Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

DETECTION OF BIOFILM PRODUCTION FOR ORAL BACTERIAL ISOLATES AND IT'S IMPACT ON DENTAL CARIES OCCURRENCE

Basheer Hamed Hamood Al-Shameri[1], Rassam Abdo Saleh Alsubari[3], Ahmed Abdullah Howilah[3], Fatima Mohammed Abdullah Al-Rohmi[1], Khaled A AL-Haddad[2], Nesreen Fadel Al-Sanabani[3,4], Hassan Abdulwahab Al-Shamahy[4]

[1]*Department of Restorative and Esthetic Dentistry, Faculty of Dentistry, Sana'a University, Republic of Yemen.*
[2]*Orthodontics, Pedodontics and Prevention Department Faculty of Dentistry, Sana'a University, Yemen.*
[3]*Department of Prosthodontics, Faculty of Dentistry, Sana'a University, Republic of Yemen.*
[4]*Departement of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.*

Article Info:

Article History:
Received: 6 December 2023
Reviewed: 30 January 2024
Accepted: 26 February 2024
Published: 15 March 2024

Cite this article:
Al-Shameri BHH, Alsubari RAS, Howilah AA, Al-Rohmi FMA, AL-Haddad KA, Al-Sanabani NF, Al-Shamahy HA. Detection of biofilm production for oral bacterial isolates and it's impact on dental caries occurrence. Universal Journal of Pharmaceutical Research 2024; 9(1): 1-7. ***http://doi.org/10.22270/ujpr.v9i1.1055***

***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Department of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen. Tel: +967-770299847; E-mail: *shmahe@yemen.net.ye*

Abstract

Background and aims: Microorganisms are known to be involved in the formation of biofilms. These biofilms are often seen in the oral cavity, tooth surfaces, prosthesis surfaces, attached oral mucosa, dental implants, etc. These are capable of causing dental caries or gingivitis, and most of them are also known to possess a higher ability to cause dental caries than non-biofilm-producing bacteria. This study was conducted to detect the biofilm formation in bacterial isolates from the oral cavity of patients attaining dental clinics in the Faculty of Dentistry, Sana'a University. In addition, the study examined the association between DMFT, decay, missing teeth due to caries, and filled teeth in the permanent teeth and the degree of bacteria ability to produce biofilm.
Materials and Methods: Biofilm production was performed on 294 oral bacteria isolates from 100 patients who visited dental clinics run by Sana'a University's Faculty of Dentistry and private dental clinics. Biofilm-forming oral bacteria were detected by the tissue culture plate (TCA) method. The impact of biofilm production was correlated with the DMFT index of the tested patients.
Results: Out of 294 isolates, biofilm formation was seen in 285 isolates (96.9%) by the TCP method. 21.4% of the iosolates showed a weak ability to produce biofilms, 72.4% showed moderate positivity, and only 3.1% showed strong positivity for biofilm production. Most *S. aureus* strains showed moderate and strong biofilm production (93.8% and 4.2%, respectively); *S. mutans* had 86.6% of strains with moderate biofilm production and 1.2% with strong biofilm production, while other streptococci had less biofilm production capacity. The DMFT index for the Yemeni patients included in the study was 5.9±2.4; there was a higher mean±SD (6.2±1.9) of DMFT for weak biofilm-producing bacteria with a difference equal to 3.5, 95% CI=1.4-4.2 (p=0.0001); for moderate biofilm-producing bacteria (6.9±2.5, difference=3.5, 95% CI=1.8-5.2, p=0.0001); and for strong-producing biofilms (mean±SD=6.2±2.9, p=0.03).
Conclusion: In conclusion, a higher DMFT was observed in biofilm producers than in non-biofilm producers. The prime biofilm producers were *S. aureus, S. mutans*, and *E. coli*.
Keywords: Biofilm formation, dental caries, DMFT, decayed, missing, filled teeth, oral cavity bacteria, Yemen.

INTRODUCTION

According to one estimate, biofilms are responsible for 80% of all infections in the body and have been linked to a wide variety of microbial illnesses[1]. Common issues like bacterial vaginosis, urinary tract infections, catheter infections, middle ear infections, dental plaque formation, gingivitis, and contact lens inflammation are among the infectious processes in which biofilms are involved[2,3]. Additionally, biofilms are involved in fatal processes like endocarditis and inflammation in cystic fibrosis, as well as permanent static devices like prostheses, heart valves, and intervertebral discs[4-6]. A biofilm is characterized as a microbial population made up of bacterial cell clusters that are attached to a surface and encased in an extracellular matrix that the

bacteria manufacture on their own[7]. Biofilm serves as a crucial component of virulence and offers an environment that is conducive to the survival of organisms[8]. Significant alterations in protein metabolism and gene expression accompany an organism's adaptation to surface-associated growth within a biofilm, which gives rise to the capacity to cause diseases and dysfunctions like dental caries, resistance to antibiotic treatment, and resistance to the host immune response[9]. It has been shown that pathogenic bacteria can form biofilms in both the natural environment and affected tissues, where they coexist as polymicrobial communities[7]. Numerous chronic illnesses, including necrotizing fasciitis, cellulitis, diabetic foot ulcers, and chronic stomatitis, have been linked primarily to the production of biofilms[10]. Biofilms are linked to 65% of nosocomial infections and have a significant influence on healthcare environments[9]. The gene product of icaADBC, polysaccharide intracellular adhesion, facilitates cell-to-cell adhesion and controls the formation of biofilms by expressing itself[11].

Biofilms can lead to gum disease and tooth decay since they are found on dental plaque in the human body. These biofilms can be either uncalcified, which can be removed with dental instruments, or calcified, which is more difficult to remove and requires the use of antimicrobials along with other removal approaches[12]. Dental plaque is an oral biofilm that sticks to teeth and is made up of several gram-positive and gram-negative bacterial and fungal species, including Candida albicans, Enterobactericiae, *S. mutans*, and other gram-positive cocci, embedded in salivary polymers and extracellular microbial products. Dental disease is caused by the buildup of germs, which exposes the teeth and gingival tissues to elevated levels of bacterial metabolites[13]. Oxidative stress and acid stress typically affect the biofilm on the surface of teeth[14,15]. At 37°C, a pH of 4 can cause depurination of DNA, leaving apurinic (AP) sites in DNA[16], specifically loss of guanine[17]. Dietary carbohydrates can also cause a substantial reduction in pH in oral biofilms to values of 4 and lower (acid stress)[15]. If dental plaque biofilm is allowed to grow over time, it may eventually lead to dental caries. When specific (cariogenic) micro-biological populations start to predominate in an environment that supports them, there is an ecologic shift away from balanced populations within the tooth biofilm. Frequent ingestion of fermentable dietary carbohydrates is necessary for the development and maintenance of the shift to an acidogenic, aciduric, and cariogenic microbial community. A carious lesion, or cavity, is the symptom of this activity shift in the biofilm, which is linked to an imbalance of demineralization over remineralization. This causes net mineral loss within the dental hard tissues (dentin and enamel first), which is then followed by acid production within the biofilm at the tooth surface[13,]. By preventing the dental plaque biofilm from maturing or by returning it to a non-cariogenic state, dental caries can be prevented and arrested[18,19]. This can be achieved through an understanding of bacteria's ability to produce biofilms and an appropriate biotechnique to prevent and remove the biofilm[18]. There is limited data on the association of biofilm production by bacteria with the occurrence of dental caries, so this study was conducted to detect the biofilm formation in bacterial isolates from the oral cavity of patients attaining dental clinics in the Faculty of Dentistry, Sana'a University, and study the association of DMFT, decay, missing teeth due to caries, and filled teeth in the permanent teeth with the degree of bacteria's ability to produce biofilm.

MATERIALS AND METHODS

Biofilm production was performed on 294 oral bacteriaisolates from 100 patients who visited dental clinics run by Sana'a University's Faculty of Dentistry and private dental clinics, over the course of one year, starting in September 2022 and ending in September 2023. The ensuing phenotypic identification of isolated bacteria was performed by standard methods following the Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines. Biofilm-forming oral bacteria were detected by the tissue culture plate (TCA) method. The impact of biofilm production was correlated with the DMFT index of the tested patients.

Recording of dental caries

The same examiner performed the examinations on each of the study adults. The caries diagnostic criteria were taken into consideration when doing the intra-examiner calibration. The adult Silness-Loe plague index was completed. This index is based not only on the simple counting of the number of decayed, missing (due to caries solely), and treated teeth, but also on the field clinical evaluation of the research participants using a probe, mirror, and cotton rolls.

Biofilm production detection*:*

Tissue culture/microtiter plate approach (TCA) was used to identify biofilm[20,21]. After being inoculated with 2 ml of BHI broth, the bacterial isolates from fresh agar plates were cultured for 24 hours at 37°C. Following a 1:40 dilution with fresh medium (BHI broth supplemented with 1% glucose), 200 µl of the sample was added to each individual microtitration plate, and the plates were incubated for an additional 24 hours at 37°C. After lightly tapping the contents, free floating sessile bacteria were eliminated by repeatedly rinsing it with phosphate buffer saline (pH 7.2). For ten to fifteen minutes, adhering bacteria that produced biofilm were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). After removing the unbound crystal violet solution in three separate PBS washes, the plate was set aside to dry. In order to release the dye, 200 µl of 95% ethanol was added to each well, and an optical density (OD) reading at 630 nm was taken. Each test strain's OD value as well as that of the negative control were computed, and OD cutoff values (ODc) were evaluated in accordance with earlier instructions[21].

Statistical Analysis: Epi-Info Statistics version 7 was utilized to examine the information. Statistical analysis was performed to consider the degree of biofilm production of 294 bacterial isolates with the mean of DMFT, decayed, missing due to caries, and filled teeth

in permanent teeth by calculating the difference, 95% CI, and *p*-value of the level of weak, moderate, and strong biofilm production.

Ethical Consideration: The Contract No. 217 project received ethical authorization on August 21, 2022, from the Medical Ethics and Research Committee of Sana'a University's Faculty of Medicine and Health Sciences. The review committee's established ethical guidelines were constantly adhered to. The selected individuals gave their written and informed consent.

RESULTS

Table 1 shows the interpretation of biofilm production by the tested bacteria based on the optical density values of the tissue culture plate method for average biofilm production with an OD value. Nine bacteria (3.1%) showed a negative ability to produce biofilms (OD < 0.17), and 21.4% of the tested bacteria showed a weak ability to produce biofilms (OD=0.17–0.34). Most of the orally isolated bacteria showed moderate positivity (OD=0.35-0.68), which amounted to 72.4%, while only 3.1% of the tested bacteria showed strong positivity for biofilm production (OD > 0.68) (Table 1).

Table 1: Interpretation of biofilm production by bacterial isolates based on optical density values of tissue culture plate method average value of OD* biofilm production.

OD value	N (%)
<0.17; Negative	9 (3.1)
0.17-0.34; Weak positive	63 (21.4)
0.35-0.68; Moderate positive	213 (72.4)
>0.68; Strong positive	9 (3.1)
Total	294 (100)

Table 2 shows the detection of biofilms by the TCA method among 294 different bacterial species isolated in the oral cavity. Most *S. aureus* strains showed moderate and strong biofilm production (93.8% and 4.2%, respectively), while the other 64.3% of *Staphylococcus* coagulase-negative strains showed only moderate positive biofilm production. Considering the *Streptococcus viridans* group, *S. mutans* had 86.6% strains with moderate biofilm production and 1.2% with strong biofilm production, while other streptococci had less biofilm production capacity (Table 2). For *Neisseria* species, 72% showed a moderate level of biofilm production.

Table 2: Biofilm detection by TCA method among different species of oral cavity isolated Bacteria n=294.

Bacteria	Biofilm production by TCA			
	Negative No (%)	Weak No (%)	Moderate No (%)	Strong No (%)
S. aureus, n=48	0 (0.0)	1(2.1)	45 (93.8)	2 (4.2)
Coagulase-negative, n=14	1(7.1)	4 (28.6)	9 (64.3)	0 (0.0)
Streptococci				
S. pyogens, n=3	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)
S. mitior, n=18	0 (0.0)	7 (38.9)	11 (61.1)	0 (0.0)
S. sanguis, n=17	1 (5.9)	9 (52.9)	6 (35.3)	1 (5.9)
S. mutans, n=82	0 (0.0)	10 (12.2)	71 (86.6)	1 (1.2)
S. alivarius, n=25	1 (4)	7 (28)	17 (68)	0 (0.0)
S. milleri, n=4	1 (25)	1 (25)	2 (25)	0 (0.0)
Neisseria species, n=50	1(2)	13 (26)	36 (72)	0 (0.0)
Haemophilus influenza, n=1	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
H. parainfluenzae, n=12	0 (0.0)	5 (41.7)	7 (58.3)	0 (0.0)
Enterobacteriaceae spp.				
E. coli, n=7	1 (14.3)	1 (14.3)	5 (71.4)	0 (0.0)
K. pneumoniae, n=4	1 (25)	0 (0.0)	2 (50)	1 (25)
Morganella morganii, n=1	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
Citrobacter freundii, n=2	0 (0.0)	0 (0.0)	1 (50)	1 (50)
P. aeruginosa, n=3	1 (33.3)	1(33.3)	0 (0.0)	1 (33.3)
Proteus species, n=2	0 (0.0)	1 (50)	0 (0.0)	1 (50)
Enterobacter species, n=1	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
Total n=294	9 (3.1)	63 (21.4)	213 (72.4)	9 (3.1)

Table 3 shows the prevalence of DMFT in total, decayed, lost teeth due to caries, and filled teeth in permanent dentition by sex. Dental caries was recorded in 96% of all patients; missing permanent teeth were recorded in 57% of patients; 69% had filled teeth; and 98% showed a DMFT index of more than one. Males also had higher rates of the previous indications than female patients. Only 4% of participants are caries-free. The rate of cavity-free adults with zero DMFT was 2%.

The DMFT index for the Yemeni patients included in the study was 5.9±2.4, for caries 3.7±2.0, for missing teeth 1.7±2.7, and for filled teeth 2.6±2.4. Considering the relationship between the DMFT index and the ability of bacteria to produce biofilms, there was a higher mean±SD (6.2±1.9) of DMFT for weak biofilm-producing bacteria with a difference equal to 3.5, 95% CI=1.4-4.2, (p=0.0001).

Table 3: Prevalence of DMFT the sum, decayed, missing due to caries, and filled teeth in the permanent teeth per sex.

Variables	Male N (%)	Female N (%)	Total N (%)
Decayed	39 (100)	57 (93.4)	96 (96)
Missed	27 (69.2)	30 (49.2)	57 (57)
Filled	33 (84.6)	36 (59)	69 (69)
DMFT	39 (100)	59 (96.7)	98 (98)

Table 4: Comparison among the DMFT index and biofilm production of total oral isolated bacteria.

Biofilm results	Mean±SD			
	DMFT	Decayed	Missed	Filled
Negative , n=9 (control)	3.4±2.1	3.0 ±2.3	1.8±2.9	1.2±2.1
Weak, n=63	6.2±1.9	3.8±1.4	1.1±2.4	2.3±2.4
Difference	2.8	0.8	-0.7	1.1
95% CI	1.4-4.2	0.3-1.9	-2.4-1.05	-0.58-2.7
Significance level	*p*=0.0001	*p*=0.14	*p*=0.42	*p*=0.196
Moderate, n=213	6.9±2.5	3.8±2.1	2.9 ±2.8	3.3 ±1.9
Difference	3.5	0.8	1.1	2.1
95% CI	1.8-5.2	0.6-2.2	-0.78-2.9	0.8-3.3
Significance level	*p*=0.0001	*p*=0.27	*p*=0.25	*p*=0.001
Strong, n=9	6.2±2.9	3±1.5	1±1.2	6.6±2.1
Difference	2.8	0.0	-0.8	5.4
95%, CI	0.26-5.3	-1.9-1.9	-3.0-1.4	3.3-7.5
Significance level	*p*=0.03	*p*=1.00	*p*=0.41	*p*=0.0001
Total, n=294	5.9±2.4	3.7±2.0	1.7±2.7	2.6±2.4
range	1-9	0-9	0-9	0-9

This procedure calculates the difference between the observed means in two independent samples. A significance value (*p*-value) and 95% Confidence Interval (CI) of the difference is reported. The *p*-value is the probability of obtaining the observed difference between the samples if the null hypothesis were true. The null hypothesis is the hypothesis that the difference is 0.

This suggests that biofilm production is associated with tooth decay. There was a higher mean±SD (6.9±2.5) of DMFT for moderate biofilm-producing bacteria compared to non-biofilm-producing bacteria, with a difference equal to 3.5, 95% CI=1.8–5.2, and this result is highly significant (*p*=0.0001). Also, there was a higher mean±SD (6.2±2.9) of DMFT for biofilm-producing bacteria with a difference of 2.8, and this result is significant (*p*=0.03).

DISCUSSION

Human illnesses caused by bacteria are known to take many different forms. The majority of these bacteria are known to exhibit specific virulence factors, which contribute to their pathogenicity. These virulence factors include the creation of biofilms, toxin synthesis, fimbriae, and pili. The majority of recalcitrant infections are caused by biofilm formation, which is one of the virulence factors that are hardest to treat since the organisms involved are extremely resistant to antibiotics[10]. In the present study, most of the oral isolated bacteria showed moderate positivity (OD=0.35 -0.68) amounting to 72.4%, while only 3.1% of the tested bacteria showed strong positivity for biofilm production (OD > 0.68) (Table 1). This ability can be explained by the fact that biofilm formation begins with the attachment of free-floating microorganisms to the surface[22,23]. The first colonizing bacteria of biofilms may initially adhere to the surface by weak van der Waals forces and hydrophobic effects[24,25]. If colonies are not immediately detached from the surface, they can attach themselves more permanently using cell adhesion structures such as pili[24]. Hydrophobicity can also affect the ability of bacteria to form biofilms. Bacteria with increased hydrophobicity led to reduced repulsion between the substrate and bacteria[26]. Some bacteria are unable to successfully adhere to a surface on their own due to their limited motility but are instead able to attach themselves to the matrix or directly to previous colonies of other bacteria[26]. Bacterial cells can interact with one another during surface colonization by employing quorum sensing (QS) products such N-acyl homoserine lactone (AHL). After colonization starts, a combination of cell division and recruitment leads to the biofilm's growth. Bacterial biofilms are usually enclosed by polysaccharide matrices. To shield the biofilm from predators and guarantee bacterial survival, the matrix exopoly-saccharides can ensnab QS autoinducers[27]. These matrices may also include elements from the surrounding environment, such as minerals, soil particles, and blood components like fibrin and erythrocytes, in addition to the polysaccharides[26]. Development, the last stage of biofilm creation, is when the biofilm is formed and can only alter in size and shape. Dental plaque is an oral biofilm that sticks to the teeth and is made up of numerous bacterial and fungal species, including *C. albicans* and *S. mutans*, that are enmeshed in salivary polymers and extracellular microbial products. Dental disease is caused by the buildup of germs, which exposes the teeth and gingival tissues to elevated levels of bacterial metabolites[13].

Most *S. aureus* strains showed moderate and strong biofilm production (93.8% and 4.2%, respectively), in the present study. *S. aureus* pathogens can attack the skin and lungs, leading to skin infection and pneumonia[28,29]. Moreover, the *S. aureus* biofilm infection network plays a crucial role in preventing immune cells, such as macrophages, from eliminating and destroying bacterial cells[30]. Moreover, biofilm formation by bacteria, such as *S. aureus*, not only develops resistance against antibiotic drugs but also develops intrinsic resistance toward antimicrobial peptides (AMPs), thus preventing pathogen inactivation and maintaining its survival[31].

Regarding the *S. viridans* group in the current investigation, *S. mutans* strains exhibited 86.6% moderate biofilm production and 1.2% strong biofilm production, whereas other *streptococci* shown lower biofilm production capacities (Table 2). Dental plaque, an oral biofilm that sticks to teeth, is made up of several types of bacteria and fungus, including *C. albicans* and *S. mutans*, which are embedded in salivary polymers and extracellular microbial products.

Dental disorders are caused by the accumulation of microorganisms that expose teeth and gingival tissues to excessive quantities of bacterial metabolites[13]. Moreover, acid and oxidative stress commonly affect the biofilm that covers teeth[15]. Acid stress is the result of dietary carbohydrates, which can cause oral biofilm pH levels to drop sharply to 4 and below. DNA becomes depurinated at 37°C body temperature, leaving apurinic (AP) sites in the DNA, particularly guanine loss[16,17].

The traditional DMF (decay/missing/filled) index is one of the most widely used tools for determining the prevalence of dental caries and the need for dental treatment in various populations. This indicator is based on clinical examinations conducted on-site on individuals utilizing a mirror, cotton rolls, and probe. The DMF index underrepresents the prevalence of caries and the requirement for treatment because it is calculated without using X-ray imaging[32]. In the current study, dental caries was noted in 96% of patients, permanent teeth missing were noted in 57% of patients, teeth filled in 69% of patients, and a DMFT index of more than one was seen in 98% of patients. Males also had higher rates of the previous indications than female patients. Only 4% of participants did not suffer from tooth decay. The rate of cavity-free or zero DMFT adults was only 2%. These results of tooth decay are considered among the highest rates in the world. Worldwide, approximately 3.6 billion people suffer from tooth decay in their permanent teeth[33]. Tooth decay is more common in Latin American countries, the Middle East, and South Asia compared to the rest of the world, while the least prevalence of tooth decay is in China[34]. In the United States, dental caries is the most common chronic childhood disease, being at least five times more common than asthma[35].

The current study indicates that the 96% of positive dental caries cases in adults (mean age=40.1 years) is higher than those reported elsewhere in the world, where between 29% and 59% of adults over the age of 50 suffer from Tooth decay[36]. The high rate of tooth decay in Yemen may be related to poverty, poor oral hygiene practices, and the absence of preventive measures and government policy to prevent and control tooth decay. In contrast, the number of cases has decreased in some developed countries, and this decline is usually due to increasingly excellent oral hygiene practices and the application of preventive measures[37].

However, countries that have experienced an overall decline in the incidence of dental caries still have uneven disease distribution[36]. Among children in the United States and Europe, twenty percent of the population suffers from sixty to eighty percent cases of tooth decay[38]. A similar skewed distribution of the disease has been found worldwide, with some children having no or very few caries and others having a large number[36]. Australia, Nepal and Sweden (where children receive government-paid dental care) have a low incidence of tooth decay among children, while cases are more numerous in Costa Rica, Slovakia and Yemen[39-44].

Considering the relationship between the DMFT index and the ability of bacteria to produce biofilms, there was a higher mean±SD of DMFT for weak biofilm-producing bacteria, modrate and strong levels (Table 4), and this result is highly significant (p=0.0001), suggesting that biofilm production is associated with tooth decay. If dental plaque biofilm is allowed to grow over time, dental caries may result. In the dental biofilm, some (cariogenic) microbiological communities start to predominate when the conditions are right, causing an ecologic shift away from balanced populations. Frequent consumption of fermentable dietary carbohydrates promotes and sustains the shift in the microbiological population toward one that is acidogenic, aciduric, and cariogenic. A carious lesion, or cavity, is the symptom of a balance between demineralization and remineralization, which causes a shift in the biofilm's activity and consequent acid production at the tooth surface. This causes net mineral loss in the dental hard tissues, namely the enamel and dentin. By preventing the dental plaque biofilm from maturing or returning it back to a non-cancerous state, tooth decay can be prevented and stopped[18,19]. This can be achieved through the behavioral step of reducing the supply of fermentable carbohydrates (i.e. sugar intake) and frequent removal of biofilms (i.e. tooth brushing)[18]. It is expected that the rates of bacteria capable of forming biofilm will increase and the rate of tooth decay will increase. Therefore, oral health research programs must be established that aim to find safe compounds to be added to toothpaste that dissolve the biofilm from the surfaces of the teeth and prevent its re-formation again, thus preserving the teeth for a longer period of function.

Limitation of the study

There hasn't been nearly enough research done on this topic in Yemen or the rest of the world to compare aerobic bacteria with anaerobic types and confirm their ability to form biofilms, among other virulence factors that lead to dental caries, oral infections, and systemic infections. Other research with a greater number of patients, anaerobic species, and fungus is necessary to identify other virulence factors of these microorganisms.

CONCLUSIONS

The studied subjects in current study showed very high DMFT scores for adults; thereby reaching WHO approved targets for oral health in Yemen cannot be achieved. Also, a high DMFT was observed in biofilm producers isolated bacteria than non-biofilm producers bacteria isolates. Prime biofilm producers in oral cavity were *S. aureus*, *S. mutans*, and *E. coli*.

ACKNOWLEDGEMENTS

The authors express their gratitude to Yemen and the Sana'a University Faculty of Dentistry for their kind assistance.

AUTHOR'S CONTRIBUTION

Al-Sanabani NF: fieldwork for this study as part of a PhD in the department of Medical Microbiology. **Al-Shameri BHH:** writing original draft, conceptualization. **Alsubari RAS:** methodology, investigation. **Howilah AA:** Writing, review. **Al-Rohmi FMA:** and editing, supervision. **AL-Haddad KA:** conceptualization, methodology, investigation. **Al-Shamahy HA:** data curation, supervision. All authors revised the article and approved the final version.

DATA AVILIABILITY

The data will be available to anyone upon request from the corresponding author.

CONFLICT OF INTEREST

Regarding this project, there is no conflict of interest.

REFERENCES

1. Landini P, Valle J. Microbial Biofilms: From Molecular Mechanisms and Structure to Antimicrobial Therapy. Microorganisms 2022; 10(8):1638. *https://doi.org/10.3390/microorganisms10081638*
2. Saleh AM, Al-Shamahy HA, Al-Hrazi RMA, Jaadan BM, AL-Magrami RTF, Al-Sharani AA. Biofilm formation and antibiotic susceptibility of uro-pathogens in patients with catheter associated urinary tract infections in Ibb city-Yemen. Universal J Pharm Res 2019; 4(6):1-7. *https://doi.org/10.22270/ujpr.v4i6.329*
3. Imamura Y, Chandra J, Mukherjee PK, *et al*. Fusarium and *Candida albicans* biofilms on soft contact lenses: Model development, influence of lens type, and susceptibility to lens care solutions. Antimicr Agents Chemo 2008; 52 (1): 171–82. *https://doi.org/10.1128/AAC.00387-07*
4. Capoor, Manu N, Ruzicka F, Schmitz, Jonathan E, *et al*. Propionibacterium acnes biofilm is present in intervertebral discs of patients undergoing microdiscectomy. PLOS ONE 2017; 12 (4): e0174518. *https://doi.org/10.1371/journal.pone.0174518*
5. Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. J Glob Infect Dis 2020; 12(3):129-134. *https://doi.org/10.4103/jgid.jgid_150_19*
6. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics (Basel) 2020; 9(2):59. *https://doi.org/10.3390/antibiotics9020059*
7. Sanchez CJ, Mende K, Beckius ML, *et al*. Biofilm formation by clinical isolates and the implications in chronic infections. BMC Infect Dis 2013; 13: 47. *https://doi.org/10.1186/1471-2334-13-47*
8. Shashikala, Ali FA, Lokare N, Matew J. Diabetic foot ulcers and biofilm formation- The culprits. Int J Biomed Adv Res 7 (2016): 428-433. *https://doi.org/10.7439/IJBAR.V7I9.3594*
9. CharanKaur D, Khare AS. Biofilm formation and antibiotic susceptibility pattern in MRSA strains in a tertiary care hospital. Indian J Basic Appl Med Res 2013; 1:37-44.
10. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 2011; 15(4):305-11. PMID: 21860999.
11. Abdel Halim RM, Kassem NN, Mahmoud BS. Detection of biofilm producing staphylococci among different clinical isolates and its relation to Methicillin Susceptibility. Open Access Maced J Med Sci 2018;6(8):1335-1341. *https://doi.org/10.3889/oamjms.2018.246*
12. Chandki R, Banthia P, Banthia R. Biofilms: A microbial home. J Indian Soc Periodontol 2011; 15 (2): 111–4. *https://doi.org/10.4103/0972-124X.84377*
13. Augustin M, Chifiriuc CB, Lazăr V, Stănescu R, Burlibaşa M, Ispas DC. Microbial biofilms in dental medicine in reference to implanto-prostethic rehabilitation. J Oral-Maxillo-Facial Surg Implantol 2010; 1 (1): 9-13.
14. Marquis RE. Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms. J Indust Microbiol 1995; 15 (3): 198-207. *https://doi.org/10.1007/bf01569826*
15. Lemos JA, Abranches J, Burne RA. Responses of cariogenic streptococci to environmental stresses. Current Issues in Molecular Biology 2005; 7 (1): 95-107. PMID 15580782.
16. Tamm C, Hodes ME, Chargaff E. The formation apurinic acid from the desoxyribonucleic acid of calf thymus. The J Biol Chem 1955; 195 (1): 49–63. *https://doi.org/10.1016/S0021-9258(19)50874-2*
17. Freese EB. Transitions and transversions induced by depurinating agents. Proceedings of the National Academy of Sciences of the United States of America 1961; 47 (4): 540-5. *https://doi.org/10.1073/pnas.47.4.540*
18. Pennwell. Toothbrush technology, dentifrices and dental biofilm removal. Dental Academy of CE Accessed 1 January 2024.
19. Fejerskov O. Pathology of dental caries. In: Dental caries: the disease and its clinical management. Oxford (UK): Wiley Blackwell 2015; 7–9. ISBN 978-1405138895.
20. Christensen GD, Simpson WA, Bisno AL, Beachley EH. Adherence of slim-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immunity 1982; 37(1):318–326. PMID: 6179880.
21. Stepanovic S, Vukovic D, Hola V, *et al*. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *Staphylococci*. Acta Patho Micro 2007; 115(8):891–899. *https://doi.org/10.1111/j.1600-0463.2007.apm_630.x*
22. Penesyan A, Paulsen IT, Kjelleberg S, *et al*. Three faces of biofilms: A microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. NPJ Biofilms Microbiomes 2021; 7: 80. *https://doi.org/10.1038/s41522-021-00251-2*
23. O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. Molecular Microbiol 1998; 28 (3): 449-61. *https://doi.org/10.1046/j.1365-2958.1998.00797.x*
24. Briandet R, Herry J, Bellon-Fontaine M. Determination of the van der Waals, electron donor and electron acceptor surface tension components of static Gram-positive microbial biofilms. Colloids Surf B Biointerfaces 2001; 21 (4): 299–310. *https://doi.org/10.1016/S0927-7765(00)00213-7*
25. Takahashi H, Suda T, Tanaka Y, Kimura B. Cellular hydrophobicity of Listeria monocytogenes involves initial attachment and biofilm formation on the surface of polyvinyl chloride. Lett Appl Microbiol 2020; 50 (6): 618–25. *https://doi.org/10.1111/j.1472-765X.2010.02842.x*
26. Donlan RM. Biofilms: Microbial Life on Surfaces. Emerging Infect Dis 2002; 8 (9): 881–890. *https://doi.org/10.3201/eid0809.020063*
27. Li S, Liu SY, Chan SY, Chua SL. Biofilm matrix cloaks bacterial quorum sensing chemoattractants from predator detection. The ISME J 2022; 16 (5): 1388–1396. *https://doi.org/10.1038/s41396-022-01190-2*

28. Kobayashi SD, Malachowa N, Whitney AR, *et al*. Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. The J Infect Dis 2011; 204 (6): 937-941. *https://doi.org/10.1093/infdis/jir441*
29. Kitur K, Parker D, Nieto P, *et al*. Toxin-induced necroptosis is a major mechanism of *S. aureus* lung damage. PLOS Pathogens 2015; 11 (4): e1004820. *https://doi.org/10.1371/journal.ppat.1004820*
30. Thurlow LR, Hanke ML, Fritz T, *et al*. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation *in vivo*. J Immunol 2011; 186 (11): 6585-6596. *https://doi.org/10.4049/jimmunol.1002794*
31. Craft KM, Nguyen JM, Berg LJ, Townsend SD. Methicillin-resistant *Staphylococcus aureus* (MRSA): antibiotic-resistance and the biofilm phenotype. Med Chem Comm 2019; 10 (8): 1231–1241. *https://doi.org/10.1039/c9md00044e*
32. Zadik Yehuda; Bechor Ron. "Hidden Occlusal Caries – Challenge for the Dentist" (PDF). The New York State Dental J 2008; 74 (4): 46–50. PMID 18788181.
33. WHO- "Oral health". World Health Organization. Retrieved 2024-01-01.
34. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century – The approach of the WHO Global Oral Health Programme Archived 2006-09-27.
35. Healthy People: 2010 Archived 2006-08-13 at the Wayback Machine. Html version hosted on Healthy People.gov Archived 2017-03-10 at the Wayback Machine website. accessed 01-01-2024.
36. Dental caries Archived 2008-12-07 at the Wayback Machine, from the Disease Control Priorities Project.
37. World Health Organization Archived 2006-03-26 at the Wayback Machine website, "World Water Day 2001: Oral health", p. 2.
38. Moynihan P. Sugars and Dental Caries: Evidence for Setting a Recommended Threshold for Intake. Adv Nutr 2016 Jan 15; 7(1):149-56. *https://doi.org/10.3945/an.115.009365*
39. The Disease Control Priorities Project : Mean DMFT and SiC Index of 12-Year-Olds for Some Countries, by Ascending Order of DMFT Archived 2008-12-07 at the Wayback Machine", from the Disease Control Priorities Project.
40. Sharafuddin AH, Alshameri BH, AL-Haddad KA, Al-Najhi MMA, Al-Shamahy HA. The effect of dental implants on increasing the colonization rate of aerobic bacteria in the oral cavity. Universal J Pharm Res 2023; 8(3):1-8. *https://doi.org/10.22270/ujpr.v8i3.944*
41. Alsamhari MMA, Al-Najhi MMA, Al-Shamahy HA, Al-dossary OAI. Analysis of biofilms for *Streptococcus mutans* from dental root surfaces of adult patients with root caries. Universal J Pharm Res 2021; 6 (5): 1-6. *https://doi.org/10.22270/ujpr.v6i5.668*
42. Alhasani AH, Ishag RA, Al-Akwa AAY, Al-Shamahy HA, Al-labani MAA. Association between the *Streptococcus mutans* biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal J Pharm Res 2021; 5(6):1-6. *https://doi.org/10.22270/ujpr.v5i6.507*
43. Bamashmoos KAO, Alhasani AH, Al-Akwa AAY, Zabara AQM, Al-Shamahy HA, Al-deen HS, and Al-labani MA. Prevalence of premature loss of primary teeth at the age of 6-10 years in Sana'a city, Yemen. Universal J Pharm Res 2020; 5(4):1-6. *https://doi.org/10.22270/ujpr.v5i4.439*
44. Al-Haddad KA, Al-dossary OA, and Al-Shamahy HA. Prevalence and associated factors of oral non-candida albicans candida carriage in denture wearers in Sana'a city- Yemen. Universal J Pharm Res 2018; 3(4): 1-6. *https://doi.org/10.22270/ujpr.v3i4.176*