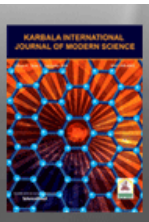
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Methods: In this study, 150 bacterial isolates were obtained from tooth and gum surface swabs of male and female patients of different ages who visited specialized centers and dental clinics in Babylon City after they were clinically diagnosed with oral (dental) infections by specialist doctors. This study examines the biofilm-forming ability of gingivitis and periodontitis oral bacterial isolates. A qualitative crystal violet correlation assay and quantitative microtiter plate examined biofilm development. The biofilm-developing genes were subsequently identified using polymerase chain reaction (PCR).

Results: The present data revealed that the isolated bacterial isolates from the oral cavity were *Sphingomonas paucimobilis, Streptococcus spp*. (with *Streptococcus mutants* exhibiting the highest prevalence at 54%), *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*, all of which underwent biofilm formation assays. Biochemical and Vitek 2 identification of bacterial isolates showed that *Streptococcus mutans* had the greatest rate (54%). Results showed that 80% of isolates could produce biofilm, ranging from weak to moderate across species. These showed that most isolates were able to colonize and adhere to oral surfaces, which is crucial to chronic oral illnesses.

Keywords

biofilm formation; Streptococcus mutans; Oral Cavity; Bacterial Pathogen

RESEARCH PAPER

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Keywords: Biofilm formation, *Streptococcus mutans*, Oral cavity, Bacterial pathogen, Polymerase chain reaction

1. Introduction

Periodontal disease is a chronic inflammatory disorder that affects 20−50 % of the population worldwide [1]. Gingivitis is produced by a microbial blaque accumulation on the clean surfaces of the teeth, which triggers a response of inflammation in the surrounding gum tissue. The clinical symptoms of gingivitis are represented by redness, swelling, pain, and a high fever [2]. Chronic inflammation that effected the gum can proceed to periodontitis, a more serious condition that destroys supporting structures of the teeth, eventually leading to loss of the tooth [3].

It is worth noting that non-oral bacteria, such as *P. aeruginosa, S. aureus, Streptococcus spp.* and *E. coli,* are microorganisms that do not naturally belong to the oral microbiome, leading to an imbalance in the oral cavity [4]. The alteration of the "equilibrium" (due to medicinal treatments, biological changes, or inadequate hygiene) between commensal bacteria and the host immune system may be the explanation for the transition of non-oral bacteria from transitory species to colonizers and thus playing a role in causing gingivitis and periodontitis when present in the oral cavity [5,6]. The emergence of antibiotic resistance has become a major worldwide health concern that affects the treatment of

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bacterial infections [7]. Horizontal gene transfer (HGT), which has been demonstrated to be driven by three main mechanisms, conjugation, natural transformation, and bacteriophage infection, allows antibiotic resistance genes (ARGs) to be rapidly spread [7–9]. HGT is mostly facilitated by plasmid conjugation, and plasmids that encode antibiotic resistance are the primary cause of the dissemination of antibiotic resistance [10,11]. A review of [9] has illustrated all factors that affected the rates of plasmid transfer.

It is commonly stated that biofilm is a microbial-derived sessile community characterized by cells that are permanently attached to a substrate or interface with one to another, immersed in a matrix of extracellular polymeric substances that they have manufactured. Biofilms play a significant role in the spread of antibiotic resistance. Consequently resistance and virulence genes are efficiently transferred across the dense of bacterial population [12]. Biofilm formation enhances the ability of bacteria to adhere, increasing their resistance to antibiotics. To support this, several studies have confirmed that biofilms enhance the transfer of resistance plasmids, which may significantly contribute to the rapid spread of these plasmids compared to planktonic bacteria [7,8,10,13,14]. In comparison to planktonic conditions, trial with *Klebsiella pneumoniae* demonstrated a higher rate of plasmid transfer for the carbapenem resistance gene in biofilm [11]. Indded, the scientific explanation for why antibiotic resistance activity is higher in biofilm-forming communities compared to planktonic cells could be illustrated. As a result of the close proximity of bacteria in a biofilm increases cell-to-cell contact and, consequently, the probability of genetic exchange, biofilms are thought to be a driving force behind the dissemination of ARGs [7,9]. Genes such as *icaA* and *icaD* implicated in the biofilm formation of *S. aureus*, by the production of an enzyme that contributes to bacterial cell adhesion and biofilm formation [15].

On the other hand, *pslA* gene is a neutral-charge exopolysaccharide composed of D-mannose, D-glucose, and L-rhamnose that is organized in pentasaccharide repeats and provides structural support during biofilm development, playing a role in both cell-to-cell and cell-to-substrate adhesion [16].

A unique group of *GTF* (glucosyltransferase) genes found in *Streptococcus mutans* expresses enzymes with glucosyltransferase activity. For instance, *GtfB* is distinguished by its capacity to bind tightly to bacterial surfaces, causing tight cellular aggregation and improving plaque cohesiveness. It creates insoluble glucans, which are an important element of a extracellular matrix of the plaque [17].

Therefore, the aim of the current study is to isolate and identify the bacterial community in the oral cavity of patients and examine their ability for biofilm formation phenotypically or genotypically.

2. Material and method

2.1. Collection of sample

Using sterile swabs immersed in a carrier medium, 150 samples were collected from people who suffered from gingivitis and periodontitis and who visited the Specialized Dental Center\ Department of Periodontology and Specialized Clinics in Babylon Governorate over four-months from September to December 2024, following protocols to ensure the accuracy of results and sample safety. Swabs were passed over the affected regions in case of gingivitis, and a periodontal probe was utilized to collect samples from the depths of the periodontal pockets in the case of periodontitis. Samples were then transferred to the lab for isolation and microbiological diagnosis.

2.2. Bacterial isolation and identification

Samples were streaked on the selective media such as Cetrimide, Eosin methylene blue, Mannitol and Mitis Salivarius Agar for culturing *P. aeruginosa*, *E. coli*, *S. aureus* and *Streptoccus mutants*, respectively and then incubated at 37 °C overnight. A single colony was picked up with a sterile loop and transferred repeatedly to the same media until a single pure colony was obtained. These colonies were used for their identification, gene detection and biofilm formation. Initial diagnosis and susceptibility tests were performed with the Vitek 2 system. Further identification was performed using specific primers to amplify *Sm479* and *pheA* for identification of *Streptococcus mutans* and *Streptococcus mitis*, respectively employed PCR method as explained in Section 2.4.

2.3. Biofilm assay

2.3.1. Qualitative detection of biofilms

The ability of the bacterial isolates, including *P.aeruginosa*, *E. coli*, *S. aureus*, *Streptococcus spp*. and *Sphingomonas spp*., to form biofilm was examined using the tube method. The trial was commenced by growing bacterial isolates individually in tubes containing 10 % brain heart infusion broth, where each tube was inoculated with a full ring of isolates and then incubated at 37 °C for 24 h. After the incubation period ended, the bacterial growth was

eliminated by washing the tube with a volume of normal saline solution and allowing it to dry completely. Crystal violet in a concentration of 0.1 % solution was then added to the tubes and left for 10 min to ensure that the dye interacted with the remaining cells. Dye was then drained and the tubes were left to dry completely. The ability of the bacterial isolates to form a biofilm was categorized based on the deposition of the violet layer on the inner walls and bottom of the tube into the following categories: poorly adhesive, moderately adhesive, strongly adhesive, and inconsistent [18].

2.3.2. Microtiter plates method

Bacterial isolates were inoculated in tubes containing 5 % brain heart infusion broth, then incubated at 37 °C for 24 h to ensure the isolates reached the growth phase. The bacterial suspension was then diluted and equilibrated with 0.5 % McFarland's standard solution to check that the concentrations were consistent. Later 200 µl of the bacterial suspension was dispensed into each well of a 96-well microtiter plate in triplicate for each bacterial isolate, with uncultured medium used as a negative control. The plate was incubated at 37 °C for 24 h for insurance of biofilm formation. Following incubation, the bacterial suspension was removed, and the wells were washed three times with normal saline solution before drying at 60 °C for 30 min to stabilize adherent cells. Then, 200 µL of crystal violet dye (5 %) was added and left for 15 min, and then the wells were washed to remove excess dye. Finally, 200 µL of ice-cold acetic acid (33 %) was added to extract the remaining dye, after which the optical density was measured at 630 nm using an ELISA Reader to evaluate the biofilm formation [19]. The optical density (OD) of the samples was compared to that of the control samples to ascertain the isolates' capacity to form biofilms, as indicated in Table 1.

2.4. Detection of genes encoded for biofilm formation by PCR

Some bacterial isolates, including 22 of *S. aureus*, 13 of *Streptococcus mutans*, and 7 of *P. aeruginosa*, were selected based on the literature to investigate their ability to form biolfilm Table 2.

Table 1. Evaluation method of biofilm formation.

Mean OD value	Biofilm formation
OD ≤ ODc	None
OD ≥ ODc	Weak
OD ≥ 2 × ODc	Moderate
OD ≥ 4 × ODc	Strong

2.4.1. DNA extraction

Pure colonies grown on the selective media for each of the tested bacteria were suspended overnight in 1 ml of distilled water and centrifuged for 1 min at 14,000–16,000 rpm in a 1.5 ml Eppendorf tube. After adding 200 µL of GT buffer, the sample was vortexed for 5 s. The supernatant was discarded, and 200 µL of lysozyme buffer was added to the positive culture (without for negative culture). Then, 20 µL of Proteinase K was added, and the mixture was incubated at 60 °C for 10 min, mixing every 3 min. Next, 200 µL of GT buffer was added and the mixture was mixed well, then the mixture was incubated at 70 °C for 10 min with intermittent shaking. Then, 200 µL of 100 % ethanol was added and mixed well. The mixture was transferred to a GD column placed in a 2-ml collection tube and centrifuged at 14,000–16,000 rpm for 2 min. The column was washed with 400 µL of WI buffer and centrifuged for 30 s, then washed again with 600 µL of wash buffer and centrifuged. The column was dried by centrifugation for 3 min, transferred to a 1.5-ml sterile tube, and 100 µL of preheated centrifugation buffer was added. After 5 min, the tube was centrifuged for 30 s at 16,000 rpm, and the extracted DNA was stored at −20 °C until use.

2.4.2. PCR method

The conditions of amplification of each gene which encoded for biofilm formation using specific pair of primers by PCR method are elucidated in Table 3.

2.4.3. Agarose gel electrophoresis

An agarose gel electrophoresis test was applied to check PCR amplification. A 1.5 % agarose gel was prepared in 1 × TAE solution, and ethidium bromide (10 mg/mL) was added for DNA detection. The solution was heated until complete dissolution, then allowed to cool to 50–60 °C before pouring into the gel mold and fixing for 30 min.

Five µl of the PCR product was loaded into the wells, along with the DNA ladder. Electrophoresis was performed at 100 V for 60 min, with the DNA molecules moving from the negative electrode towards the positive electrode. The gel was visualized using a gel imager, and the ethidium bromide-stained bands were clearly visible, confirming the success of the amplification Following the same aforementioned steps, molecular identification of the *Sm479* and *pheA* genes specific for *Streptococcus mutans* and *Streptococcus mitis*, respectively, was performed.

Table 2. Primers used for amplification of the tested genes.

Primer Type	Nitrogen base sequence		Size product (pb)	Reference
icaA	TCTCTTGCAGGAGCAATCAA	F	188 pb	[13]
	TCAGGCACTAACATCCAGCA	R		
icaD	ATGGTCAAGCCCAGACAGAG	F	198 pb	[14]
	CGTGTTTTCAACATTTAATGCAA	R		
pslA	CACTGGACGTCTACTCCGACGATAT	F	1119 pb	[15]
	GTTTCTTGATCTTGTGCAGGGTGTC	R		
gtfB	ACTACACTTTCGGGTGGCTTGG	F	517 bp	[16]
	CAGTATAAGCGCCAGTTTCATC	R		
gbpA	GGTGGTTCTGTGCCTGATGA	F	162 bp	[17]
	TTGCCAGCCTGATACACGTT	R		
pheA	TGGCTTATCCTTCCTAGATGG	F	557 bp	[18]
	GATTGCGGTCGACAAA	R		
Sm479	TCGCGAAAAAGATAAACAAACA	F	435 bp	[19]

Table 3. Primers sequence used to amplify biofilm genes.

Primer Type	Nitrogen base sequence		Size product (pb)	Reference
icaA	TCTCTTGCAGGAGCAATCAA	F	188 pb	[20]
	TCAGGCACTAACATCCAGCA	R		
icaD	ATGGTCAAGCCCAGACAGAG	F	198 pb	[21]
	CGTGTTTTCAACATTTAATGCAA	R		
pslA	CACTGGACGTCTACTCCGACGATAT	F	1119 pb	[22]
	GTTTCTTGATCTTGTGCAGGGTGTC	R		
gtfB	ACTACACTTTCGGGTGGCTTGG	F	517 bp	[23]
	CAGTATAAGCGCCAGTTTCATC	R		
gbpA	GGTGGTTCTGTGCCTGATGA	F	162 bp	[24]
	TTGCCAGCCTGATACACGTT	R		
pheA	TGGCTTATCCTTCCTAGATGG	F	557 bp	[25]
	GATTGCGGTCGACAAA	R		
Sm479	TCGCGAAAAAGATAAACAAACA	F	435 bp	[26]
	GCCCCTTCACAGTTGGT TAG	R		

3. Statistical analysis

Data if possible were assessed for any significant differences using t-test. Data were presented as M ± S.D. The accepted level of significance was $P \leq 0.05$. (Mini Tab statistical software version 17; PA, USA) was applied for performing the statistical analysis.

4. Results and discussion

4.1. Patients

Out of 150 samples collected from gingivitis and periodontitis cases, 55 were male and 95 were female, demonstrating that females were more likely to develop these disorders which revealed significant differences among them ($P \leq 0.05$).When the type of inflammation was examined, 20 of the total samples showed periodontitis (60 % female, 40 % male), accounting for 13.3 % of the cases, and 86.6 % had gingivitis Fig. 1.

It is worthwhile to state gingivitis has been linked to habits such as the use of braces or insufficient dental care. This finding can be possible to the hormonal changes that occur throughout puberty in teenage girls. Hormonal fluctuations, particularly those of estrogen and progesterone, have a significant impact on the oral health. These changes can increase blood flow to the gums, making them more

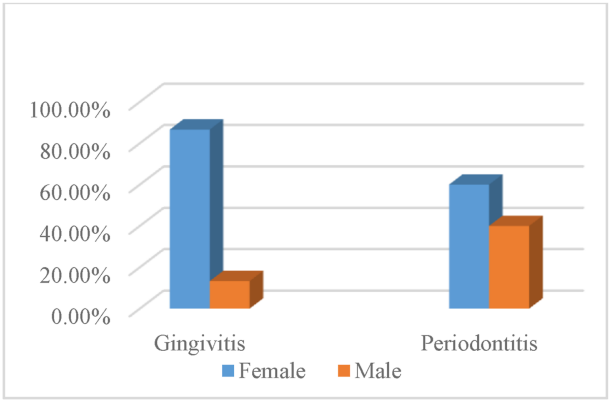

Fig. 1. Number of oral samples collected from male and female participants.

sensitive and prone to irritation, hence raising the risk of gingivitis and plaque diseases [27]. It is common knowledge that females were more susceptible to periodontitis due to a decrease in estrogen concentration, especially during menopause, which causes osteoporosis and low bone density, this is particularly true in the jawbone, which increases the risk of severe periodontal disease and tooth loss due to poor bone support tooth loss due to poor bone support. This causes osteoporosis and low bone density, particularly in the jawbone, which raises the risk of severe periodontal disease and tooth loss due to inadequate bone structure [28]. The present data are not in agreement to an Indian study found that men were more likely to develop gum disease due to hormonal differences, increased tobacco use, and increased exposure to sunshine [29].

4.2. Bacterial isolation

The microbial community causing gum disease and dental inflammation was analyzed, and the results showed the presence of 43 isolates of *Sphingomonas paucimobilis*, 43 isolates of *S. epidermidis*, 23 isolates of Streptococcus (13 of *S. mutans*, 6 of *S. pseudoporcinus*, 2 of *S. pneumoniae*, 1 of *S. sanguinis*, 1 of *S. salivarius*, 1 of *S. pluranimalium*), 22 isolates of *S. aureus*, 7 isolates of *P. aeruginosa*, and 6 isolates of *E. coli* Fig. 2.

The results of detecting the *sm479* gene using PCR confirmed that 13 samples were *Streptococcus mutans* for carrying this gene which has 435 base pairs, as shown in Fig. 4 A. Confirming the current findings, a prior study, found that all *Streptococcus mutans* isolates detected using conventional methods were 90 % positive for this gene [30]. On the other hand, according to amplify the *pheA gene* using specific primers for detection of *S. mitis*, there are no isolates representing these bacteria among the identified bacteria in the present study.

The results of the current study were consistent with a local study [31]. Similar bacterial species were identified in the patients with periodontal disease and tooth inflammation. This substantiates the concept that these microbes contribute similarly to the progression of oral illnesses.

4.3. Biofilm assay

4.3.1. Microtiter plates method

Ten isolates of *S. aureus*, 10 isolates of *Streptococcus spp.*, 7 isolates of *P. aeruginosa*, 6 of *E.coli*, and 10 of *Sphingomonas paucimobilis* were subjected to the biofilm examination according to the microtiter method Fig. 3.

The current study found that all tested bacterial genera, including *S.aureus, Streptococcus spp.* (including *S. mutans, S. pneumoniae, S. sanguinis, and*

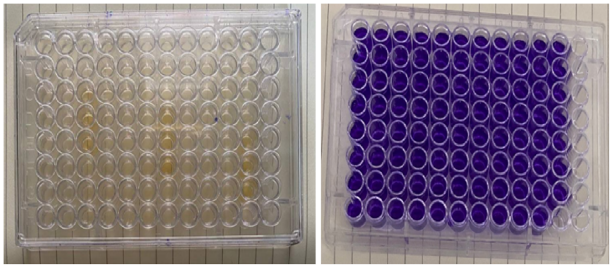

Fig. 3. Biofilm formation by bacterial isolates using crystal violet staining method.

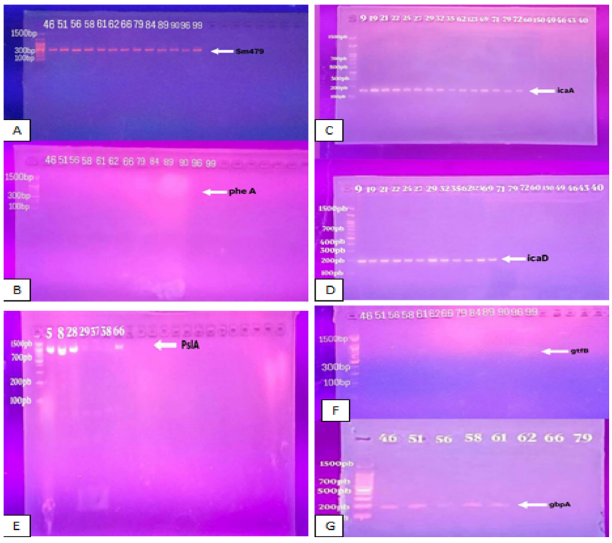

Fig. 4. Electrophoresis of PCR reaction products of bacteria isolated from oral cavity. Sample code, A-Sm479, B-Phe A, C-icaA, D-icaD, E-PslA, F-gtfB and G-gbpA.

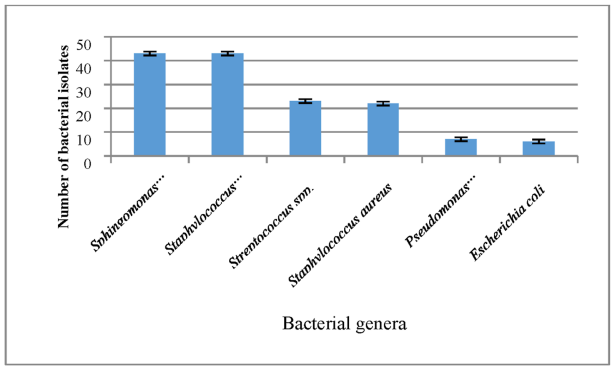

Fig. 2. Distribution of bacterial isolates isolated from the oral cavity.

Table 4. Distribution of the studied bacterial isolates according to their biofilm production by both quantitative and qualitative methods.

Type of bacteria	Qualitative		Quantitative		Number Of Isolates
	Poor	Moderate	Poor	Moderate	
S.aureus	60 %	40 %	70 %	30 %	10
Streptpcoccus spp.	100 %	0	100 %	0	10
P.aeruginosa	71.4 %	28.6 %	100 %	0	7
E.coli	100 %	0	89.3 %	13.7 %	6
Sph.pucimobilis	100 %	0	100 %	0	10

S. salivarius), *P.aeruginosa, E.coli,* and *Sphingomonas paucimobilis,* were able to form biofilm. Among *S. aureus* isolates, 30 % were intermediate biofilm producer, whereas 70 % were weaker biofilm formers. All *Streptococcus spp.* isolates showed a limited ability to produce biofilms Table 4. In addition, *P.aeruginosa* and *Sphingomonas spp*. isolates formed mild biofilms in 100 % of cases. In the case of *E. coli*, 13.7 % of isolates exhibited a moderate biofilm-forming ability, whereas 83.3 % displayed a weak activity. These findings are consistent with a prior study indicating that clinical isolates produce biofilms. For example, *S. aureus* isolates identified from various clinical sources were shown to be 100 % biofilm-producing [32], while *S. mutans, S. pneumoniae, S. sanguinis*, and *S. salivarius* were all demonstrated to create biofilms at 100% [33,34]. Similarly, *P. aeruginosa* isolates revealed total biofilm-forming capabilities [35], whereas *Sphingomonas paucimobilis* isolates produced an ability of 100 % biofilm, with varying capacity [36]. A study conducted in Mexico supported the present results about *E. coli*, which demonstrated that all clinical isolates could form biofilms to variable degrees [37]. A study examined the oral *S. aureus* isolates discovered that 87 % of the oral microbes produced biofilms, indicating a greater incidence of biofilm formation than found in the current study [38]. Furthermore, contrasting research reported that only 69 % of *P.aeruginosa* isolates formed biofilms, wheras 31 % did not [39].

Biofilms are structured microbial communities that develop through significant changes in bacterial physiology, this development results in increased resistance to unfavourable environmental conditions, as well as to treatments with antibiotics and other biocides [40,41]. In addition, the bacterial community can benefit from biofilm formation in different ways, such as cell signalling, symbiosis with plants, adherence to the substrate, nutrition compartmentation, genetic exchange, and cell aggregation [42]. Wang et al., has summarized the mechanisms of bacterial biofilm formation by three steps as encouraging the synthesis of eDNA and EPS to promote adhesion, strengthening the biofilm through improving intercellular communication and managing the biofilm's initial colonization by regulating of its movement speed [43].

4.3.2. Qualitative detection of biofilms

To confirm these results, the ability of the bacterial isolates to form biofilms of *S. aureus*, *Streptococcus* spp., *Sphingomonas pucimobillis, E. coli*, and finally *P. aeruginosa* was verified by the density and thickness of biofilms adhering to the tube wall. All isolates of *Sphingomonas* spp. and *Streptococcus* spp. showed consistent results with the quantitative method with the exception of *S. aureus*, *P. aeruginosa,* and *E. coli*. However, *S.aureus* showed a variation in their ability to form biofilm, with 60 % of them were weakly productive and 40 % being moderately productive. Futhermore, *P. aeruginosa* showed that 71.4 % of the isolates exhibited poor production of bioflims, while 28.6 % were moderately productive as presented in Table 4. Finally, all isolates of *E. coli* revealed poor production of biofilms. The reason behind this difference between the quantitative and qualitative results is not clear, but it could be attributed to the sensitivity and efficiency of the quantitative method in determining the yield of the isolates [44]. Indeed, literature demonstrated that environmental factors could improved the ability of bacteria to produce biofilm including a temperature, pH and sources of carbon and nitrogen [45,46]. A previous study mentioned that other environmental factors that may impact initial attachment include temperature, oxygen tension, osmolarity, pH, and iron availability [47].

In addition, both biotic such as high osmolarity and abiotic surfaces can facilitate the formation of biofilms by bacteria in both natural and therapeutic circumstances nitrogen [48]. In contrast, various biofilms producer bacteria were found to inhibit their ability for biofilms formation including *S. aureus* [49], and *P. aeruginosa* [50] for a review, see Ref. [46].

4.3.3. Detection of genes encoded for biofilm

The current trial employed PCR technique to discover the presence of the *icaA* and *icaD* genes in 22 samples of *S. aerues*, *pslA* gene in 7 samples of *P. aeruginosa*, and the *gbpA*, *gtfB* genes in 13 samples of

S. mutans, showed that the isolates were biofilm producers. Bands obtained after running of PCR products during electrophoresis revealed that *icaA* and *icaD* had product sizes of 188 and 198 bp, Fig. 4 (C&D). Bands of the PCR products of *pslA* gene were at a size of 1119 bp Fig. 4 (E), whereas the *gbpA* and *gtfB* genes were sizes of 162 and 517 bp Fig. 4 (F&G), respectively, which showed a distinct amplification band.

The current data showed that 15 out of 22 and 12 out of 22 of *S. aureus,* which constitute 68 % and 54.5 %, had the *icaA* and *icaD* genes, respectively. These results clearly indicate that the frequency of icaA gene was significantly higher in *S. aureus* in comparison to frequency of *icaD* in the same bacteria ($P \leq 0.05$; Figs. 5 and 6). It is, however, these results could mean that the *icaA* gene may have a stronger role in the ability of bacteria to form a biofilm. These results are consistent with those reported in Refs. [51,52], where the percentages were 65 % and 54.8, respectively. However, they differ from the results reported in Refs. [53,54], which recorded percentages of 47 % and 20 %, respectively. In the present study, molecular methods were more reliable for detect the ability of the tested bacteria to produce biofilm other than the quantitative and qualitative methods.

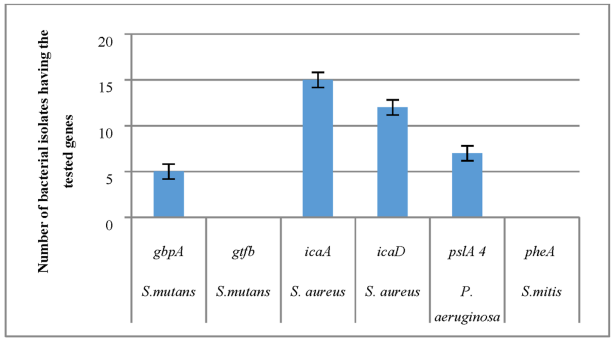

Fig. 5. Rate of frequency of the tested genes in isolates of bacteria identified from the oral cavity of patients.

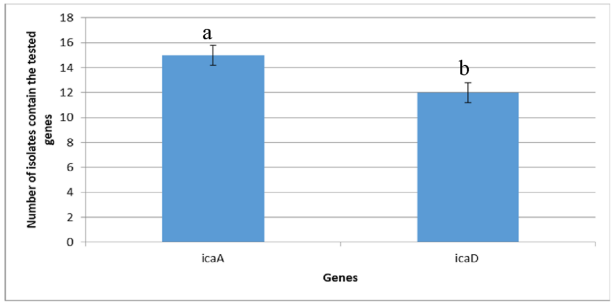

Fig. 6. Rate of frequency of icaA and icaD genes in isolates of S. aureus. Columns having different letter are significantly different ($P \leq 0.05$).

Concerning the *pslA* gene, Fig. 5 illustrates the electrophoresis results of *P. aeruginosa* isolates, indicating that 57.1 % of these isolates possessed this gene. The findings of the present study align with research conducted in Iran, which indicated that 58 % of *P. aeruginosa* isolates possessed the *pslA* gene [55]. The current results demonstrated that 38 % of *Streptococcus mutans* carried the *gbpA* gene. Finally, the opposite was none of the oral bacterial isolates of *Streptococcus mutans* possessed the *gtfB* gene. However, a previous study found that 77.3 of *S. mutans* possessed the *gbpA* [56]. Supporting the present findings, a local study revealed that 45 % of *Streptococcus mutans* isolates also lacked this gene [57], whereas a previous study found that 46.9 % of *Streptococcus spp*. did not possess the gene in question, implying that the *gtfB* gene is not universally conserved across all strains [58].

The divergence between phenotypic and genetic identification of biofilm genes in *S. aureus* isolates indicates the existence of several *icaADCB* genes that facilitate the synthesis of polysaccharide intercellular adhesion molecules essential for biofilm formation [59]. However, the ability to generate biofilms in the absence of the tested biofilm genes suggests the presence of other genetic factors affecting biofilm matrix formation in *P. aeruginosa*. The lack of biofilm production observed using phenotypic approaches, despite the presence of biofilm-forming genes, may result from chromosomal changes affecting the synthesis of biofilm-associated proteins [60]. Although the current study provides solid evidence for the ability of *Streptococcus spp.* isolates to produce biofilms, there is a difference between the results of genetic and phenotypic analysis. This is partly due to the study's use of polymerase chain reaction (PCR) detection to target a single gene rather than whole-genome sequencing analysis, which may provide a more thorough picture of the genetic diversity among the isolated strains [61]. Furthermore, *S. mutans* have many glucosyltransferases (GTFs), including the *gtfB*, *gtfC*, and *gtfD* genes, which facilitate biofilm formation and enhance the aggregation of *S. mutans* and other bacteria on dental surfaces [62]. Bacteria utilize biofilms as a survival strategy, which increases their resistance to treatment due to their natural ability to withstand antibiotics and immune responses [63].

It is widely accepted horizontal gene transfer, alternate sigma factors, toxin–antitoxin systems, and regulatory pathways all play a role in the molecular mechanisms that mediate biofilm development [64]. In this context, horizontal gene transfer is important in biological processes by using modified

microbes with genetic engineering (65). These biological application are bioremediation, waste water treatment, degradation of many environmental pollutants, waste management, bio mining, biofuels and compounds production [64,66,67]. Additionally, a range of biological processes, including photosynthesis, nitrogen fixation, and fermentation, have been observed to be carried out by biofilm-producing bacteria in Antarctica's "desert-like" lake ice cover [68]. Furthermore, it has been found that biofilm producer bacteria are responsible for the cycling of nitrogen, sulphur, and other metals [46]. A recent study demonstrated that biofilm engineering targets quorum sensing molecules, functional protein, structural protein, polysaccharides, c-di-GMP signalling and the stringent response [65]. The ability of recombinant plastic-degrading *E. coli* to generate biofilms has been reported to enhance when cyclic-di-GMP is targeted [69]. Additionally, QS contributes to the release of a significant quantity of eDNA as a result of bacterial subpopulation autolysis during the late stage of biofilm production [70]. For more information review to see Refs. [34,46,64,70]. Biofilm infections are now challenging to treat in clinical settings. There is an urgent need to develop a novel and effective treatment approach to tackle this significant issue. Important insights against antibiotic resistance due to bacterial biofilms, could be accomplished by a comprehensive understanding of bacterial biofilm production and regulation mechanisms. In fact, it should be taken into consideration to design strategies that use antimicrobial drugs with anti-biofilm and antibacterial capabilities to prevent biofilm [71]. Therefore, genes that encode for biofilm formation will provide light on the distinct process of biofilm formation and could help in the creation of treatments for diseases caused by biofilms, which the present study aims to do.

5. Conclusions

Based on the results, although the majority of isolated bacteria considered as pathogenic, other bacteria with no ability to from biofilm could be pathogenic as well. The present data revealed that the isolated bacterial isolates from the oral cavity were *Sphingomonas paucimobilis, Streptococcus spp*. (with *Streptococcus mutants* exhibiting the highest prevalence at 54 %), *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*, all of which underwent biofilm formation assays. Results showed that 80 % of isolates could produce biofilm, ranging from weak to moderate across species. The present results demonstrated that genes such as icaA and icaD were detected in the majority of *S. aureus* isolates, which employ that these genes could be responsible for the ability of these bacteria to produce biofilm. In the same context, *pslA* and gbpA genes could encode for biofilm formation in *P. aureginosa and S.mutans,* respectively. However, both quantitative and qualitative methods showed that the majority of the tested bacterial genea were able to produce biofilm. There is no harmony between the phenotype and genotype of the ability of bacteria for biofilm formation indicating that several genes could be considered as encoding biofilm formation. Although some benefits were obtained, the present study had several drawbacks, in particular the limited sample size and the number of genes investigated. In spite of that, several biofilm-encoding genes from the genera of *Streptococcus mutans*, *Streptococcus mitis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were examined in this work.When comparing the current study to earlier research, this could be the primary improvement. As an instance, virulence factor genes have been assessed in individual microbes such as *Streptococcus mutans* [43,63,67,69–71], *Staphylococcus spp.* [21,26,42,49,57,58,60,61], *Pseudomonas aeruginosa* [28,45,49,50,69], *Streptococcus mitis* [36], *and Sphingomonas paucimobilis* [46]. Further research is needed for covering all encoding genes for biofilm formation in each genus of the tested bacteria. In addition, other clinical isolates can be tested for future work to provide better clinical importance. Furthermore, it could be the production and regulatory mechanism of biofilm is a good idea to evaluated in order to find out and create anti-biofilm medications that prevent biofilm formation.

Ethics approval

The present study was carried out in accordance with the ethics committee at Karbala University, College of Science in number of 164 on 3/8/2025 and Ministry of Health, Bayblon Health Directoratein number of 48 on 22/5/2025.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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