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Khat Chewing Induces a Floral Shift in Dental Material-Associated Microbiota: A Preliminary Study

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Background: The habit of khat (*Catha Edulis Forskal*) chewing is widely practiced in the southern regions of Saudi Arabia, Yemen, and East Africa. This social habit has tremendous effects on oral and general health of khat chewers. Khat may affect bacterial species in plaque biofilms on oral rehabilitation materials. This preliminary case-controlled study aimed to assess and compare the effect of khat chewing on bacterial biodiversity between non-khat chewers (NKC) and khat chewers (KC) in oral biofilms on oral rehabilitation materials using polymerase chain reaction (PCR).

Material/Methods: Fifty participants were organized into 2 equal groups of NKC and KC, each containing 5 subgroups related to filling material type. Some participants had amalgam (A) or composite (C) restorations, while others had feldspathic porcelain (FP), nickel chromium (NC), and zirconia ceramic (ZC) crowns or bridges. Oral biofilm samples were collected from all participants, DNA was extracted, and samples were subjected to PCR. Bacterial species were then identified and counted. PCR products were sequenced to detect similarity. Partial 16S rRNA gene sequences of the current study samples were compared with 16S rRNA gene sequences from GenBank using BLAST on the National Center for Biotechnology Information website.

Results: The *Streptococcus* sp. was the most common bacterial species among our participants (40; 80%), followed by *Lactobacillus* and *Veillonella* spp., accounting for 12% (6) and 8% (4), respectively. *Streptococcus* sp. was observed equally among NKC and KC, but *Lactobacillus* and *Veillonella* spp. were higher in KC and NKC, respectively. *Lactobacillus* was associated mainly with prosthetic materials, and *Streptococcus* was found among all examined dental restorative materials.

Conclusions: This research concluded that khat chewing significantly affects bacterial biodiversity in oral biofilms in the presence of different restorative and prosthetic dental materials.

MeSH Keywords: Biofilms • Catha • Dental Materials • RNA, Ribosomal, 16S

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Background

Bacterial dental biofilms are complex 3D structures in which bacteria are embedded in a matrix composed primarily of exopolysaccharides. These biofilms may be found on hard and soft tissues of the oral cavity too and are associated with dental caries and periodontal diseases; hence, various biomaterials are used for oral rehabilitation [1]. All biofilms have extracellular polysaccharide matrices [2]. *Streptococcus* sp. virulence is due to the species' superior capability to produce an insoluble polymer matrix with a high affinity for solid surfaces [3]. Many other endogenous microbes, such as *Lactobacillus* sp. and *Veillonella* sp., persist in oral cavity dental plaque biofilms; these microbes are potent acid producers and are acid tolerant, and they ferment carbohydrates and produce weak organic acids as byproducts [4–7]. However, *Streptococcus* sp. can assemble an insoluble polysaccharide matrix [8–10]. This matrix decreases the local pH to below the critical level, resulting in tooth demineralization and secondary caries [5,7,11]. These organisms are found mostly along crown margins and on the surfaces of different types of fillings that harbor microbial colonization [12]. Therefore, streptococcal biofilms on the surfaces of restorative and prosthetic dental materials are important for the attachment of other microorganisms that cause recurrent caries [8,11].

Khat (also qat; *Catha edulis*) chewing is a popular habit in many regions, especially in Jazan Province, Saudi Arabia. People often chew khat for more than 5 hours daily because of its amphetamine-like effects. Khat chewing is associated with salivary gland enlargement, inflammation, and xerostomia [13,14]. However, in the literature no information is available on the possible effects of khat chewing habit on salivary parameters. This unhealthy social habit has recently appeared in North America and Europe, particularly among immigrants and refugees [14]. Khat chewing has many deleterious effects on oral tissues in addition to the sympathomimetic and euphoric effects on those who chew it [14,15]. Khat can also affect salivary properties and consequently contribute to oral biofilm formation, thus contributing to recurrent dental caries [13,15].

Previous *in vivo* studies revealed a benefit of khat chewing on oral cavity health. The pathogenic burden is reduced among khat chewers, as evidenced by relatively small bacterial proportions and oral biofilm counts from healthy and periodontitis sites [16,17]. These findings are consistent with those of previous results indicating that khat has a prebiotic-like effect and selective antimicrobial properties against major oral pathogens. Khat produces bacterial shifts that are compatible with periodontal health and foster the growth of several health-compatible species [18].

The surface characteristics, morphology, particle size, polymerization degree, and survival period of the dental materials and bacterial strains enable determining the number of adhered bacterial colonies versus bacteria showing mere attachment. Materials used in oral rehabilitation are expected to have high surface roughness, which facilitates bacterial attachment and position retention [19].

Motevasselian et al. [20] examined *Streptococcus* sp. adhesion on amalgam and different composites types with different polishing protocols. These authors found no significant differences between the examined restorative materials in terms of the adhered colony rate. Cazzaniga et al. [21] reported that the finishing and polishing of different composite resin types can significantly alter a composite but does not remarkably affect biofilm formation.

Viitaniemi et al. [22] found no difference in young biofilm formation on fully or partially polished zirconia ceramic (ZC) materials. However, low plaque accumulation with reduced microbial vitality on dental ceramics was recorded in the interdental space. Shemesh et al. [23], reported that a thick *streptococcal* biofilm formed after 3 days. Laosuwan et al. [24], found no differences in the adhesive growth of supragingival streptococci to enamel or titanium alloy miniscrews. Georgiev et al. [25] established a novel clinical approach to investigate biofilm formation on composite resin overlay surfaces after 72 hours, one week, and 2 weeks. These authors detected a multilayered biofilm covering the proximal surfaces at the junction between the composite resin and tooth structure. In addition, these authors identified a mature biofilm at the gingival margin of the prostheses.

No previous studies have explored the effect of local khat chewing habits on oral biofilm formation on prosthetic and restorative materials. Hence, this preliminary study assessed the effect of khat on bacterial biodiversity, specifically on that of *Streptococcus*, *Lactobacillus*, and *Veillonella* species, in the plaque biofilms on different dental materials used in oral rehabilitation.

Material and Methods

Study participants and grouping

This preliminary case-controlled study was conducted among patients who reported to the outpatient section of the examination unit of the Faculty of Dentistry, Jazan University. Data were collected from October 2017 to May 2018. The Ethics Committee at the Deanship of Research of Jazan University approved the study. All participants signed informed consent forms after having been informed about the study's objectives,

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procedures, and safety, and the confidentiality of the collected data. The study was performed in accordance with the ethical principles of the Declaration of Helsinki.

The sample size consisted of 50 participants who were divided into 2 equal groups (25 NKC and 25 KC), consisting of 33 males and 17 females. Each group was further divided into 5 subgroups according to the restorative materials used. The first group, non-khat chewers (NKCs), consisted of 5 subgroups with 5 participants each. Some participants had amalgam (A), composite (C) restorations, while others had feldspathic porcelain (FP), nickel chromium (NC), and zircon ceramic (ZC) crowns or bridges. The second group, khat chewers (KCs), also consisted of 5 similar subgroups with 5 participants each. The participants had amalgam (A-K), and composite (C-K) restorations, while others had feldspathic porcelain (FP-K), nickel chromium (NC-K), and zirconia ceramic (ZC-K) crowns or bridges.

Inclusion and exclusion criteria

Participants were included in the current study if they fulfilled the previously reported inclusion criteria [3,13,25–30], as follows: participants from both genders, 18 years or older; with one type of filling or prostheses on the khat chewing side, minimum khat chewing time of twice weekly for 3 years or more [13,31]; ≥20 remaining teeth; good oral and dental health and under no treatment with any topical or systemic antibiotics, anti-inflammatory drugs, or corticosteroids within 3–6 months prior to the study; and no systemic or salivary gland disease that can affect salivary flow. Participants were asked not to eat 2 hours before sampling. Participants with dental oral lesions or infections or any registered periodontal pocket were excluded from the study.

Sample collection and DNA extraction

Biofilm materials were sampled using sterile absorbent paper points (size 80; Suredent Corporation, Korea). Amalgam and composite (A, C) restorative-material biofilm samples were collected with paper points from the occlusal surfaces of the participant's teeth at the junction between the restoration and tooth surfaces. Samples for the prosthetic materials (FP, NC, and ZC) were collected from the buccal surfaces at the finish point on the cervical area of the crown or bridge and the surface on the khat chewing side. All samples were collected from posterior areas (premolar and molar areas only) to prevent the tongue or cheek from affecting the buffer flow [32]. Samples for each case were obtained from 2 sites and stored in Tris-EDTA (TE) buffer (Invitrogen, Carlsbad, CA, USA) at 20°C for subsequent laboratory analysis.

DNA extraction for 16S ribosomal DNA (rDNA) analysis

Before DNA extraction, samples were thawed and centrifuged at 15 000 g for 1 min. Next, the resultant pellet from each sample was suspended in 180 µL lysozyme digestion buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) containing 20 mg/mL lysozyme and incubated at 37°C overnight. The DNA was finally purified from the digestion using a Purelink Genomic DNA extraction kit (Invitrogen, Carlsbad, CA, USA), eluted in 100 µL of the supplied buffer, and stored at –80°C [16–18].

DNA amplification

PCR experiments were performed under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 60 s, annealing at 60°C for 60 s, extension at 72°C for 120 s, and a final extension for 5 min. PCR experiments were carried out in a total reaction volume of 25 µL, each containing 12.5 µL master mix and 25 µM of each primer. The 16S ribosomal RNA gene sequences were amplified by PCR using universal primers 27F and 1492R [8,33], and the primer sequences were 27F, 5'-AGA GTT TGA TCM TGG CTC AG-3' and 1492R, 5'-TAC GGY TAC CTT GTT ACG ACTT-3'. PCR products were analyzed via gel electrophoresis and stained with ethidium bromide. A gel documentation system was used for visualization.

Sequencing and information analysis

PCR products were sequenced by Microgen Company (Seoul, Korea). Sequencing reactions of the samples were performed in an MJ Research PTC-225 Peltier Thermal Cycler using ABI PRISM® Big Dye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems) according to the manufacturer's instructions. The 27F and 1492R primers were used to sequence both strands (at least 1000 bp), and fluorescence-labeled fragments were purified from the unincorporated terminators following the Big Dye®X Terminator™ purification protocol.

Bacterial identification

Samples were suspended in deionized water for electrophoresis in a sequencer, which searched for sequence similarity. We compared 16S rRNA gene sequences with 16S rRNA gene sequences from GenBank using the BLAST search program on the National Center for Biotechnology Information website [33].

Statistical analysis

Microbiological data included the log-transformed absolute counts and relative counts (% total bacteria) of each tested

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Table 1. Participant demographic data.

Parameter	Group type		Gender		Chewing khat		Smoking		Using oral hygiene aids		
	NKC	KC	Male	Female	Yes (M: F)	No	Yes	No	Toothbrush	Miswak	No
Number	25	25	33	17	25 (15: 10)	25	35	15	24	11	15
Percentage	50	50	66	34	50 (30: 20)	50	70	30	48	22	30
Mean	1.42		1.34		.480		.700		.920	.425	–
St. deviation	.498		.478		.505		.463		.724	.348	–
P value	.050*		0.551		1.000		.746		.373	1.000	0.533

* Significant.

Table 2. Frequency and percentage of total bacterial type with group type (NKC and KC).

Bacteria type	Group type				Total N (%)		P value
	Non-khat chewer N (%)		Khat chewer N (%)				
Streptococcus sp.	19	(76%)	21	(84%)	40	(80%)	0.042*
Lactobacillus sp.	2	(8%)	4	(16%)	6	(12%)	
Veillonella sp.	4	(16%)	0	(0%)	4	(8%)	
Total	25	(50%)	25	(50%)	50	(100%)	

* Significant.

species/phenotype. Questionnaire data were pooled for analysis. The IBM Statistical Package for the Social Sciences V 20.1 (SPSS IBM, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive analyses that included the frequency and percentage were performed for all parameters. Different parameters were compared using the chi-square/Fisher's exact test. Statistical significance was set at p>0.05.

Results

The frequency and percentage of KC were 15 (30%) and 10 (20%) for males and females, respectively. Participants' ages were between 20 and 55 years. The mean age was 33.54 years. Among the examined participants, 35 (70%) were smokers, 24 (48%) were regular toothbrush users, 11 (22%) were miswak (a traditional tooth-cleaning stick) users, and 15 (30%) did not use any oral hygiene instrument. No significant differences in these variables were detected with Fisher's exact test, and the p values were ≥0.050 (Table 1).

The results in Table 2 illustrate the presence of many isolated bacterial species, such as *Streptococcus* sp. with a frequency and percentage of 40 (80%), followed by *Lactobacillus* sp. and *Veillonella* Sp. with frequencies and percentages of 6 (12%) and 4 (8%), respectively. The frequency and percentage of *Streptococcus* spp. were 19 (76%) among NKC and 21 (84.0%) among KC. *Lactobacillus* sp. was high among KC (4 samples out of 6), and *Veillonella* sp. accounted for 4 samples among NKC. The p value was significant at 0.42 among the bacterial and group type's variables.

The frequency and percentage of amalgam and feldspathic porcelain dental material samples were the highest in *Streptococcus* sp. with 10 (25%) samples for each, followed by composite and nickel chromium with 8 (20%) samples for each. Zirconia ceramic dental material was the lowest with 4 (10%) only. *Lactobacillus* sp. were 5 (83.3%) in ZC and 1 (16.7%) in NC, while the *Veillonella* sp was higher among composite with 2 (50%) and 1 (25%) for both NC and ZC. The p value was significant at 0.005 (Table 3).

The association between group types with smoking, brushing, or miswak users is shown in Table 4. The smoking participants were higher in frequency and percentages with 16 (64%) and 19 (76%) among the NKC and KC group, respectively. The non- brush and non-miswak users were the higher in frequency and percentage with 17 (68%) and 21 (84%) among the NKCs group. In the KC group, the non-miswak had 18 (72%) and the brushes users had 16 (64%), respectively. All of the variables showed no significant differences except in using the brush, in which the p value was 0.046. Also, the association between the *Streptococcus* sp, smoking, non-brushes, and non-miswak participants were the highest in the

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Table 3. Frequency and percentage of different dental materials and bacterial type (Fishers exact test).

Dental material/ bacterial type	Amalgam N%	Composite N%	Feldspathic porcelain N%	Nickel chromium N%	Zirconia ceramic N%	Total N%	P value
Streptococcus sp.	10 (25.0%)	8 (20.0%)	10 (25.0%)	8 (20.0%)	4 (10.0%)	40 (80.0%)	0.005*
Lactobacillus sp.	0 (0.00%)	0 (00.0%)	0 (00.0%)	1 (16.7%)	5 (83.3%)	6 (12.0%)	
Veillonella sp.	0 (0.00%)	2 (50.0%)	0 (00.0%)	1 (25.0%)	1 (25.0%)	4 (8.0%)	
Total	10	10	10	10	10	50 (100%)	

* Significant.

Table 4. Association between group types and bacterial types with smoking, using brushes, or Miswak (Fisher's exact test).

	Non-smoking N (%)	Smoking N (%)	Non-brush using N (%)	Brush using N (%)	Non-miswak using N (%)	Miswak using N (%)	Total N%
Group type							
Non-khat chewer	9 (36%)	16 (64%)	17 (68%)	8 (32%)	21 (84%)	4 (16%)	25 (50%)
Khat chewer	6 (24%)	19 (76%)	9 (36%)	16 (64%)	18 (72%)	7 (28%)	25 (50%)
Total	15	35	26	24	39	11	50 (100%)
P value	0.538		0.046*		0.496		
Bacteria type							
Streptococcus sp.	12 (30%)	28 (70%)	21 (52.5%)	19 (47.5%)	32 (80%)	8 (20%)	40 (80%)
Lactobacillus sp.	1 (16.7%)	5 (83.3%)	2 (33.3%)	4 (66.7%)	4 (66.7%)	2 (33.3%)	12 (16%)
Veillonella sp.	2 (50%)	2 (50%)	3 (75%)	1 (25%)	3 (75%)	1 (25%)	4 (8%)
Total	15	35	26	24	39	11	50 (100%)
P value	0.530		0.418		0.750		

* Significant.

frequency and percentages, with 28 (70%), 21 (52.5%), and 32 (80%), respectively. None of the variables were significantly different, with P value ≤0.50 (Table 4).

Sequencing phylogenetic analysis

Bacterial identity was accomplished mainly via DNA sequence analysis. To confirm the serotype-specific results, we performed partial sequencing on 50 PCR product samples representing positive bacterial species. The sequences and identity of *Streptococcus* sp., *Veillonella* sp., and *Lactobacillus* sp. and their strains detected among our samples reached 98%, 99%, and 100%, with scores of 1881, 1095, and 2034 bits, respectively. The BLAST search demonstrated that the sequences and identity of our samples agreed with other published sequences of bacteria, as shown in (Figure 1), which provides the GenBank accession numbers and the country of isolates.

Discussion

The objectives of this case-controlled study were to conduct preliminary evaluation on the effect of khat chewing on the bacterial flora and biofilm formation on different types of dental materials (A, C, FP, NC, and ZC) used for oral rehabilitation in the oral cavity.

Oral biofilm and periodontal parameters are reported to be influenced by all the appliances that are in contact with mucosa for many hours per day, such as prosthodontic frameworks [34] and orthodontic appliances [35] or continuous chewing of substances [36].

The dental restorative biomaterials used for oral rehabilitation create different junctions between the tooth and restoration or prosthesis [11,25]. This junction affects the biofilm composition in many ways, such as in the steps, grooves, and gaps formed between the restoration and the tooth surfaces. These

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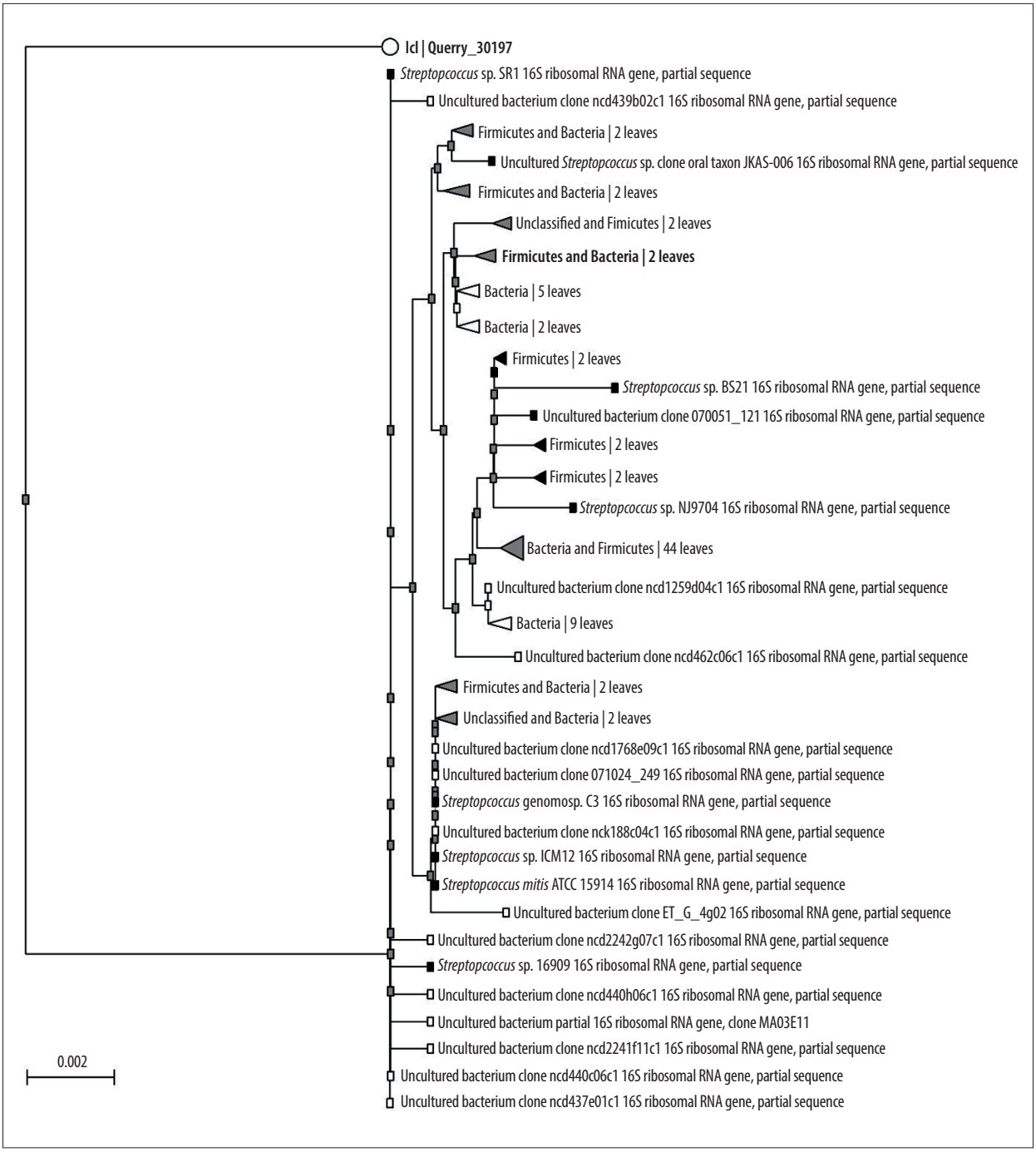

Figure 1. Similarity tree of *Streptococcus* sp. Identities of *Streptococcus* sp. from Jazan and other *Streptococcus* sp. SR1 16S ribosomal RNA gene, partial sequence ID: JQ612587.1, Length: 1250, Number of Matches: 1; Score 1881 bits (1018), Expect=0.0, Identities=1069/1093 (98%), Gaps=6/1093 (0%), Strand=Plus/Plus. Subject; Reference; Query: Sample.

junctions may complicate mechanical oral biofilm removal and alter the chemical balance of this biofilm in that area [37].

Habitual Khat chewing may result in several changes to the oral mucosa and dentition. The mechanical and chemical irritation caused by chewing khat, together with xerostomia, may result in an extrinsic brown stain on the chewing side of the mouth and can increase the development of dental caries [13,15]. These stains may contribute to colonization by *Streptococcus* sp. [38,39] and favor the presence of chromogenic bacteria found in the oral cavity, such as *Lactobacillus* and *Veillonella* sp. [8].

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Table 2 and Figure 1 show that *Streptococcus* sp. was the most common strain in the 2 group types, and it was represented in 40 (80%) of the examined cases. This result fully agrees with those of previous studies [21,38], which indicated that *Streptococcus* sp. accumulate at high levels. This study followed the recommendation of Al-Alimi et al. [16], who demonstrated that quantifying *Streptococcus* sp. allows for a highly reliable assessment of the prebiotic activity of khat. In the present study, this species exhibited equal percentages among NKC and KC in different restorative materials, consistent with the results of previous studies [21,24] in which high levels of *Streptococcus* sp. were detected in their tested samples.

In clinical examinations, most patients have at least 1 dental filling or prosthesis, and the role of biofilm-related infections in filling as opposed to main oral infections is not easily distinguished [8,11]. To comprehensively understand the relationship between the nature of the microbial flora and each of the various filling/restoration types, we excluded subjects who had more than 1 restoration type.

Composite resin restorations are usually adhesively attached to tooth surfaces, with no gaps in between. These gaps should be avoided, science it they cause microleakage and dye penetration in dentine margins, which alter the microbiota along restoration margins and lead to secondary caries formation [11,40]. This condition requires that the dental restoration is cured and placed by a special technique to avoid gaps caused by polymerization shrinkage resulting from the attraction of the restorations to the center of the filling [11]. In our work, a space was formed at the junction between the tooth surface and restoration (the placement of our sample collections for composite and amalgam, which is considered a favorite location for biofilm formation). This result coincides with previous studies [20–21,23], indicating the absence of significant differences in terms of biofilm formation or adhesion colony rate in different areas. Also, our samples showed that khat chewing has a slight effect on biofilm formation in composite samples, which is in agreement with an *in vitro* study suggesting that khat chewing is associated with dental caries [31].

The remarkable influence of the different composite resins tested on *Streptococcus* sp. biofilm formation suggests that, compared with surface roughness, material characteristics and composition play a minor role [21,25]. The claim that a dental composite resin restorative material accumulates more biofilm than amalgam is not true [41], and this is also supported by the results obtained from our samples. Here, composite samples with *Streptococcus* sp. were equal between the 2 groups (2 NKC and 3 KC samples); this result can be explained by the issue of the composite related to its surface wearing, which results in a rough surface that facilitates biofilm formation.

Amalgam is commonly used for filling teeth and it is not directly attached to the tooth. This gap is a potential area for ingrowth of bacterial biofilm, thus causing secondary caries [7]. In the present preliminary study, a slightly higher amount of *Streptococcus* sp. was detected in amalgam samples (3 NKC and 2 KC) compared with that in NKC, which may be due to washout by saliva from the oral cavity and the continuous corrosion of amalgam.

A prosthesis is cemented to tooth surfaces after a significant amount of enamel and dentine reduction. Dental luting agents that fill the cement space between the tooth and restoration retain the prosthesis. Exposure of the luting agent to oral saliva leads to washout and wear, thus causing an increase in the space over time, which can result in secondary caries [11]. Souza et al. [42] found a higher accumulation of oral biofilms on cobalt-chromium-based material alloy and FP than that on zirconia or titanium used for prostheses fabrication at different time intervals. Their results agree with our findings because FP and NC cases were among the highest in our samples (Table 3). This finding can be attributed to the mechanical and chemical effects of khat on NC, which were greater than those on zirconia crowns [15], as well as the cause of the effect of the oral cavity on the surfaces of FP and NC prostheses. This condition was shown clearly in our zirconia crown (ZC) samples, which contained only 4 samples with *Streptococcus* sp (Table 3). The ZC samples accumulated fewer biofilm bacteria. This result strongly coincides with a previous study [22] that found less *Streptococcus* sp. biofilm adhesion on lithium disilicate glass-ceramic compared with that on partially or fully stabilized zirconia.

Total *Streptococcus* sp., *Lactobacillus* sp., and *Veillonella* sp. were counted, as reported previously [8,10–11]. Aas et al. [8] studied the species in intact enamel, white spot lesions, dentine lesions, and deep dentine lesions, concluding that bacterial species of *Streptococcus* sp., *Veillonella* sp., and Lactobacillus sp. are likely the key elements in facilitating the succession of species in developing dental biofilm *in vivo* and play an important role in caries progression [8,10]. These findings agree with the results of the present preliminary study. *Lactobacillus* sp. was found mainly in participants with prosthetic materials, specifically in areas after teeth preparations (dentine and deep dentine). This result is in agreement with Aas et al. [8], who concluded that *Lactobacillus* sp. is abundant in this region of the tooth surface.

In our study, equal numbers of samples with different restorative materials contained *Streptococcus* sp. in the NKC and KC groups (19 and 21 samples), but *Lactobacillus* sp. was more common among KC (4 samples out of 6). Only the NKC samples contained *Veillonella* sp., which was highly prevalent among all the NKC samples. Interestingly, this result conflicts with the

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findings of Al-Hebshi et al. [18], who detected a significantly higher total bacteria number of *Veillonella* sp. among KC, specifically on the khat-chewing side. Our results suggest that there is no such relationship between the existence of *Veillonella* sp. and khat chewing habit (Table 2). *Veillonella* sp. use lactic acid for growth in saliva, and they communicate metabolically with initial, early, middle, and late colonizers to establish multi-bacterial communities in all stages, from intact enamel to deep dentine cavities, in all dentitions [8,10].

Miswak use is an effective aid in oral hygiene and has antibacterial activity against oral bacteria formation. The total oral biofilm survival rate on miswak was significantly reduced as compared with the toothbrush. Thus, the use of miswak can help to maintain proper dental hygiene by limiting the risk for oral biofilm contamination and translocation [43–45]. According to several studies [46–49], Miswak as a brushing tool or as a mouth wash has been reported have important antimicrobial, antibacterial, and preventive roles, particularly on cariogenic bacteria such as *Streptococcus* and *Lactobacillus* sp. Table 4 shows that there were no significant differences or association observed among subjects that used miswak (11 participant only), brushes, or smoking with group type; which might be due to the relatively small sample size. An association and significant differences were detected between the tooth-brushing subjects (p value 0.046), despite the small number of subjects (24 among NKC and KC). Thirty-five participants in this study were smokers and smoking might be a major factor in biofilm formation and enhanced periodontal or dental disease (Table 4). This supported the conclusions of Halboub et al. [50] that there is no relationship between smoking and KC. Moreover, as shown in Table 4, we found no association between bacterial type and the habits of smoking, brushing, or miswak use (p value ≥0.050).

Many studies have documented *Streptococcus*, *Lactobacillus,* and *Veillonella* sp. are isolated principally from clinical subjects in intact enamel, white spot lesions, and cavities in deep dentine. However, no study has been conducted among the Saudi Jazan population, which has a widespread social habit of khat chewing. The present study is the first of its type to assess the presence of various oral cavity bacterial species in Saudi people living in the Jazan region and supports the scientific literature on the genetic presence of these species. The outcome can predict existing genes in the community. The PCR results were confirmed via partial sequencing for samples, as previously reported [8,10,33]. The BLAST search showed that the sequence obtained from our samples is in full agreement with many published sequences of the same species, as shown in Figure 1, which provides the GenBank accession numbers and country of isolates. Further clinical case-control studies are highly recommended for each material and its types in the presence of various other social habits.

Moreover, further clinical longitudinal studies with a higher number of samples and different dental materials could be useful to confirm the results of the present preliminary report.

Conclusions

Within the scope of this preliminary clinical study, we concluded that khat chewing significantly affects the examined bacterial species. Almost equal percentages of *Streptococcus* sp. were detected among NKC and KC. However, more *Lactobacilli* sp. were detected in KC, while more *Veillonella* sp. was found in NKC. Lactobacilli were mainly associated with prosthetic materials, while streptococci were found among all tested dental restorative materials.

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Conflict of interests

None.

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