Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5545 - 5562
Received 05 March 2021; Accepted 01 April 2021.

Study of Dental Plaque Isolates, Their Properties of Biofilm Formation and Susceptibility to Antibiotics

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ABSTRACT

The aim of this study is to determine the microorganisms' profile of the supragingival dental plaque with their properties of biofilm formation, to test the antibiotics resistance ability and to reviewing the role of *C. albicans* in caries using an *in vitro* dual-species cariogenic oral biofilm model. The target microorganisms were isolated from attached plaque of the teeth from the patients attending Dental Clinic, Health Clinic Centre Mosul University. The ability of formation biofilm using different surfaces were tested. The antibiotic susceptibility and an *in vitro* dual-species cariogenic oral biofilm model were conducted. A total of 39 isolates were effectively acquired. To screen the selected isolates according to its biofilm formation ability, three methods were used for that. The results showed that almost all the isolates can form visible growing biofilms. Antibiotic susceptibility assays results showed that most of the isolates were resistant to a wide range of antibiotics.The pH values of dual-species biofilms after 48 h of growth, were above the critical demineralization zone of pH 5.3–5.5. It was concluded that almost all the isolates from patients attending Dental Clinic, can form visible growing biofilms. Further, the study revealed that antimicrobial resistance of isolates for the patients with dental caries conditions is associated with biofilm formation.Our finding suggests that *C. albicans* are not a microorganism of cariogenesis, it is by actively increasing pH, could prevent caries.

Key words
Teeth biofilm formation, dental plaque, antibiotic resistance, *C. albicans* in caries.

1. Introduction

A biofilm is a total of microorganism where cell are adhered to one another. These disciple cells are often installed inside a self-delivered grid of extracellular polymeric substance (EPS). Biofilm EPS, which is additionally alluded to as (sludge), is a polymeric clutter of DNA, proteins and polysaccharides. 80% of all microbial contamination are accepted to be brought about by biofilm. Biofilms are liable for a few ceaseless sickness that are hard to treat; the basic antimicrobial specialist are regularly incapable on the grounds that microorganism in biofilm deferent from their planktonic partner (same space yet free-living). Characteristic spot of biofilm in teeth is called dental plaque (1). Dental plaque is a yellowish biofilm that build up on the teeth. If not removed regular, it can lead to dental caries.

Supra gingival plaque originates from saliva and sub gingival plaque comprises gingival crevicular fluid, which contains many substances that can be used as nutrients by the bacteria.

Dental plaque composition consists of micro - organisms, intra - cellular matrix, 20 percent solids, 80 percent water, cells containing primarily bacteria that make up 35 percent of dry weight and extracellular matrix components that make up 65 percent of dry weight. Non-bacterial species except for bacteria include yeast, viruses, epithelial cells, host cells, leukocytes, and macrophages.(2).

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The present of teeth organisms

Minuscule perspective on dental plaque, show a set of microbes that generally appear to include Gram-negative bacilli and Gram-positive cocci. The mouth condition change for the length of the day depending on the levels of salivation, nutrition and oxygen. Such vacillation of oral conditions induces a similar change in the number of occupants inside the mouth of the biofilm as it affects the microbial relationships of the microbial organisms. Despite the fact that reviews show that the usual microflora at the end still shows signs of change due to natural conditions and area, the most well-known microscopic organisms that are found in the supragingival plaque are: gram positive cocci *St. oralis, St. mitis, St. mutans, St. sanguis,* and *Staph. epidermidis, Staph. aureus,* gram negative cocci and poles Fusobacteria and *Neisseria sicca* and gram positive bars and fibers, *Actinomyces* spp. *Corynebacterium* spp., *Prevotella* spp. and lactobacilli. Due to their collaboration with each other and the tooth surface, these prominent microorganisms are responsible for plaque growth.

Candida species are natural oral pit mucosal surface colonizers and are talented in keeping fast to tooth surfaces, taking part in biofilm arrangement, and sugar aging proceeds. In comparison to buccal mucosa and tongue, *C. albicans* was present in a higher recurrence on occlusal tooth surfaces, as it has slightly more partiality to the carious tooth structure. Colonization of the oral pit by this yeast occurs by parasitism, without causing ailment. A groundbreaking dimorphic growth has been suggested to have an acidogenic and cariogenic potential for the most prevalent species, *C. albicans*. The shift from the saprophytic to the parasitic structure is linked to its harmful effects and its related components, the most important of which is the modification of the oral microbiota balance. This transition can occur due to, for eg, mouth ventilation or orthodontic devices. *Candida albicans* may turn back and forth as individual yeast cells, pseudohyphae or enraptured fiber among growth.

It is essential for the foundation of the disease, these three morphotypes and the switch between them. Hyphae, in any case, are deemed the most harmful form of morph. This morphotype has been suggested to penetrate polish breaks and dentinal tubules from outside of the teeth as well as from the root canal. (3).

As described before, various factors have been suggested to be virulence factors of this fungi, including persistence, attachment, dimorphism or / and germ tube development, interaction with the host defense mechanisms, phenotypic switching, synergism with bacteria and production of other metabolites or hydrolases. *Streptococcus mutans* is extremely common in dental biofilms inhabited by *C.albicans*, making it likely that they have a symbiotic relationship(4). In recent years numerous studies have investigated the interaction between such microbes (5, 6).

It is thought that the fungus is believed to provide *S. mutans* with adhesion sites in the biofilm, that is proposed to be facilitated by *S. mutans* developed dextran. Direct adhesion, in which *S. mutans* adheres to *C. albicans* on the hyphal cell wall and attaches to the one of the several adhesions, would be another possible binding mechanism. *C.*

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albicans is known to bind to *Staphylococcus gordonii* and *Staphylococcus aureus* through the hyphal adhesion Als3p. (7). *C. albicans* can benefiting from the lactic acid that *S. mutans* releases for the own metabolism in addition to adhesion. This effect reduces oxygen stress to levels that are favorable for *C. albicans* and *S. mutans* then provides conditions that stimulate *S. mutans*. (4).

In the scope of the ecological hypothesis of plaque, to the increasing interest for a polymicrobial cause of dental caries identified, The objectives of this research is to determine the bacterial profile of the supragingival dental plaque with their properties of biofilm formation, to test the antibiotics resistance ability of strong biofilm former isolates and to reviewing the role of *C. albicans* in caries using an *in vitro* dual-species cariogenic oral biofilm model.

2. Materials and methods

2.1 Subject groups

From patients visiting the Dental Clinic, University of Mosul / Mosul Health Clinic Center, ten teeth were taken. Male and female patients were randomly selected and they did not use antibiotics during the last three months and all patients had different dental and periodontal problems. A special dentist takes all of the samples.

2.2 Isolation and Identification

Isolation and identification pattern of facultative aero-anaerobes bacteria were done employing standard bacteriologic techniques.

2.3 Clinical sample collection

In this research, teeth samples were collected aseptically from each patient and transported to the microbiology laboratory/ biology department for processing. From the ten teeth, six teeth only were chosen for the following assays depending on the worst teeth decays statute. Supragingival plaque samples were collected by sterile cotton rolls and scraping the supragingival plaque. The tubes containing samples (teeth) were pre-incubated for 30 minutes at 37°C, and shaken vigorously in a vortex mixer for the 60s (8, 9).

Direct swap from teeth supragingival plaque (attached bacteria) and the residues from the washing teeth were all inoculated on to Nutrient agar (NA) and Brain heart infusion agar (BHI). For determination of the total bacteria (CFU/ mL), serial dilutions of the samples were prepared in peptone water (Bacto peptone, Difco, Detroit MI, USA). On a prepared media NA and BHI, 100 µL of third fold dilution was spread. All plates were incubated aerobically at 37 °C for 1-5 days. Following incubation, the number of viable colonies (CFU/ml) was counted using total viable plate count method. The selected isolates were purified by sub-culturing on the selective media. Mannitol Salt Agar (MSA) media for the staphylococci isolates, Sabouraud Chloramphenicol Agar for the Candida isolates and blood agar for the streptococci isolates.

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2.4 Morphological Characterisation

All pure isolates were determined based on Bergeys manual of determinative bacteriology (10), this included morphological characteristic (cell shape and Gram staining) and to check the growth pattern, different media including NA, BHI, MSA and Streptococcus Selective agar (Difco) were used after incubation at 37 °C for 24 hours.

2.5 Biochemical tests

For selected bacterial isolates, oxidase and catalase (Sigma-Aldrich Company) test were conducted.

2.6 Candida tube germination assay:

Approximately 0.5 ml of human serum was inoculated into a pure yeast colony in a separate sterile test tube and incubated for 2 h. On a microscope slide, a drop of the yeast and serum mixture was mounted, covered with a cover slip, and microscopically observed, using x10 and x40 objective lenses. The development of germ tubes was supported by the existence of thin filaments extending from the cell surface (11) .

2.7 Antibiotic susceptibility assay:

Antibiotic resistance of the all the isolates were determined. Mueller-Hinton agar for the staphylococci, Gram-negative bacilli and yeast, Chocolate agar with micro-aerophilic condition for streptococci were used, following the method of Kirby Bauer disk diffusion assay (12). Antimicrobial agents' disks were obtained from Bioanalyse co., Turkey. The explanatory standards for resistance/ sensitive of each antibiotic were depended on criteria determined by the NCCLS. The antibiotics selected for the bacterial isolates comprised of: Kanamycin K (30µg), Amoxycillin AML (10µg), Vancomycin VA (30µg), Erythromycin E (30µg), Ampicillin AMP (10µg) and for the yeasts isolates, Fluconazole (25 µg), and Voriconazole (1µg).

2.8 Biofilm forming assay:

2.8.1 Microtiter plate biofilm forming assay

All 39 isolate grown in brain-heart infusion (BHI, 35.9 g/L) broth overnight at 37◦C. were evaluated for their biofilm forming using 96-well microtiter plate in a method that was described in (13). Using 10 mL of BHI broth, each isolate was grown at 37 °C overnight. Later, 200 µL of these cultures were then moved into wells of a microtiter plate. To serve as controls, some wells only had sterile BHI broth. Aerobic incubation of the plates was done for 24 h at 37 °C. The well culture was aspirated out before the plates were washed three times using 200 µL of 0.9% saline in order to get rid of non-adherent cells. The plates were then dried in an inverted position. Using 200 µL of 1% crystal violet solution in water, each well was stained for 20 min. Aspiration of the unbound crystal violet was done before washing the wells thrice with 200 µL 0.9% saline. Bound

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dye was released by the addition of 200 μL of 95% ethanol. Quantitative analysis of biofilm production was then performed. From each well, one hundred microliters was transferred to a new microtiter plate and, using a microtiter plate reader, each well's optical density (OD) was measured at 595 nm and classified as strong, moderate and weak biofilm former. This experiment was done in triplicate wells, Based on strongest biofilm formation and multi-drug resistance, three isolates identified as *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* were chosen for following assays.

2.8.2 Teeth Biofilm Forming Assay:

Teeth biofilm forming assay was done according to (14) with some modification. The teeth were crushed using the turbine into 1 cm pieces and immersed in each well of 96-well microtiter plate that contained BHI broth. Incubation of the isolates took place at 37 °C for 24 hours. After incubation, the teeth were treated using a steps assay described in previous Section (2.7.1).

2.8.3 Tube method:

A loop of test organism from overnight culture on nutrient agar was individually inoculated with ten ml of BHI broth. For 24 hours, broths were incubated at 37°C. The cultures were decanted and phosphate buffer saline rinsed the tubes (pH 7.3). The tubes were 0.1 percent crystal violet, dried and stained. Washed the remaining stain with deionized water. Tubes in an inverted position were dried. A noticeable stained film was seen lining the wall and bottom of the tube during positive biofilm formation. Experiments were performed three times in triplicate and read as absent, weak, moderate and strong. (15).

2.9 The double-species formation of biofilms

Following the method of (16) with some modifications, 1.5 mL Brain heart infusion broth was inoculated with a combination of *S. mutans* and *C. albicans*, with up to 10% fetal bovine serum and 0.2 percent sucrose added to promote hyphal development. The biofilms of two animals were inoculated with a 20-fold diluted night culture of *S. mutans* and *C. albicans* in Brain heart infusion broth containing 10% fetal bovine serum. The culture was then incubated aerobically for 24 hours at 37 ° C. After that, the media was re-freshed every 24 hours before the biofilm was completed in 48 hours. After incubation, the biofilm was treated using a step assay described in Section above (2.7.1). Biofilm formation of *S. mutans*, *C. albicans* and fresh BHI broth were used as controls.

2.10 PH estimation of the medium expended

The pH of biofilms that had been grown for 24 and 48 hours was measured by measuring pH meter at every medium refreshment.

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3. Statistical analysis

The standard deviations of the means were calculated using the Excel analytics program. One-way ANOVA was used to compare the findings statistically employing SPSS version 19. Statistically important differences (P 0.05). For each set of data, the average of six replicate samples was used to determine the effects.

4. Results

Microbial populations on the (NA) and (BHI) agar plates represented different densities for each tooth (Table 1). Varied colony morphologies were observed on the agar plates. A total of 39 isolates were effectively acquired from each culture plate and a microscopic examination determined that 4 yeast and 35 bacterial isolates. Furthermore, the morphological characterisation indicated that the Gr^{+} bacteria were 5 isolates clusters 2 isolates bacilli 3 isolates filament 8 isolates diplococci. For the Gr^{-} bacteria were 9 isolates bacilli 8 isolates coccobacilli (Figure 1). In addition to morphological characterisation the oxidase, catalase tests and growth pattern using differential mannitol salt agar indicated that 5 isolates were *Staphylococcus aureus* 8 isolates were Streptococcus spp.

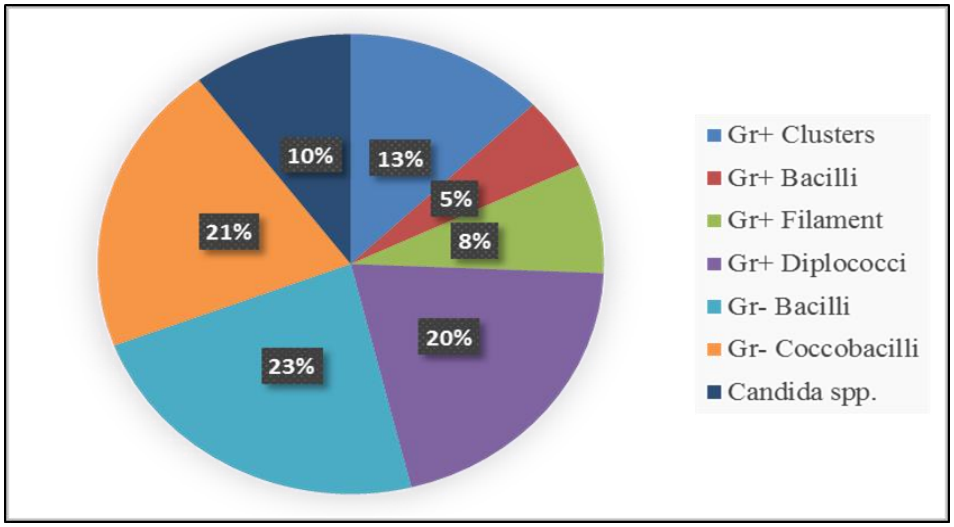

Figure 1: Percentage of microbial isolates from teeth

Table 1: Population densities of bacterial isolate from teeth

Samples	**Total count (CFU/ mL)**	
(tooth/ patient)	**Nutrient agar**	**Brain heart infusion agar**
(A)	7.7×10^6	9.6×10^6
(B)	8.6×10^6	9.8×10^6
(C)	8.7×10^6	7.6×10^6

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(D)	5.9×10^6	9.2×10^6
(E)	7.2×10^6	8.2×10^6
(F)	6.7×10^6	9.5×10^6

Table 2: Profile of antimicrobial resistance of isolates, K= kanamycin (30µg), VA= vancomycin (30µg), AML= amoxycillin (10µg), E= erythromycin (30µg), AMP= ampicillin (10µg), F=Fluconazole (25 µg) and V=voriconazole (1µg).

No		**Gram positive bacteria**				
	Name of Isolates	**Antibiotics**				
	Clusters bacteria	**K 30 µg**	**VA 30 µg**	**AML 10 µg**	**E 30 µg**	**AMP10 µg**
1	T1	R	R	S	R	R
2	T2	R	R	S	R	R
3	T3	R	R	S	R	R
4	T4	R	R	R	R	R
5	T5	R	R	R	R	R
	Bacilli bacteria	R	R	S	R	R
6	T6	R	R	S	R	R
7	T7	R	R	R	R	R
	Filament bacteria	R	R	S	R	R
8	T8	R	R	S	R	R
9	T9	R	R	S	R	R
10	T10	R	S	S	R	R
	Diplococci bacteria	R	R	S	R	R
11	T11	R	R	S	R	R
12	T12	R	S	S	R	R
13	T13	R	R	S	R	R
14	T14	R	R	S	R	R

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15	T15	R	R	I	R	R
16	T16	R	R	S	R	R
17	T17	R	R	S	R	R
18	T18	R	R	S	R	R
Gram Negative Bacteria						
	Bacilli bacteria					
19	T19	R	R	S	R	R
20	T20	R	R	S	R	R
21	T21	R	R	S	R	R
22	T22	R	R	R	R	R
23	T23	R	R	S	R	R
24	T24	R	R	S	S	R
25	T25	R	R	R	R	R
26	T26	R	R	S	R	R
27	T27	R	R	R	R	R
	Coccobacilli bacteria					
28	T28	R	R	S	R	R
29	T29	R	R	S	R	R
30	T30	R	R	S	R	R
31	T31	R	R	R	R	R
32	T32	R	R	R	R	R
33	T33					
34	T34					
35	T35					
Yeast						
Fluconazole (25 µg)					**Voriconazole(1µ)**	

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36	T36	R	I
37	T37	R	R
38	T38	I	R
39	T39	R	R

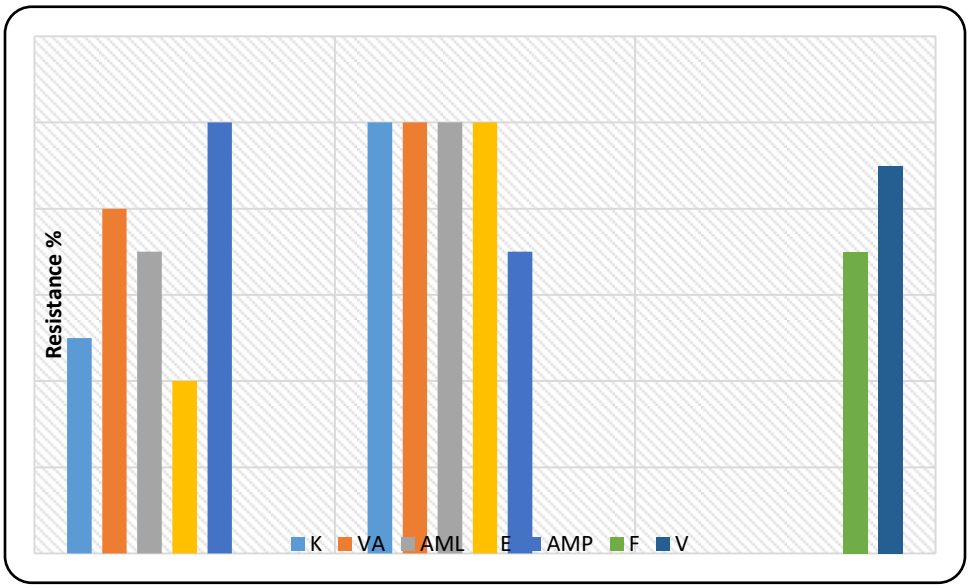

Figure 2: Profile of antimicrobial resistance of isolates, K= kanamycin (30µg), VA= vancomycin (30µg), AML= amoxycillin (10µg), E= erythromycin (30µg), AMP= ampicillin (10µg), F=Fluconazole (25 µg) and V=voriconazole (1µg).

Regarding resistance profile among isolates (Figure 2) (Table 2), resistance to a wide range of beta- and non-beta-lactam antibiotics presented the highest percentages and most of the isolates were multi-drugs resistant.

For quantitative detection of biofilm formation, the findings from tests of the development of biofilms utilising microtiter plates showed that almost all of the isolates have the abilities to form visible growing biofilms when compared to the controls (fresh broth), further analyses were conducted to classify isolates according to strong, moderate, and weak forming of biofilms. Of the 39 biofilm-forming isolates, 20% showed strong forming, 10% moderate forming and 8% showed weak biofilm forming, as illustrated in (Table 2). The three strongest biofilm formation and multi-drug resistance isolates, identified as *Staph. aureus, Streptococcus mutans* and *Candida albicans* were chosen for the development of biofilms utilising pre-sterilised teeth and following assays.

When compared with the controls, the findings of the biofilm development assay using pre-sterilized teeth showed that the selected isolates have a stronger capacity for adhesion than microtiter plate, and that is the first step in the complex process of biofilms on

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various surfaces (fresh broth), though with differences in the degree of adhesions (Figure 3 & 4) (Table 3). The more biofilm formation using microtiter plates and pre-sterilised teeth was by *C. albicans* followed by *Staph. aureus* and *S. mutans.* (Table 4) with significant difference $P \leq 0.05$.

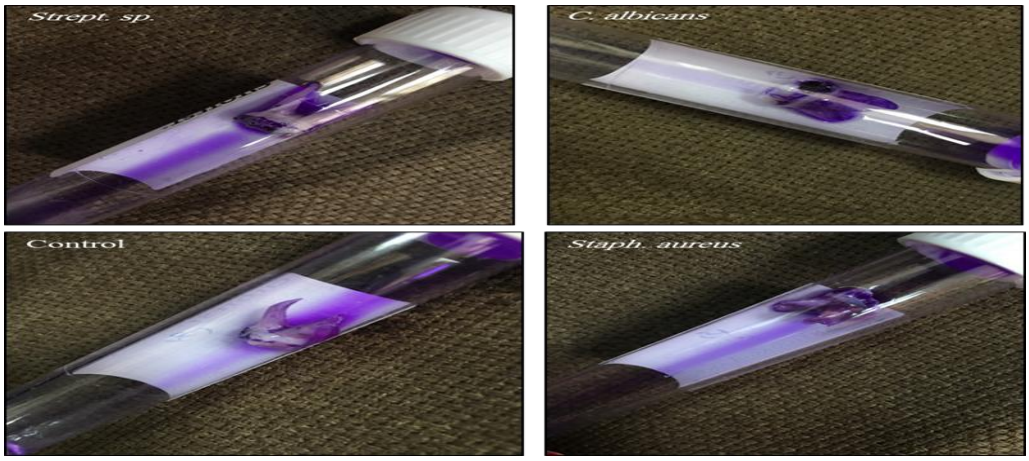

Figure 3: Biofilm formation on teeth surfaces

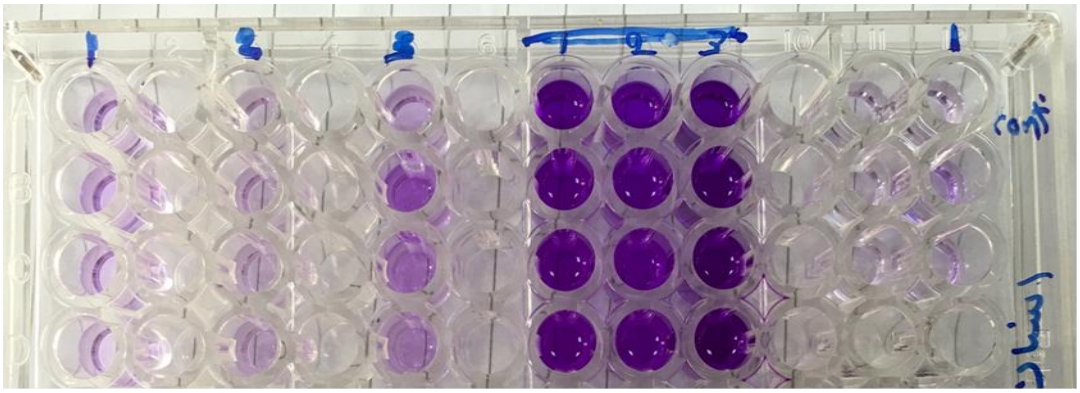

Figure 4: Quantitative detection of biofilm formation of the isolates using MTP and teeth surfaces.1- *Staph. aureus* 2-*Streptococcus mutans* 3- *C. albicans*

Table 3: Evaluation of biofilm formation for the isolates using microtiter plates.

Isolates, (%)			
	Weak Biofilm	**Moderate Biofilm**	**Strong Biofilm**
Isolates (39)	8	10	20

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Table 4: Biofilm development capability on microtiter plates and sterilised teeth surface areas using microtiter-plate reader, Optical Density (OD) at 595 nm. Significant difference $P \leq 0.05$ between biofilm formations of the selected isolates.

Isolates	**Surface type**	**No.**	**Mean ± SD**	**Sig(2-tailed)**	
C. albicans	Microplate	6	0.6590 ± 0.00608	0.000	
	Teeth	6	2.8360 ± 0.05635	0.000	
Staph. aureus	Microplate	6	0.4170 ± 0.00954	0.000	P≤ 0.05
	Teeth	6	2.1247 ± 0.01305	0.000	
S. mutans	Microplate	6	0.42320 ± 0.00844	0.000	
	Teeth	6	1.0050 ± 0.00100	0.000	
Control	Microplate	6	0.0020 ± 0.00100	0.000	
	Teeth	6	0.0030 ± 0.00100	0.000	

Germ tube test is a screening test which is used to differentiate *C. albicans* from other yeast and for pathogenicity. When Candida is grown in human or sheep serum at 37°C for 3 hours, they forms a germ tubes, which can be detected as filamentous outgrowth extending from yeast cells (Figure 5).

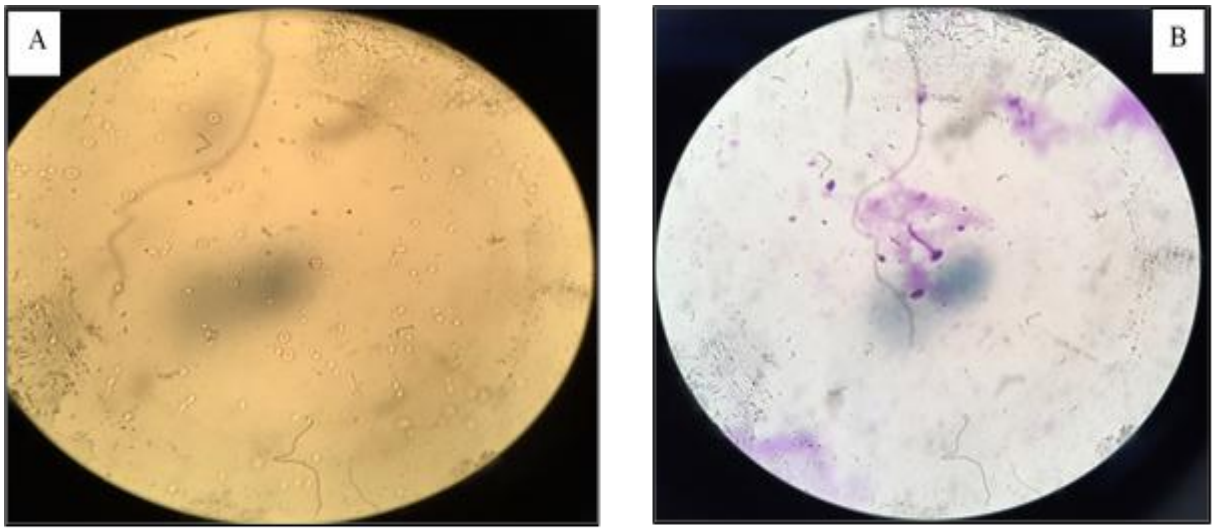

Figure 5: Germ tube test for *C. albicans*: A: germ tube of *C. albicans* , B: crystal violet stained smear of *C. albicans* germ tube.

For Qualitative biofilm detection, the adhesion abilities of the selected isolates on glass

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surface showed different results, *Staph. aureus* was the more biofilm formation (strong) followed by *Strept. mutans* (moderate) and *C. albicans* (weak) (Figure 6).

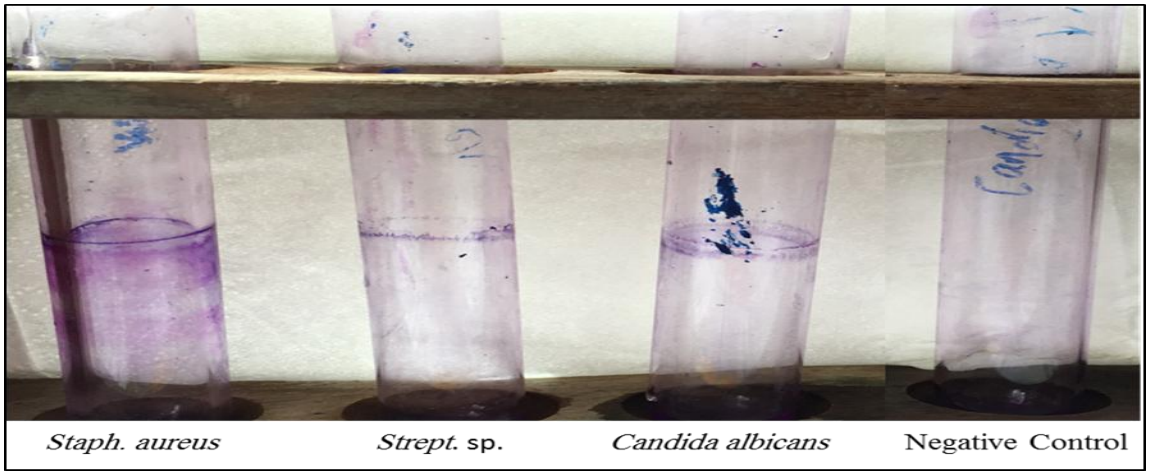

Figure 6: Qualitative biofilm detection: Tubes showing various degrees of biofilm production using crystal violet stain after 24 h of aerobic incubation.

pH of the spent medium

Because acidification is a crucial step in that process to dental demineralization, the pH of the media of the three biofilms (individual biofilms of *S. mutans* and *C. albicans* and the mixed species of biofilm) was determined. On the other hand, *S. mutans* at all time points assessed, acidified environment to a pH of 4.6. The pH of a dual organisms biofilms was similar to that of *S. mutans* biofilms after 24 hours of growth. Nevertheless, after 48 hours, the pH has been less acidic, via a mean pH of 5.7, with significant difference $P \leq 0.05$ (Table 5) that is above the sensitive area of de-mineralization of pH 5.3-5.5. (17).

Table 5: pH value calculated in wasted BHI media of various biofilms, within 24 and 48 h of aerobic incubation, Significant difference $P \leq 0.05$ between mixed biofilms and single-species biofilms (the results were reproduced in 6 experiments).

Time / h	Isolates	No.	Mean ± S. D.	Sig	
24	*C. albicans*	6	7.200 ± 0.1000	0.1000	
	S. mutans	6	4.6000 ± 0.10000	0.0000	
	Mixed biofilm	6	4.5000 ± 0.10000	0.0000	
48	*C. albicans*	6	7.2000 ± 0.10000	0.0354	$P \leq 0.05$
	S. mutans	6	4.5667 ± 0.11547	0.0000	
	Mixed biofilm	6	5.000 ± 0.10000	0.0000	

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	BHI (Control)	6	7.0000 ± 0.0000	0.0001	

5. Discussion

Oral microscopic organisms are indivisibly entwined with sicknesses that will influence each human sooner or later throughout everyday life: gum disease, periodontal maladies and dental caries. Treatment and aversion of these ailments lays principally on the mechanical expulsion of the microscopic organisms. Rather than most other bacterially intervened illnesses, anti-microbial treatment alone may not be viable (18), for the accompanying reasons: (i) oral bacterial ailments are polymicrobial (for example no single life form is the etiological specialist); and (ii) as the pathogens are likewise normally happening individuals from the oral microflora in solid people, reinfection is unavoidable (19). In the present investigation, Microbial populations on the (NA) and (BHI) agar plates represented different densities for each tooth.

Regarding resistance profile among isolates (Figure 2), resistance to a wide range of beta- and non-beta-lactam antibiotics presented the highest percentages and most of the isolates were multidrug resistant, this is similar to other studies (20, 21), it is apparent that all the isolates bacteria and yeast were resistant to the antimicrobials currently used in clinical practice using a diverse number of genetic strategies. The observed increase in antibiotic resistance among isolates could be attributed to the strong biofilm formation ability of these isolates science many studies reported the number of genetic factors known to be involved in biofilm production (22), and could be the ability of the isolates to repertoire of mechanisms of antibiotic resistance including, inhibition of therapeutic agents, drug target alteration, a sophisticated adaptive response of the cell walls that promotes survival in the human host and overexpression of efflux pumps (22).

Resistance mechanisms are typically based on slow absorption of the antibacterial agents, shifts in the growth rate of microbes or other physiological changes associated with biofilm production. (22). Yeast isolates was found resistant to both voriconazole and fluconazole, these resistant has also been reported by others (23, 24) for *Candida* spp. isolates from patients admitted to intensive care medical units.

Microbial biofilm has long been regarded as a virulence factor that contributes to numerous health device-related infections and causes nosocomial infections. (25). All the isolates showed the ability of producing biofilm on microtiter plate surface with different in the adhesions (strong, moderate and weak) (Table 3). Biofilm production clearly increases bacterial resistance, its infections are characterized by persisting and progressive and play a major role in microorganism colonization during infection, providing an opportunity for bacteria to develop drug resistance. For quantitative detection of biofilm formation, the more biofilm formation using microtiter plates and pre-sterilised teeth was by *C. albicans* followed by *Staph. aureus* and *Strept*. *mutans* and that the selected isolates possess a strongest capacity of adhesion to teeth than microtiter plate surface. These data clearly showed the delay in the initiation or onset of biofilm formation on polystyrene microtiter plates compared to teeth. This finding may relate to

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differences in adsorption patterns and adhesion therefore, such as substratum-dependent conformational changes, seem to account for the effect. Also, although that microtiter plate being hydrophilic, the selected isolates were adherent easier on biotic surface than on abiotic surface. Our findings for the ability of biofilm formation corroborate with the findings of (26) and (27) for the bacterial isolates and (28) for yeast isolate.

The creation of germ tubes plays a major role in pathogenesis and the production of plaque biofilms for pathogens. Consequently, interactions between *C. albicans* and microorganisms can occur at chemical, physical and metabolic levels. (29). Physical contact, such as binding, occurs with the hyphaea in certain cases (30). Hyphae provides bonding places for various microorganisms that interact with *C. albicans*. It may be natural that aerobic incubation would prevent the colonization of anaerobic microscopic species. In any case, this didn't occur within the sight of *C. albicans*. Furthermore arrangement of hyphae give anaerobic conditions to presence strict anaerobic bacteria (31).

For Qualitative biofilm recognition, the bond capacities of the chosen isolates on glass surface demonstrated different outcomes, *Staph. aureus* was the more biofilm formation (strong) followed by *Strept*. *mutans* (moderate) and *C. albicans* (weak). Apart from providing extended surface for initial adhesion, a rough surface also increases the surface area available for bacteria accumulation compared to a smooth surface. It was found both *in vivo* and *in vitro* that bacteria accumulated to a greater degree on rough surfaces than on a highly polished surface (32).

There were some studies describing *C. albicans* as an organism able to synergize the initiation and development of caries induced by *S. mutans* (33) (6). An alternatives perspective is shared by this in vitro biofilm analysis. The results of this study demonstrating that the presence of *C. albicans* in a dual biofilm with *S. mutans* does not contribute to strengthening the cariogenic capability in term of acidity, presence of *C. albicans* tend to lower the biofilm's cariogenic ability. We noticed that, probably because of the presence of *C. abicans*, mixed biofilms become less acidic over time. The calculated pH stays above the crucial demineralization area of 5.3–5.5 after 72 hours of medium in the presence of *C. albicans*. This indicates that *C. albicans* has a significant effect at pH rates. Since continued exposure to low pH levels lead to cavity formation, this information could suggest that *C. albicans* play a constructive role in the cavity prevention process. Within growing mixed biofilms, lactic acid production has increased, indicating that *C. albicans* does not inhibit the production of acid through *S. mutans*, After all, it is logical to assume, that *C. albicans*, independently of *S. mutans*, influence pH. in line with recognized metabolic processes, *C. albicans* lack L-lactate dehydrogenase, the enzyme needed to convert lactic acid to pyruvate. The final product of the metabolization of sucrose by *C. albicans* are ethanol. However, ethanol does not influence the medium's pH. Even, under hypoxic conditions, lactic acid seems to be the most important source of carbon for the fungus. As for lactic acid, supplied by *S. mutans*, exist in the media of growth, *C. albicans* might use lactic acid as an energy source. Saccharolytic bacteria are thought to rapidly transform all stored sucrose to lactic acid, causing *C. albicans* depend on lactic acid as its source of carbon. The resulting

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 5545 - 5562
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decrease in acids by *C. albicans* lead to less acidic conditions in the process.

6. Conclusions

It was concluded that almost all the isolates from patients attending Dental Clinic, Health Clinic Centre Mosul University, can develop observable growing biofilms on the different types of surfaces and the strongest was using teeth surface compared to the controls, with variations in the degree of adhesions (strong, moderate and weak). The findings revealed that antimicrobial resistance of bacteria isolated from plaque is associated with biofilm formation. The selected isolates were adherent easier on biotic surface than on abiotic surface. This in vitro analysis shows, in safe oral environments, *C.albicans* should not be ignored, since they have the ability to greatly influence bacterial plaque. Further, the study revealed that, the oral *C. albicans* isolates were able to adhere to materials and biomaterials with different degree. In overview, biofilms of dual species show strong variations in terms of as compared to both single-species biofilms, lactic acid-producing ability and pH. Such results indicate that *C. albicans*, in this dual-species biofilm, are not by definition a cariogenic organism. The existence, however, of *C. albicans* and in the *S. mutans* biofilm tends to reduce the acidity of the cariogenic capacity of the biofilm.

7. Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8. Acknowledgment

The author would like to thank the University of Mosul for supporting this research. We also acknowledge the Biology department and Iraqi Ministry of Higher Education and Scientific Research for providing the tools.

9. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

10. Conflict of Interest

The author have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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