامل ممرض شائع بسبب قابليتها على التسبب بأنواع مختلفة من الاصابات الخمجية امتدادا من الإصابات الخمجية الجلدية البسيطة الى الامراض الجهازية الشديدة. احد اهم العوامل لضراوةهذه البكتيريا هوقابليتها على انتاج غشاء حياتي على السطوح الصلبة من خلال تثبيت الخلايا العائمة وإنتاج طبقة حماية من مواد متبلمرة خارج خلوية. انتاج الغشاء الحياتي والسيطرة عليه تتم من خلال عدة جينات، *أهمها icaA وicaD*.اطقم الاسنان هي أجهزة مصنعة من مواد مختلفة لاستبدال الاسنان المفقودة. الغرض من هذه الدراسة هو اختبار قابلية المواد المختلفة الي يصنع منها طقم الاسنان على دعم بناء الغشاء الحياتي للمكورات العنقودية على الصعيد المظهري من خلال الكشف عن نمو البكتيريا على الطقم باستعمال صبغة البنفسج البلوري والمجهر الالكتروني الماسح، وعلى الصعيد الجيني تم الكشف وحساب التعبير الجيني

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للجينين *icaA*و*icaD*. كشفت النتائج ان مواد أطقم الاسنان تدعم تكوين الغشاء الحياتي وان هنالك ارتفاعا بالتعبير الجيني لكل من *icaA*و*icaD*.

Introduction

In the past, bacteriologists thought that colonization of the oral cavity with *Staphylococcus aureus* does not affect mouth's condition. However, in the recent decade conducted studies, results conducted suggest that *S. aureus* resides in the oral cavity more frequently than expected (more often than the nasal vestibule) [1], and that may result in various mouth and systemic infections [2]. *Staphylococcus aureus* produces an extracellular polymeric substance (EPS) within a sessile community known as biofilm which helps the microbe resistance or reduction of the antibacterial effect [3]. Like any other bacterial biofilm, *S. aureus* biofilm is composed of two distinct components, water (about 97%) and the organic matter which constitutes of EPS and micro colonies [4]. The major component of *S. aureus* biofilm EPS is the polysaccharide-intercellular-adhesin (PIA), also named poly-(1-6)-N-acetylglucosamine (PNAG) due to its chemical composition. PIA is positively charged which encourages colonization, biofilm formation and biofilm-based infections, evasion of immune system, antimicrobials and phagocytosis resistance [5].

One important element in the process of biofilm formation is the *ica* (intercellular adhesion) operon; a gene cluster encoding the production of PIA. The operon is composed of five genes: *icaA, icaD, icaB, icaC* and *icaR.* However, *IcaA* and *icaD* code for the transferases, they are also responsible for the production of PIA [6].

Dentures are prosthetic devices made to replace lost teeth and are supported by the oral cavity tissues. Denture bases are made mainly of polymethylmethacrylate, metallic or plastic [7]. Polymethylmethacrylate (acrylic) is the most widely used denture base material due to its cost, aesthetics, easy to process and maintenance properties. It is a combination of advantages rather than one excellent aspect that accounts for its wide usage [8]. Plastic (flexible) dentures are less rigid than acrylic and are mostly custom made for those who suffer with the conventional acrylic base dentures irritating the gums, induce allergic reactions or generally fail to provide a comfortable result [9]. Metal dentures are frameworks usually casted from chrome cobalt and may have a longer life span than acrylic and plastic dentures. There are two advantages of this type of denture. They can be designed to be thinner and stronger and they provide better support for the remaining teeth [10].

Staphylococcus aureus is frequently encountered as oral normal flora of denture wearers more than in non-wearers [11]. Including dental prostheses, *S. aureus* can adhere to many different surfaces of oral cavity [12]. Denture is a non-shedding oral surface that is particularly easily colonized by staphylococcal biofilm [13]. *S. aureus* may cause many forms of infections. Among them are buccal infections, such as angular cheilitis, periodontitis, mucositis and infections that are associated with dental implants [14]. Most adverse complications can occur when an infectious agent is inhaled and passed to the lower respiratory system causing pneumonia [15].

The importance of this study is to assess the types of denture materials that may encourage *S. aureus* biofilm formation.

Materials and Methods

Staphylococcus Isolates

A total of 93 *S. aureus* isolates were obtained from the Microbiology lab at Department of Biology, College of Science, University of Baghdad. These isolates were originally isolated from different specimens (comprising blood, skin infection swabs, denture swabs, sputum, and urine) and showed the highest polysaccharides production on Congo red agar. These isolates were reidentified by the amplification of *16SrRNA*.

Fabrication of Denture Pieces

Polymethylmethacrylate, plastic and metallic were chosen for this study. A total of 60 pieces of each type were fabricated, each of which had identical dimensions (10mm × 10mm × 1mm) and equal surface roughness [16].

Roughness of the Pieces

In order to get equal surface roughness, the pieces were smoothened using a machine (Parkside®, Germany). Then the roughness was measured by a specialized (Positector®, USA). The roughness of all pieces was set at 200 – 210 µm [17].

The Affinity of Denture Materials for Water

A distilled water (DW) droplet was placed on the denture material pieces (in sextuplicate). The hydrophobicity was determined by measuring the contact angle (θ) of water on the surfaces (Figure 1), if $\theta < 90^o$ then the surface is hydrophilic and if the $\theta > 90^o$ it is hydrophobic [18].

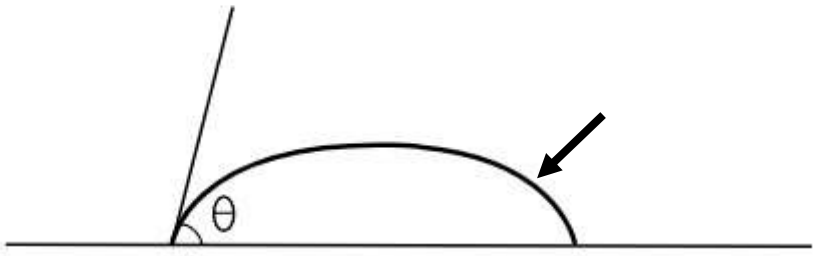

Figure 1: Contact angle of a water droplet (arrow) and a solid surface.

Biofilm Formation Assay

Each of the 13 selected isolates were tested for the biofilm formation by allowing the bacteria to grow in 30 ml of Tryptone soy broth (HiMedia, India), supplemented with 1% glucose (Pioneer, Iraq) in the presence of the pieces in a sterile container. Thereafter, all containers were inoculated with 50 µl from bacterial suspension of 0.5 OD_{600} and then incubated in a shaker incubator for 24 hours at 37ºC. Each of isolate was tested on three pieces of the same type of denture. Similar denture type in a bacteria-free medium was considered as control.

After incubation, all pieces were gently washed with DW to remove unattached cells and were left to dry on a filter paper. Subsequently, all pieces were submerged in absolute methanol for 10 minutes, washed, and left to dry. They were then placed in a Petrie dish filled with 0.5% crystal violet stain for 10 minutes, washed, and left to dry. Separately, each piece was placed in a tube that contained 2ml of ethanol-glacial acetic acid mixture (1:1 vol/vol) for 10 minutes. A volume of 200 µl of the resolubilized stain was removed into a microtiter plate where the optical density was read at 630 nm using a microplate reader (BioTek, USA).

The biofilm intensity was calculated using the criteria developed by Stepanovic *et al*. [19] as shown in Table 1.

Table 1: The criteria for biofilm intensity [19]

Average O.D Value	Biofilm's intensity
O.D. ≤ O. Dc	Non-producer
O. Dc > O.D. ≤ 2×O.Dc	Weak
2×O.Dc > O. D ≤ 4× O.D	Moderate
4×O.Dc < O. D	Strong

***Optical density cut off Value (O.Dc) =Average of Negative Control + 3×Standard deviation of the Negative Control.**

Scanning Electron Microscopy

The biofilm was assayed as mentioned previously, except for staining steps. The pieces were then submitted into the scanning electron microscope (SEM) imaging using field emission electron microscope (FE-SEM) (Thermo Fisher Inspect F50, USA) [20].

Molecular Study

Primer Reconstitution

All oligonucleotide primers (Scientific researcher laboratories, Iraq) were dissolved in sterile deionized DW to produce a solution with a final concentration of 100 picomoles/µl. Nevertheless, a working primer solution 10 picomoles/µl was prepared. The primer sequences are listed in Table 2.

Table 2: Primers involved in this research

Primer Name	Sequence (5′ – 3′)	Amplicon Size (bp)	Reference
16SrRNA	F- ACGGTCTTGCTGTCACTTATA	257	[21]
	R- TACACATATGTTCTTCCCTAATAA		
***ica*A**	F- CAATACTATTTCGGGTGTCTTCACTCT	102	[22]
	R- CAAGAAACTGCAATATCTTCGGTAATCAT		
***ica*D**	F- TCAAGCCCAGACAGAGGGAATA	83	[22]
	R- ACACGATATAGCGATAAGTGCTGTTT		
***mec*A**	F- GTAGAAATGACTGAACGTCCGATAA	310	[23]
	R- CCAATTCCACATTGTTTCGGTCTAA		
***rpo*B**	F- CAGCTGACGAAGAAGATAGCTATGT	82	[22]
	R- ACTTCATCATCCATGAAACGACCAT		

PCR Technique

Extractions of Genomic-DNA

DNA extraction for all isolates was done following the manufacturing procedure of bacterial DNA extraction Kit (Promega®, USA). The concentration and purity of extracted DNA were carried out using Nanodrop (Quawell, USA). The samples with purity ratio 1.8-2 were considered free from protein contamination [24].

Detection of *16SrRNA*

Using the procedure described by Matsuda *et al*. [21], the *S. aureus 16SrRNA* gene was screened in each isolate. The *S. aureus 16SrRNA* was amplified by using primers listed in Table 2. PCR was done by a thermocycler (Qiagen, Germany) using a 25l reaction mixture containing12.5 µl of 2x master mix (Promega, USA), 1µl each of the forward and reverse primers, 7.5 µl of nuclease-free water and 3 µl of theDNA template. The thermocycling conditions included initial denaturation at 94°C for 4 minutes, 37 cycles of 94°C for 1 minute,

50°C for30 seconds, and 72°C for 1 minute. A final extension at 72ºC for 5 minutes. The PCR products (10 µl) were evaluated on a 1.5% (w/v) agarose gel. Negative control reaction contained all components without DNA template.

Detection of *mecA*

The *mecA* gene was investigated in study isolates following the procedure described by Zhang *et al.* [23]. Thermocycling was done using 25 µl final reaction volume containing 1 µl of each of the primers specific for the *mecA* gene (Table 2), 3 µl of template DNA, 12.5 µl of master mix with 7.5 µl of nuclease-free water. Thermocycling conditions were set as 94°C for 10 minutes. 12 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 75 seconds, 25 cycles of 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 75 seconds. A final extension was done for 5 minutes at 72°C. The amplified PCR products (10 µl) were resolved on a 1.5% (w/v) agarose gel. Negative control reaction contained all components with no DNA template.

Detection of *rpoB, icaA and icaD*

The *rpoB, icaA* and *icaD* were screened in the study isolates following the procedure described by Kot *et al.* [22]. The thermocycling was done using 25 µl final reaction volume containing 1 µl of each of the primers specific for the *rpoB* genes (Table 2), 3 µl of template DNA, 12.5 µl of master mix with 7.5µl of nuclease-free water. Thermocycling conditions were set as primary denaturation at 94ºC for 10 minutes, 40 cycles: 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 45 seconds. Final extension was done at 72°C for 5 minutes. The amplified PCR products (10µl) were resolved on a 1.5% (w/v) agarose gel. Negative control reaction contained all components with no DNA template.

Gene Expression for *icaA* and *icaD*

Sample Preparation for RT-qPCR

An isolate with strongest biofilm for each type of the denture material under test was selected for RT-qPCR. Three containers contained 50 ml of TSB supplemented with 1% glucose, each container was inoculated with one isolate. Afterwards, three pieces of the dentures were added and incubated at 37ºC for 24 hours. Thereafter, the pieces were removed from the medium and rinsed gently with DW. After that the biofilm cells developed on the denture pieces were collected via scraping in sterile sample-cubs containing 10ml of normal saline [16].

RNA Extraction

The total RNA extraction was carried out using Genezol® reagent according to the protocol provided by the manufacturer company (Geneaid/ Korea).

Synthesis of Complementary DNA

The procedure was performed in a volume of reaction (20µl) according to manufacturer instructions (Bioneer/Korea). Each of the mixtures contained 10 µl of the sample (purified RNA), 2 µl reverse transcriptase, 2 µl of oligo dT enzyme and 6 µl of nuclease-free water. Subsequently, thermocycling steps were set at 37ºC for 10 minutes, 42ºC for 60 minutes and finally at 95ºC for 5 minutes.

Quantitative Real Time-PCR Technique

Quantitative Real Time-PCR was done using thermal cycler real time PCR system (Qiagen/Germany). The components of the reaction mix are mentioned in Table 3. Whereas the thermocycling protocol was programmed for the following optimized cycles and according to the thermal profile as demonstrated in Table 4.

Table 3: The components and volume reaction for qPCR

Component	Volume (µl)
qPCR master mix	10
Primer-F (10 pmol)	1
Primer-R (10 pmol)	1
cDNA	5
Nuclease-free water	3

Table 4: Thermal Program setting for *icaA*, *icaD*, and *rpo*B

Cycles step	Temperature ºC	Time	cycles
Initial denaturation	95	5 minutes	1
Denaturation	95	30 seconds	50
Annealing	50	30 seconds	
Extensions	72	30 seconds	

Depending on the real time cycler software, the threshold cycle ($\mathbf{C_t}$) was calculated for each sample. Expression data of selected genes was normalized against the housekeeping gene (*rpo*B). Analysis of q-PCR products was according to Livak method [25]. The results were expressed as folding change in gene expression as follow:

$$\Delta C_t = C_t \text{ (gene)} - C_t \text{ (HKG)} \quad \ldots\ldots (1)$$

$$\text{Gene expression} = 2^{(-\Delta Ct)} \quad \ldots\ldots (2)$$

Statistical Analysis

All biofilm experiments were performed in triplicate. Whereas hydrophobicity experiments were done in sextuplicate. The data is expressed as mean and standard deviation. Normality test was assessed using Shapiro-Wilk test. The differences in biofilm and hydrophobicity values were analysed using the Kruskal-Wallis test.

Results

The Affinity of Denture Materials for Water

According to the results of contact angle of water (θ), except for the metallic, the other two materials were hydrophilic. The types of dentures show different wet-ability degrees ordered from hydrophilic to hydrophobic as follow: Acrylic > Plastic > Metallic (Table 6).

Table 6: Readings of contact angle of denture pieces

Material	Contact angle (º)	SD	Affinity for water
Acrylic	43.8	1.067	**Hydrophilic**
Plastic	47.1	0.966	**Hydrophilic**
Metallic	93.7	2.299	**Hydrophobic**

SD = Standard deviation

Biofilm-forming Capacity of *S. aureus*

Based on findings presented in Table 7, it can be noticed that the acrylic and plastic are more supportive for the biofilm formation than the metallic denture. Based on the number of isolates that can develop biofilm. Putting in mind the strength of the biofilm, the denture materials can be ordered from the highest biofilm-supportive to the lowest one as follows: Acrylic > Plastic > Metallic.

Table 7: Biofilm intensity on different denture materials

Isolate code	Plastic			Acrylic			Metallic			P value	$LSD_{0.05}$
	OD_{630}	**SD**	**Intensity**	**OD_{630}**	**SD**	**Intensity**	**OD_{630}**	**SD**	**Intensity**		
Control	0.065	0.005	-	0.102	0.012	-	0.062	0.002	-		
S 56	0.1	0.005	W	0.149	0.015	W	0.066	0.006	No	1.20×10^{-4}	1.89×10^{-2}
S 57	0.134	0.02	W	0.298	0.015	M	0.066	0.002	No	3.31×10^{-6}	2.9×10^{-2}
S 58	0.15	0.02	W	0.181	0.014	W	0.073	0.005	W	2.05×10^{-4}	2.80×10^{-2}
S 61	0.117	0.028	W	0.173	0.063	W	0.065	0.002	No	4.42×10^{-2}	7.98×10^{-2}
S 66	0.095	0.006	W	0.187	0.004	W	0.124	0.006	W	6.56×10^{-10}	8.5×10^{-3}
S 70	0.107	0.002	W	0.213	0.012	W	0.166	0.007	M	1.16×10^{-5}	1.61×10^{-2}
S 71	0.088	0.004	W	0.136	0.004	W	0.084	0.001	W	1.37×10^{-6}	6.21×10^{-3}
S 76	0.088	0.003	W	0.179	0.002	W	0.084	0.006	W	1.66×10^{-7}	7.96×10^{-3}
S 80	0.1	0.001	W	0.289	0.003	M	0.082	0.008	W	4.45×10^{-9}	9.72×10^{-3}
S 112	0.077	0.001	No	0.113	0.004	No	0.073	0.003	W	5.49×10^{-3}	5.49×10^{-3}
S 114	0.079	0.003	No	0.094	0.015	W	0.073	0.004	W	7.40×10^{-2}	1.78×10^{-2}
S 120	0.086	0.01	W	0.112	0.006	No	0.068	0.008	No	1.46×10^{-3}	1.6×10^{-2}
S 121	0.092	0.009	W	0.242	0.026	W	0.082	0.007	W	0.588868	-
Cut off value		0.05			0.138			0.068		-	-

W= weak, M= moderate, No = non-producer, SD = standard deviation

Scanning Electron Microscope Study

The examination showed that the biofilms formed on acrylic and plastic were dense with multiple water channels and covered whole surface of the pieces, while the biofilm formed on metallic piece was sparse cell aggregations separated by areas of biofilm-free spaces as shown in Figure 2.

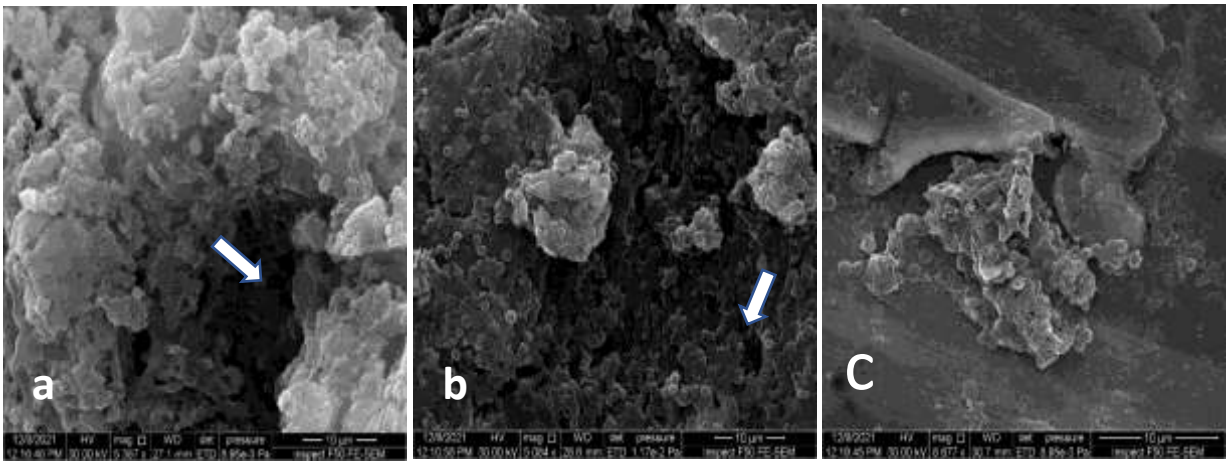

Figure 2: Field emission scanning electron microscope micrograph of *Staphylococcus aureus* biofilm on different denture materials. A: Acrylic (5387X), B: Plastic (5084X), and C: Metallic (8677X). White arrows indicate the water channels.

Detection of *rpoB icaA* and *icaD*

16SrRNA, mecA, rpoB, icaA and *icaD* were found in 13 (100%), eight (61.5%), 13 (100%), 11 (81%), and 12 (84.6%) isolates, respectively, as illustrated in Figure 3.

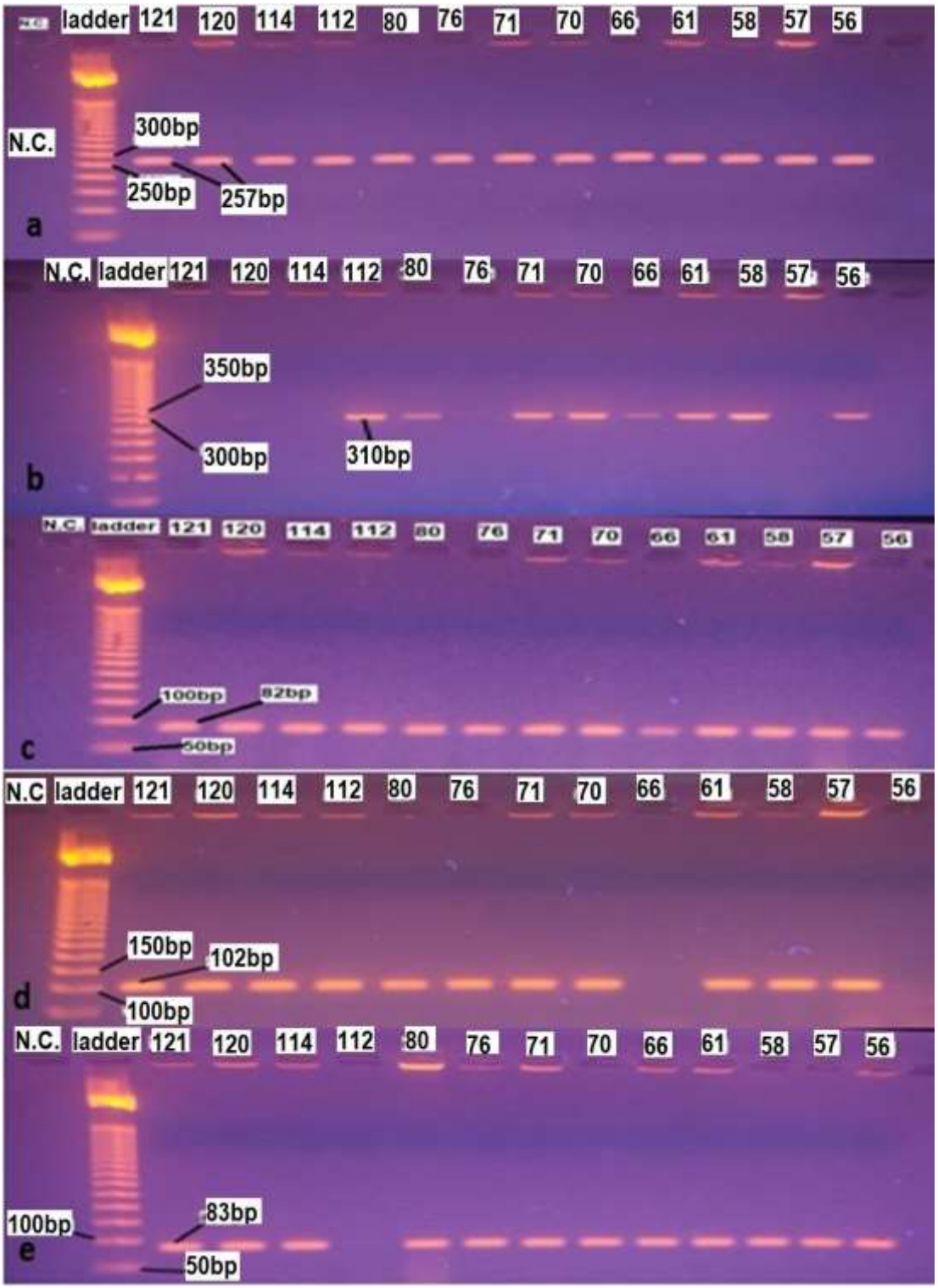

Figure 3: Agarose gel electrophoresis of the PCR products of a) *16SrRNA* at 257 bp, b) *mecA* at 310 bpc) *rpoB* at 82 bp,d) *ica*A at 102 bp,e) *ica*D at 83 pb. N. C. represents the negative control.

Quantitative Expression of *icaA* and *icaD* Genes

The chosen isolates represented the strongest biofilm produced per denture material as recalled form Table 7. The current findings presented in Table 8 indicate that the gene expression of both *ica*A was less than that of *icaD* in all denture materials, except the metallic denture.

Isolate S70 was chosen to test which denture material stimulated more gene expression than the others. The result summarized in Table 9 demonstrates that *icaA* expression decreased in the following order: Acrylic > Plastic > Metallic. Nonetheless, *icaD* expression decreased in the following order: Plastic > Acrylic > Metallic. Consequently, it can be concluded that the metallic is most biofilm-unsupportive material.

Table 8: Gene expression of *ica*A and *ica*D in biofilm of the isolate that developed the strongest biofilm on each denture materials

Denture material	Isolate code	C_t			Δ*icaA*	Δ*icaD*	Gene expression	
		rpoB	***icaA***	***icaD***			***icaA***	***icaD***
Acrylic	S57	32.12	26.66	25.41	-5.46	-6.71	44.01	104.69
Plastic	S58	30.8	26.72	25.67	-4.08	-5.13	16.91	35.01
Metallic	S70	33.45	27.51	29.68	-5.94	-3.77	61.393	13.64

Table 9: Gene expression of *icaA* and *icaD* in biofilm of the isolate S70 developed on different denture materials

Denture material	C_t			Δ*icaA*	Δ*icaD*	Gene expression	
	rpoB	***icaA***	***icaD***			***icaA***	***icaD***
Plastic	33.83	27.56	26.31	-6.27	-7.52	**77.17**	**183.55**
Acrylic	34.29	26.17	27.06	-8.12	-7.23	**278.20**	**150.12**
Metallic	33.45	27.51	29.68	-5.94	-3.77	**61.39**	**13.64**

DISCUSSION

Upon findings presented in Table 7, the denture materials can be ordered from the highest biofilm-supportive to the lowest one as follows: Acrylic > Plastic > Metallic. This notion was also confirmed from the isolate point of view; given that the acrylic was the most supportive material. Whereas the metallic was the highest non-supportive material.

Mohammed *et al*. [16] reported that the compact acrylic (by CAD-CAM technique) was less supportive material for the aggregation of *P. aeruginosa* and *S. epidermidis*) than the conventional and flexible acrylic fabricated by the conventional method.

According to the model of thermodynamics of microbial adhesion, hydrophobic bacteria prefer to colonize on hydrophobic surfaces and vice versa [26]. Consequently, the hydrophobicity of the bacterial cell surface affects the adhesion properties of the bacteria [27]. Hydrophilic surface (contact angle <90◦) attracted most of bacteria such as *S. aureus* and *Escherichia coli* [28]. Also, Maikranz *et al.* [29] found that *S. aureus* cells have many weakly

binding macromolecules that aid adherence to hydrophobic surfaces while they have few, but strongly binding macromolecules that aid adherence to hydrophilic surfaces. Recalling Table 6, metallic is considered hydrophobic because its contact angle is more than 90º [30]. Thus it may inhibit bacterial attachment. Whereas acrylic and plastic are moderate hydrophilic (θ is 43º-47º) with moderate support for biofilm formation in the current experiment.

The findings of SEM in this research concur to the findings of the crystal violet method. Leoney *et al.* [20] studied the biofilm on dentures by microtiter plate method and SEM. The results showed that SEM helps us to visualize the strength of biofilm formed and concur with the microtiter plate method of quantification of biofilms. Also, Zochniak *et al.* [31] studied the biofilm of *S. aureus* on dentures of cystic fibrosis patients using crystal violet method. The results were concurred by comparing the results with SEM imaging.

For further confirmation that isolates under test are *S. aureus*, *16S rRNA* has been detected and confirmed for all of the isolates.

The best method for detecting methicillin resistant *S. aureus* (MRSA) is through identifying *mecA* (the gene responsible for the resistance of methicillin) presence in the isolates. Using PCR for the identification of *mecA* with specific primers, the PCR yielded fragments sizes of 310 bp [23]. The current results show that 61.5% carry the gene. In a previous Japanese study of edentulous patients, it has been found that 65 out of 100 patients harbour MSSA or MRSA on their dentures [32]. Interestingly Smith *et al*. [14] found that staphylococci were less common in the mouths of subjects with prosthetic devices. The proportion of subjects harbouring MRSA on their denture varied from 1% of out-patients to 12% of in-patients in the study. In more recent research, it has been found that 27% denture wearer out-patients harbour *S. aureus* on their dentures and only one of them harbour MRSA, compared to 33% of denture wearer in-patients with 12% of the isolates were MRSA [33]. In conclusion, previously mentioned studies have demonstrated that dentures may provide a supportive surface for *S. aureus* biofilm formation and may act as a reservoir for *S. aureus*.

The *rpoB* gene has been shown to be more discriminative than the 16S ribosomal DNA (rDNA) gene. The divergence between the *rpoB* sequences of different strains is considerably higher than those between their *16SrRNA* genes. To confirm that discriminatory power is higher, twenty clinical isolates of enteric bacteria were assigned to the correct enteric species on the basis of *rpoB* sequence comparison. The trees, depending on *rpoB*, were more suitable with the presently accepted classification of Enterobacteriaceae than those that resulted with *16S rRNA*. This information indicates that *rpoB* is a potent identification tool which may help in the identifying bacteria [34]. Two previous studies in Iraq investigated *rpoB* gene of *Klebsiella pneumonia*. The first was performed by Hadi [35] in Kufa and the second was in Kirkuk by Hasan*et al.*[36] in which both studies showed that 100% PCR products were *rpoB* positive. The research findings are identical to the findings of Hadi [35] and Hasan*et al*. [36] and all were 100% positive for *rpoB*. *rpoB* is the housekeeping gene and is essential for RNA polymerase synthesis [37].

Mohammed and Radif [38] stated that *S. aureus* isolates were more frequent in wounds than those of urinary tract infections and all isolates were biofilm producers. The tissue culture plate assay shows that 46.15% of the isolates were strong biofilm producer, 46.15% had moderate ability and 7.70% were weak, where the gene expression of *icaA* using real time PCR assay revealed a significant difference in the expression level between strong biofilm producing isolates, both weak and moderate ones. Other two previous studies of non-clinical samples in Brazil and China, *icaA* was detected in about 49% of *S. aureus* isolate from cow

milk [39, 40]. Avila-Novoa *et al*. [41] found *icaA* and *icaD* genes in 52.3% of *S. aureus* isolates collected from food contact surfaces in dairy industry in Brazil. While a previous clinical study showed 17 out of 18 (94%) were *icaA* positive MRSA strains that can form strong biofilm, identified by phenotypic method [42]. While Gowrishankar and colleagues revealed that *ica* genes were present in 84.13% of *S. aureus* isolates collected from in-patients in India [43].

In this research the molecular detection of *ica*A gene was performed by using PCR technique with specific primers. This technique was applied on all 13 selected isolates of *S. Aureus*. The results revealed that 11 (84.6%) isolates possess the *icaA* gene and only two (15%) isolates (S56, S66) didn't harbour this gene.

It has been found that the *icaD* gene product is mandatory for the most proper enzymatic activity of the product of *icaA* gene [44]. Co-expression of both *icaA* and *icaD* is mandatory for full phenotypic expression of biofilm in clinical staphylococcal isolates [45].

Previous study in Mosul, Iraq showed the species *S. hominis*, *S. epidermidis*, *S. aureus* and *S. xylosus* contain the gene *icaD* in 42.8% of the isolates [46]. Another study conducted in Basra, Iraq of 150 isolates of *S. aureus* showed that 100% of the isolates harboured *icaD* gene [47]. While Mørk*et al*. [48] reported that the *icaD* was located in about 90% of isolates.

Expression of *icaA* and *icaD* genes showed different levels on different materials for different isolates. Also when tested the same isolate (S70) and measured the genes expression of *icaA* and *icaD* on the different materials, the genes showed different expressions. Overall gene expression on metallic was the lowest than the isolates that grew on acrylic and plastic.

Conclusion

Through the results obtained from this research, the following can be inferred. Most of *S. aureus* isolates can form biofilm on the different denture materials. Where all of the denture materials support biofilms with different strengths from weak to moderated, metallic denture is the most unsupportive for biofilm formation. Detection of biofilm using EMS is concurred with crystal violet method. Gene expression of *icaA* and *icaD* elevated in different levels and the metallic was the least stimulant among the three types for both of the genes. Overall, dentures are potent reservoir for *S. aureus* that can be transmitted to different sites and can cause further complications.

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