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Dental-plaque based bacterial profiling from adults and children in Al-Diwaniyah Province, Iraq

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Abstract

Background: Dental plaque, also known as dental biofilm, is a pale-yellow sticky film that is generated on teeth due to the activity of biofilm-forming bacteria. This dental health issue can frequently occur, especially in individuals with low tooth hygiene. **Objectives:** The current study was performed to understand the bacterial profile of dental plaque from adults and children in Al-Diwaniyah Province, Iraq. **Materials and methods:** Sixty dental-plaque samples were collected from 30 adults (18-56 years old) and 30 children (6-14 years old). Regular techniques (cultivation, colony morphology, and VITEK 2) were applied to identify the bacterial members in each sample. **Results:** The results uncovered that Gram-positive bacteria were dominant, in which *Streptococcus*spp was highly prevalent. *Lactobacillus*spp showed a second-place prevalence, while *Staphylococcus*spp unveiled less existence of Gram-positive isolates in the dental-plaque samples of all individual ages. For the Gram-negative bacteria, *Enterobacter*spp, *Enterococcus* spp, and *Klebsiellaaerogenes*were the most frequent bacterial isolates of the

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dental biofilm of all participant ages. **Conclusion:** The study informs the dominant presence of *Streptococcus*spp, *Lactobacillus*spp, and *Staphylococcus*spp in the dental-plaque samples of adults and children. This profile might indicate that the oral cavity is a suitable habitat for those bacteria to form the dental biofilm.

Keywords: Bacterial profiling, dental biofilm, dental plaque, tooth plaque.

Introduction

The oral cavity comprises hundreds of microbial organisms, either planktonic or biofilm-integrated. Mainly commensal bacteria are oral microbiota. Pathogenic bacteria can contribute to oral infections, which may contribute to medical conditions on occasions. The management is difficult for biofilms carrying pathogens, in which many typical antimicrobials proved ineffectual. Recent advancements in science and technology provide new techniques for pathogen management, containment, and biofilm characterization processes (1). Via biofilms creation, microbes have established a novel survival technique. Many microorganisms develop from the planktonic state to build complex matrix-like structures called biofilms, in which microorganisms live together as communities. Biofilms are thick micro-communities initiated on inactive surfaces and embrace deposited polymers. Modification in the gene expression profiles occurs due to biofilm formation as a response and its evolving environment. The systemic regulation of biofilms and the resulting alteration in gene expression can help to defend the microbial members against the disinfectants or antibiotic agents. A significant public health

concern can emerge from the eventual biofilm (2,3,18).

Diverse biofilms can be found in the oral cavity. The oral cavity is particular in terms of its microbial diversity, and up to 1000 various organism species are endorsed. Natural teeth and oral prostheses such as dentures and implants can be potential biofilm substrates. The aerobic tooth surface can turn into an anerobic environment to eventually be colonized by specific bacteria. Dental plaque can induce oral infections, including dental caries and periodontal disease. Plaque comprises several microorganisms that are integrated into the formation and secretion of extracellular polymers (4–6, 17). Plaque may also form between teeth and gingival crevice, which makes its removal much tougher. Therapeutic agents are not an easy way to treat infected spots in which antibiotic substances can not permeate to those locations. In the subgingival crevice, the significant component of nutrients for the growth of the plaques is the gingival fluids, in which the gingival fluids supply proteins and glycoproteins. Proteolytic enzymes, which can directly destroy the soft and hard tissues, are released when dental plaque is made. Additionally, the host defense mechanisms at the biofilm sites can be clashed and disrupted (7).

The current study was performed to understand the bacterial profile of dental plaque from adults and children in Al-Diwaniyah Province, Iraq, due to the importance of dental biofilms in the induction of dental and oral diseases.

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Materials and methods

Samples

Sixty dental-plaque samples were collected from 30 adults (18-56 years old) and 30 children (6-14 years old) using toothpicks. The samples were collected during the year 2020 from hospitals, dental clinics, and healthcare centers in Al-Diwaniyah Province, Iraq. Later, the collected dental plaques were transported to the microbiological Lab, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq, where they were subjected to the required study techniques.

Sample preparation and bacterial isolation

An amount of the dental-plaque sample was placed in a plastic dish. Then, the amount was diluted in the plastic dish by adding a phosphate buffer solution at 10ml and mixed-crushed until it was utterly homogenized.

The cultivation was performed using 0.1ml of the final working solution and by the agar diffusion method, including brain heart infusion (BHI) agar, blood agar, mannitol salt agar, and MacConkey agar. The cultivated plates were incubated for 24-48hrs under aerobic and jar-based microaerophilic conditions at 37˚C. Following the incubation, the morphological characteristics of the colonies were identified per methods mentioned by Blau*et al*. (8), and the

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identification was confirmed using VITEK2 cards (bioMérieux, Inc., North Carolina, USA) and following methods described by Olowe *et al*. (9).

Results

The results uncovered that Gram-positive bacteria were dominant, in which *Streptococcus*spp was highly prevalent. *Lactobacillus*spp showed a second-place prevalence, while *Staphylococcus*spp unveiled less existence of Gram-positive isolates in the dental-plaque samples of all individual ages. For the Gram-negative bacteria, *Enterobacter*spp, *Enterococcus* spp, and *Klebsiellaaerogenes*were the most frequent bacterial isolates of the dental biofilm of all participant ages. Other bacterial members, such as *Bacillus vallismortis*, *B*. *subtilis*, *Staph*. *epidermidis*, *E*. *faecalis*, and *E*. *saigonensis*. Figure (1) shows the colony morphological features on the blood agar plate.

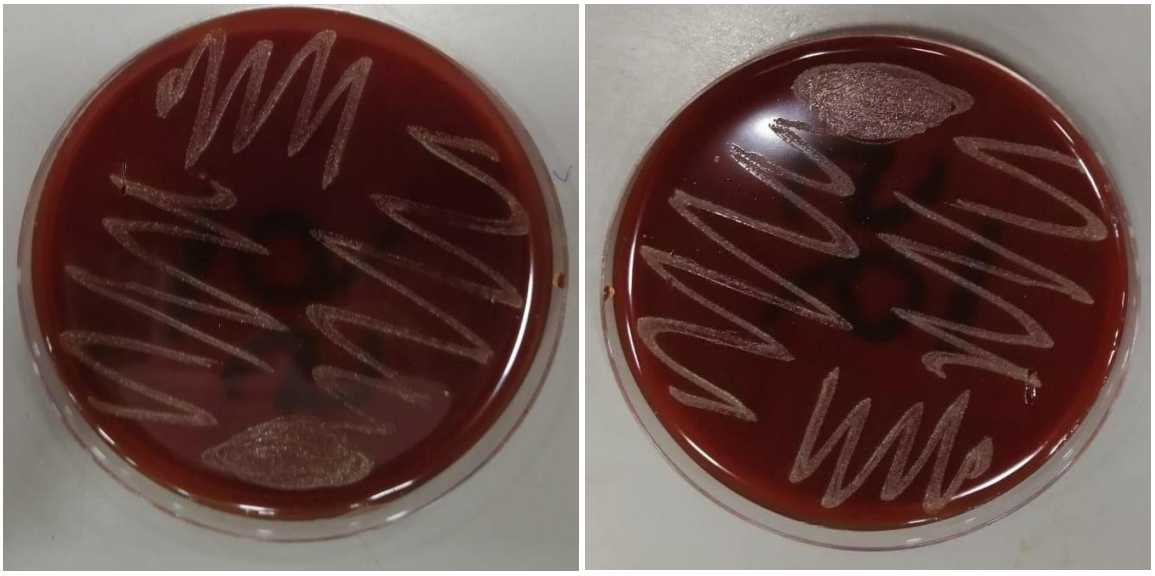

Figure 1: Blood agar based colonies of bacteria isolated from dental plaque.

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2	AMY	_	4	PIPLC	_	5	dXYL	_	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	+	14	CDEX	_	15	AspA	_	16	BGAR	+	17	AMAN	_	19	PHOS	_
20	LeuA	+	23	ProA	+	24	BGURr	_	25	AGAL	+	26	PyrA	_	27	BGUR	_
28	AlaA	+	29	TyrA	+	30	dSOR	_	31	URE	_	32	POLYB	+	37	dGAL	+
38	dRIB	_	39	ILATK	_	42	LAC	+	44	NAG	_	45	dMAL	+	46	BACI	_
47	NOVO	+	50	NC6.5	_	52	dMAN	_	53	dMNE	+	54	MBdG	_	56	PUL	_
57	dRAF	+	58	O129R	_	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	_
64	OPTO	+															

For the findings of the VITEK 2(table 1, 2, and 3) displays examples for the S. aureus, S. parasanguinis, and L. raffinolactis, respectively.

Table 1: Examples of the VITEK 2 results. A. S. aureus.

Table 2: Examples of the VITEK 2 results. *S. parasanguinis*.

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2	AMY	_	4	PIPLC	_	5	dXYL	_	8	ADH1	_	9	BGAL	_	11	AGLU	+
13	APPA	+	14	CDEX	_	15	AspA	_	16	BGAR	_	17	AMAN	_	19	PHOS	_
20	LeuA	+	23	ProA	_	24	BGURr	_	25	AGAL	+	26	PyrA	_	27	BGUR	_
28	AlaA	+	29	TyrA	+	30	dSOR	_	31	URE	_	32	POLYB	+	37	dGAL	+
38	dRIB	_	39	ILATK	_	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	_	52	dMAN	_	53	dMNE	+	54	MBdG	_	56	PUL	_
57	dRAF	+	58	O129R	_	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	_
64	OPTO	+															

2	AMY	_	4	PIPLC	_	5	dXYL	_	8	ADH1	+	9	BGAL	_	11	AGLU	_
13	APPA	_	14	CDEX	_	15	AspA	_	16	BGAR	_	17	AMAN	_	19	PHOS	_
20	LeuA	_	23	ProA	_	24	BGURr	_	25	AGAL	_	26	PyrA	+	27	BGUR	_
28	AlaA	_	29	TyrA	_	30	dSOR	_	31	URE	_	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATK	+	42	LAC	+	44	NAG	(+)	45	dMAL	+	46	BACI	+
47	NOVO	_	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	_
57	dRAF	_	58	O129R	+	59	SAL	_	60	SAC	+	62	dTRE	+	63	ADH2s	_
64	OPTO	+															

Table 3: Examples of the VITEK 2 results. L. raffinolactis.

Discussion

Dental plaque is a dental biofilm present in the oral cavity on dental surfaces that has anaerobic conditions. This biofilm has a wide range of organisms and composed of deeply

bound bacteria in a mixture of microbial and salivary natural materials. Sugar and time can help dental plaque to cause dental caries. Dental plaque is comparable to the sequences of biofilms in other natural ecosystems (10).

The findings of the present work identified the dominance of groups of Gram-positive and Gram-negative bacteria from dental plaques of adults and children. These results agree even with those from advanced techniques, such as polymerase chain reaction (PCR) and next generation sequencing. These studies demonstrated the high degree of species richness of both healthy and disease-related bacterial oral populations. A study that focussed on the establishments of extreme early childhood caries interactions using the 454-sequencing tool provided taxa correlated with caries. PCR and those advanced tools have shown the occurrence of *S. Bifidobacteriaceae spp.*, *S. mutans*,*Abiotrophiadefectiva*, *Capnocytophagagingivalis*,*Lachnospiraceae spp.*, *Streptococcus cristatus, and S. sanguinis*. In many studies of the oral bacterial profile, *Streptococcus* species were the dominant isolates (11–15).

In a study performed in Sudan by Ali *et al*. (16) to detect the microbial profile of dental plaque in children with congenital heart defects found that these children had high levels of *S*. *sanguinis* and *L*. *acidophilus*. The high levels of *Streptococci* and *lactobacilli* of the current study agree with those from (16).

The formation of the biofilms in the oral cavity can be seen in various parts of this compartment, such as tooth surface structures, gingiva, mucosa, implants, and dentures. The

dental plaque can extend to reach the root surface leading to the rapid growth of different bacterial species. The morphological features of dental plaque can take various shapes and organization according to the location. Factors, including the pH of the oral niches, the existence of nutrients, exposure to antimicrobial substances, and host defense mechanisms, can determine the generation of dental plaque (10).

Conclusion

The study informs the dominant presence of *Streptococcus*spp, *Lactobacillus*spp, and *Staphylococcus*spp in the dental-plaque samples of adults and children. This profile might indicate that the oral cavity is a suitable habitat for those bacteria to form the dental biofilm.

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