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# Caries-related factors and bacterial composition of supragingival plaques in caries free and caries active Algerian adults

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ABSTRACT

**Objective:** To compare oral hygiene practices, education and social background, food intake and oral malodor of Algerian adults suffering from dental caries with normal controls, and to determine and compare the bacterial composition of the supragingival plaques from the above-mentioned groups.

**Methods:** Participants completed a questionnaire and were clinically examined for dental caries using decayed, missing and filled teeth index according to the criteria laid down by the World Health Organization. Supragingival plaque samples were collected from 50 caries-free adults (CF) and 50 caries-active adults (CA). Standard procedures of culture and identification of aerobic and anaerobic bacteria were used. Data were analyzed using *Chi*-square test.

**Results:** A total of 117 bacterial strains were isolated from supragingival plaques in CF group subjects, 76 (64.96%) of them belonged to 9 aerobic genera, and 41 (35.04%) to 9 anaerobic genera ($P < 0.05$). While in the second group, 199 strains were isolated, 119 (59.80%) of the strains belonged to 10 aerobic genera and 80 (40.20%) to 10 anaerobic bacteria ($P < 0.05$). *Streptococcus mutans*, *Enterococcus faecium*, *Aerococcus viridans*, *Actinomyces meyeri*, *Lactobacillus acidophilus* and *Eubacterium limosum* showed a significantly higher prevalence in the CA group ($P < 0.05$). The findings revealed that CA group had a high sugar intake (80%). A significantly higher frequency of tooth brushing ($P < 0.000$) and a significantly less self-reported oral malodor ($P < 0.000$) and tooth pain ($P < 0.000$) were found in CF group, while there was no association of socioeconomic levels and intake of meal snacks with dental caries.

**Conclusions:** This study confirms the association of some aciduric bacteria with caries formation, and a direct association of sugar intake and cultural level with dental caries. Furthermore, oral hygiene practices minimize the prevalence of tooth decay.

## 1. Introduction

Dental plaque happens to be a diverse community of the microorganisms found on the tooth surface [1]. Greater than 700 microbial species inhabit the oral cavity [2]. The formation of dental plaque in a healthy individual involves an ordered pattern of colonization by a range of bacteria, and once a dynamic balance is established, the composition of the resident microbiota of each site remains relatively stable over time [3]. Dental caries is one of the most common chronic and multifactorial diseases affecting the human population [4]. It is considered by the World

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The study protocol was performed according to the Helsinki declaration and approved by the Tlemcen University Hospital Committee for research on human subjects. All participants were informed about the goals of this study and were asked to give their written consent.

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Health Organization as one of the most important global oral health burdens that affects people of various age groups all over the world.

Cariogenic plaques result when acidogenic and aciduric bacterial species increase following high frequency of exposure to carbohydrate. The microbial metabolism of such carbohydrates will result in the acidification of the biofilm, which in turn may lead to acid-induced demineralization of the dental hard tissues [5]. Moreover, the prevalence and incidence of dental caries in a population is influenced by a number of risk factor such as sex, age, socioeconomic status, dietary patterns and oral hygiene habits [6].

There are three microbial hypotheses regarding the etiology of dental caries [7], namely the specific plaque hypothesis, the non-specific plaque hypothesis, and the ecological plaque hypothesis. The specific plaque hypothesis has proposed that only a few species of the total microflora are actively involved in disease [8]. Of which the most relevant were mutans streptococci [main species: *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus*] and lactobacilli [9]. Contrary to the specific plaque hypothesis, the non-specific plaque hypothesis suggests that caries is the consequence of the overall interaction of all the groups of bacteria within plaque [10]. The ecological plaque hypothesis suggests that caries is the result of an imbalance in the microflora due to ecological stress, resulting in an enrichment of certain disease-related micro-organisms [8].

Two closely related species of mutans streptococci, namely *S. mutans* and *Streptococcus sobrinus*, are associated with dental caries in humans [11]. Based on current research, it is believed that the bacteria of the genus *Lactobacillus* are important in further caries development, especially in the dentin [12]. However, it is now recognized that a large number of species naturally present in oral plaque biofilm produce acid from dietary carbohydrate and that the consequent caries-associated microbiota is complex [13].

Since oral bacteria are considered as one of the etiologic factors involved in caries development, various microbial studies have been conducted to better understand this dental problem.

Because of a lack of data available for detecting the bacterial diversity of dental caries in the Algerian adults population, the present study was performed to determine all cultivable bacterial species associated with health and dental caries of permanent teeth in adults, to determine the associations of specific bacterial species or bacterial communities with healthy and carious teeth, and to reveal the relation of food intake, oral hygiene practices, cultural level, socioeconomic status and oral malodor with dental caries among Algerian adults.

## 2. Materials and methods

### 2.1. Subject population

The study protocol was performed according to the Helsinki declaration and approved by the Tlemcen University Hospital Committee for research on human subjects. All participants were informed about the goals of this study and were asked to give their written consent.

One hundred healthy patients, aged 20–35 years were recruited from the dental clinic at the Departments of Dentistry, University of Tlemcen. They completed a medical and dental history questionnaire and signed an informed consent document. The questionnaire consisted of two parts. The first part consisted of general information such as age, sex and educational qualification. The second part consisted of six questions related to oral health, attitude and practices.

Subjects underwent dental examination for caries prevalence by a dentist, who applied the World Health Organization's caries diagnostic criteria to determine the decayed, missing, filled teeth (DMFT) index [14]. The subjects were divided into two groups: caries-free (CF) group (DMFT = 0, $n$ = 50) and caries-active (CA) group ($4 \leq$ DMFT $\leq 8$, $n$ = 50).

Exclusion criteria included antibiotic therapy in the previous 3 months, any systemic diseases and having less than 24 permanent teeth.

### 2.2. Plaque sampling

Samples were collected in the morning, 12 h after tooth brushing and 2 h after the last food and/or drink intake.

The supragingival plaque samples of CF and CA groups were collected by isolating the teeth with sterile cotton rolls and rubbing the vestibular face of the smooth and proximal surfaces of the central and lateral incisor, canines, premolars, and the first and second molars with a sterile swab. Each sample was pooled in 1 mL of pre-reduced brain heart infusion broth, (pH 7.2, Oxoid, Basingstoke, UK) and transported immediately to microbiology laboratory located next to the dental clinic.

### 2.3. Microbiological processing

The specimens were processed in the laboratory for the cultivation of aerobic and anaerobic bacteria.

First, the samples were vortexed for 30 s and the suspension was serially diluted ($10^{-1}$–$10^{-4}$) with sterile brain heart infusion broth (pH 7.2, Oxoid, Basingstoke, UK), aliquots (100 μL) of each dilution and the undiluted suspension were inoculated onto non-selective and selective media. Enriched blood agar [Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% laked blood] was used for the isolation of facultative and anaerobic bacteria. Plates were incubated anaerobically for 5–7 days at 37 °C in an environment consisting of 80% $N_2$, 10% $CO_2$, and 10% $H_2$, and aerobically for 24–48 h at 37 °C. Selective media such as MacConkey (Oxoid, Basingstoke, UK) used for the isolation of enterobacteria and Chapman (Oxoid, Basingstoke, UK) for staphylococci were also inoculated and incubated under an aerobic condition for 24–48 h at 37 °C.

Bacterial identification was based on the colony morphology and pigmentation, Gram staining and the biochemical reactions (API 20 A, API 20 Strep, API 20 E, API 20 Staph) (Biomérieux, Marcy l'Etoile, France) [15,16]. Antibiotics sensitivity test was also used to confirm biochemical test results.

### 2.4. Statistical analysis

Statistical analysis was performed on SPSS v.20.0 statistics software (SPSS Inc., Chicago, IL, USA). Simple frequency tables and descriptive statistics were processed and analyzed by *Chi*-square test ($\chi^2$). Statistical significance was set at $P < 0.05$.

## 3. Results

A total number of 100 individuals were examined for dental caries. One half (50%) was CA, whereas the other half was CF.

The questionnaire data revealed that there were 13 males (26%) and 37 females (74%) in the CF group, in which 41 (82%) persons were aged between 20 and 25 years and 9 (18%) within 25–30 years age group. Whereas in the second group, there were 16 (32%) males and 34 (68%) females, in which 31 (62%) of

them aged between 20 and 25 years, 16 (32%) within 25–30 years and 3 (6%) were within 30–35 years (Table 1).

**Table 1**
Socio-demographic distribution frequency in adults with and without caries (%).

| General information | Without caries ($n$ = 50) | With caries ($n$ = 50) | $P$ value |
|---|---|---|---|
| Age group | | | |
| 20–25 | 82 | 62 | < 0.05 |
| 26–30 | 18 | 32 | |
| 31–35 | 0 | 6 | |
| Gender | | | |
| Male | 26 | 32 | > 0.05 |
| Female | 74 | 68 | |
| SES | | | |
| Low | 0 | 6 | > 0.05 |
| Middle | 70 | 74 | |
| High | 30 | 20 | |
| Cultural level | | | |
| Low | 4 | 26 | < 0.05 |
| High | 96 | 74 | |

SES: Socioeconomic status. $P < 0.05$: Statistically significant difference between parameter frequency for subjects with and without active caries.

In CF group, 15 (30%) persons belong to high social class and 35 (70%) to middle social class. Whereas in the CA group, 10 (20%) persons belong to a high social class, 37 (74%) to middle social class and 3 (6%) to lower social class (Table 1). Patients with lower education background had a statistically highly significant caries experience ($P < 0.05$) compared to those with higher levels of education (Table 1).

The frequency of each parameter for subjects with and without active caries is shown in Table 2. A significant difference between adults with and without active caries was observed in sugar intake, oral malodor, tooth-brushing frequency, tooth pain and inter-dental cleaning ($P < 0.05$). However, there were no significant differences in intake between meal snacks.

**Table 2**
Clinical parameters frequency in adults with and without caries (%).

| Clinical parameters | Without caries ($n$ = 50) | With caries ($n$ = 50) | $P$ value |
|---|---|---|---|
| Sugar intake | | | |
| Yes | 30 | 80 | < 0.05 |
| No | 70 | 20 | |
| Tooth-brushing frequency | | | |
| Irregular | 2 | 50 | |
| Once a day | 8 | 28 | < 0.05 |
| Twice a day | 62 | 22 | |
| More than twice a day | 28 | 0 | |
| Tooth pain | | | |
| Yes | 0 | 46 | |
| No | 100 | 54 | < 0.05 |
| Oral malodor | | | |
| Yes | 6 | 32 | < 0.05 |
| No | 94 | 68 | |
| Intake of between meal snacks | | | |
| Yes | 54 | 56 | > 0.05 |
| No | 46 | 44 | |
| Inter-dental cleaning | | | |
| Dental floss (F) | 24 | 0 | |
| Toothpick (TP) | 6 | 8 | < 0.05 |
| Inter-dental brush | 12 | 2 | |
| Combination (F + TP) | 20 | 8 | |
| Nothing used | 38 | 82 | |

$P < 0.05$: Statistically significant difference between parameter frequency for subjects with and without active caries.

The analysis of dental plaques of CA adults revealed the presence of 117 strains belonging to 18 genera of 9 aerobic and 9 anaerobic genera ($P < 0.05$). And from dental plaques of adults with caries, a total of 199 strains were isolated, with 10 aerobic and 10 anaerobic genera.

Moreover, there were more isolation of Gram-positive bacteria than Gram-negative bacteria for the two groups ($P < 0.05$).

There were no significant differences between the isolation rate of various species in examined groups, except for *S. mutans* (6% *vs.* 20%) ($P < 0.05$), *Enterococcus faecium* (*E. faecium*) (16% *vs.* 38%) ($P < 0.05$), *Aerococcus viridans* (*A. viridans*) (6% *vs.* 22%) ($P < 0.05$), *Actinomyces meyeri* (*A. meyeri*) (10% *vs.* 28%) ($P < 0.05$), *Lactobacillus acidophilus* (*L. acidophilus*) (6% *vs.* 22%) ($P < 0.05$), and *Eubacterium limosum* (*E. limosum*) (0% *vs.* 8%) ($P < 0.05$), which were statistically significant, and occured in CA subjects (Table 3).

**Table 3**
Isolation frequency of aerobic and anaerobic bacteria in supragingival plaque of CF and CA groups (%).

| Bacterial species | Supragingival plaque | | $P$ value |
|---|---|---|---|
| | CF ($n$ = 50) | CA ($n$ = 50) | |
| I. Aerobic bacteria | | | |
| Gram-positive cocci | | | |
| *Streptococcus sanguinis* | 2 | 4 | 0.558 |
| *Streptococcus acidomonimus* | 10 | 12 | 0.749 |
| *Streptococcus constellatus* | 8 | 14 | 0.338 |
| *Streptococcus agalactiae* | 10 | 10 | 1.000 |
| *Streptococcus uberis* | 4 | 6 | 0.646 |
| *S. mutans* | 6 | 20 | 0.037 |
| *Streptococcus oralis* | 4 | 6 | 0.646 |
| *Streptococcus anginosus* | 8 | 12 | 0.505 |
| *Streptococcus intermedius* | 2 | 6 | 0.307 |
| *Gemella morbillorum* | 4 | 0 | 0.153 |
| *Gemella haemolysans* | 8 | 6 | 0.695 |
| *E. faecium* | 16 | 38 | 0.013 |
| *Micrococcus* sp. | 0 | 2 | 0.315 |
| *A. viridans* | 6 | 22 | 0.021 |
| *S. aureus* | 8 | 12 | 0.505 |
| *Staphylococcus hominis* | 8 | 10 | 0.727 |
| *Lactococcus lactis* ssp. *lactis* | 8 | 10 | 0.727 |
| *Lactococcus lactis* ssp. *cremoris* | 10 | 8 | 0.727 |
| Gram-negative cocci | | | |
| *Moraxella* spp. | 14 | 16 | 0.779 |
| Gram-negative rods | | | |
| *Klebsiella pneumoniae* | 12 | 10 | 0.749 |
| *Aeromonas hydrophila* | 8 | 14 | 0.338 |
| II. Anaerobic bacteria | | | |
| Gram-positive rods | | | |
| *Actinomyces naeslundii* | 24 | 30 | 0.499 |
| *A. meyeri* | 10 | 28 | 0.022 |
| *Actinomyces israelii* | 4 | 6 | 0.646 |
| *Actinomyces viscosus* | 0 | 2 | 0.315 |
| *L. acidophilus* | 6 | 22 | 0.021 |
| *Bifidobacterium* spp. | 2 | 6 | 0.307 |
| *Propionibacterium propionicum* | 8 | 12 | 0.505 |
| *E. limosum* | 0 | 8 | 0.041 |
| Gram-negative rods | | | |
| *Prevotella* sp. | 8 | 18 | 0.137 |
| *Fusobacterium mortiforum* | 4 | 8 | 0.400 |
| *Capnocytophaga* sp. | 6 | 10 | 0.461 |
| *Bacteroides* sp. | 2 | 6 | 0.307 |
| *Porphyromonas asaccharolytica* | 4 | 4 | 1.000 |

$P < 0.05$: Statistically significant difference between species isolation frequency for subjects with and without active caries. *S. aureus*: *Staphylococcus aureus*.

For the CA group, *E. faecium* were the major isolated strains (38%) followed by *Actinomyces naeslundii* (30%) (Table 3). Whereas, *S. mutans* and *L. acidophilus* represented 20% and 22% respectively.

## 4. Discussion

The present study offers the first description of the microflora associated with supragingival plaque in CF and CA Algerian adults based on culture methods. A wide diversity of bacterial species was observed. The relationship between cultural level, socioeconomic status, the food intake, oral hygiene practices, tooth pain, and oral malodor with dental caries was revealed by the questionnaires data.

Women had higher prevalence and severity of caries compared to men, which was consistent with the findings of Lukacs [17], Doyal and Naidoo [18], and Ferraro and Vieira [19]. This higher caries prevalence in females may be due to different salivary composition and flow rate, hormonal fluctuations during pregnancy, dietary habits, and particular social roles of women among their family (caretaker, meal preparation, *etc*.) [19]. Other studies have proposed that the differential effects of genes influencing dental caries may partly explain the observed differences in the two sexes [20].

Eighty-three studies found at least one measure of caries to be significantly higher in low socioeconomic position compared with high socioeconomic position individuals, while only three studies found the opposite [21]. In the present study, there were no association between socioeconomic status and caries prevalence in Algeria ($P < 0.05$) due to the free medical services that allow access to dental care services to all socioeconomic levels.

The frequency of sugar intake was most important in CA adults (80%) than that of in CF adults (30%). Previous studies have observed a linear relationship between sugar consumption and caries [22].

It is now recognized that there are a large number of species naturally occurring in the oral plaque biofilm, which produces acid from dietary carbohydrate [13]. However, when the presence of fermentable carbohydrates is frequent, a gradual increase in such bacteria occurs in the oral environment, causing an imbalance in the demineralization/remineralization process in dental tissues [23]. A caries process progress and a lesion develops when the remineralization is not given enough time to eliminate the damage done during demineralization [24].

In this study, mutant streptococci and lactobacilli were more frequently isolated from supragingival plaques of CA subjects (20% and 22% respectively). Several studies in humans are largely based on the mathematical relationship between various streptococci, lactobacilli and dental caries [25].

Sixty-two percent of the CF group brushed their teeth twice a day, whereas 50% of the CA group brushed less than once a day. Tooth brushing with a fluoride-containing toothpaste is one of the main oral behaviors to reduce the number of bacteria in the oral cavity [26]. Fluoride application removes bacteria from the mouth, and concomitantly reduce the risk of tooth caries.

For areas between the teeth that a toothbrush can't reach, regular flossing will help prevent tooth decay by removing food particles [27], 24% of adults without tooth decay used dental floss and 38% of this group used dental floss in combination with toothpick.

The significant difference in oral malodor between subjects with and without active caries (Table 1) shows that oral malodor in subjects with active caries (32%) was significantly higher than in those without active caries (6%) ($P < 0.05$). Poor oral hygiene habits and presence of conditions such a dryness of mouth, bleeding gums, dental caries and coating or deposits over tongue tend to influence the prevalence of halitosis [28].

The anaerobic microbiota of the tongue biofilm is one of the main factor responsible for the release of sulfur-containing compounds, which are directly involved in the occurrence of halitosis [29]. Previous research showed that among the Gram-negative bacteria, *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium* species*, Tannerella forsythia*, and *Treponema denticola* are major contributors of volatile sulfur compounds [30], which may be the reason of the high frequency of *Prevotella* (18%) in supragingival plaque of CA Algerian adults.

There was no statistical significant differences between the examined groups when it comes to snacks intake between meals ($P = 0.841$). These results are in accordance with previous study [31]. Moreover, Bowen *et al*. [32] concluded that it is not the frequency of ingestion per say that is related to the development of caries, but the time that sugars are available to microorganisms in the mouth.

According to the results of the present study, it is concluded that there was no significant difference of bacterial genera composition isolated from supragingival plaques in adults with and without caries except for *Micrococcus* sp. and *E. limosum* that was absent in the CA supragingival plaque.

There were more isolations of Gram-positive than Gram-negative bacteria in plaques of CF (75.21%, 24.79%) ($P < 0.05$) and CA subjects (78.39%, 21.61%) ($P < 0.05$), which concur with those reported by Ahmed *et al*. [33] and Ziouani *et al*. [34]. In another hand, facultative anaerobes and aerobic bacteria were more predominant than anaerobic bacteria in supragingival plaques of CF (66.67%, 33.33%) ($P < 0.05$) and CA groups (59.80%, 40.20%) ($P < 0.05$), while other authors indicated that aerobic bacteria were isolated more frequently from supragingival plaque of both groups [34,35].

A wide range of the *Streptococcus* genera was isolated from the supragingival plaque of both the CF and CA Algerian subjects, which confirmed previous reports that some *Streptococcus* species are associated with healthy states [36], while others are associated with disease states [37]. *S. mutans* was more frequent in supragingival plaque of CA adults (10/50; 20%) than CF adults (3/50; 6%) ($P < 0.05$) (Table 3). A similar finding was observed in a previous study showing a positive correlation of dental caries with increasing titers of *S. mutans* [38]. In a parallel way, the incidence rate of *L. acidophilus* in CA samples (22%) were higher than in CF samples (6%) ($P < 0.05$) (Table 3). Previous researches have reported that *Lactobacillus* is considered as essential acidogenic bacteria causing caries [39].

According to these results, mutans streptococci and *Lactobacillus* spp. are the main aetiologic agents of dental caries in humans [40,41].

Previous studies indicated that the genera of *Actinomyces* in plaque are significantly associated with dental caries [42]. Several species of these genera were isolated from plaques of CF and CA Algerian adults, in which *A. meyeri* was isolated with a significant statistical difference ($P < 0.05$).

Studies on co-aggregation interactions between *Streptococcus* spp. and *Actinomyces* spp. have revealed that the inter-bacterial adhesion between these two bacteria promotes early dental plaque biofilm development [43]. More importantly, those early colonizing bacteria provide specific binding sites either directly or through salivary glycoproteins binding to the pioneer organisms for subsequent bacterial colonization and promote the development of biofilm [44].

*E. faecium* strains were more frequently isolated from supragingival plaques of CA subjects (19/50; 38%) than CF subjects (8/50; 16%) with a significant statistical difference ($P < 0.05$). They have been known to be acidogenic and aciduric and they are not commonly seen in the human oral cavity [45] until the studies of Reyes *et al*. in 2012 [46], which was the first known report of its isolation in the oral cavity of CA humans. This bacterium was also isolated with a high frequency in supragingival plaques of CF Algerian adults [34].

Another bacterium found in the CA and CF plaque samples in this study is *Prevotella* sp. with an important frequency in the CA supragingival plaques (9/50; 18%). Previous research has shown that *Prevotella* spp. and *Capnocytophaga* spp. possess similar co-aggregation properties [47]. Moreover, Gomar-Vercher *et al*. [48] found that *Prevotella* showed an increasing percentage in caries compared to healthy individuals.

The strain *A. viridans* was also isolated from the supragingival plaque samples of CF and CA (6%, 22%) groups with a significant statistical difference ($P < 0.05$). This bacterium is present only in very small numbers in the upper respiratory tract of normal persons, and they may be present in rather small numbers on normal skin [49]. But there have been no reports on the isolation of this bacterium from dental plaque until the studies of Ziouani *et al*. [34] which revealed the presence of an important frequency of *A. viridans* in supragingival plaques of CF Algerian adults. Also, previous studies have found that the majority of this α-group (aerococci) form acid in glucose broth (final pH value 5.0–5.6) [49].

Members of the genera *Fusobacterium*, *Porphyromonas* and *Bacteroides* were also isolated from the supragingival plaques of both groups. They are able to coaggregate with other bacteria in the oral cavity [50,51].

Some bacterial species which are putative respiratory pathogens [52] (*e.g. S. aureus*) colonize the supragingival plaques of CF (8%) and CA adults (12%). A similar isolation was observed in supragingival plaque of CF adults [53] and CA patients [54]. *S. aureus* are transient microorganisms in the mouth and are secondary invaders of the dental caries [55].

In the other hand, microbial investigations have shown that the bacterium *Moraxella* is an exclusive human pathogen involving the upper respiratory tract [56]. This bacterium was isolated from supragingival plaque of CF (14%) and CA subjects (16%).

Culture methods represented an important tool for the detection of bacteria from supragingival plaque and another site of the oral cavity. This is the first study that elucidates supragingival plaque bacterial diversity in CF and CA Algerian adults.

The supragingival bacterial flora of both groups was composed mainly of Gram-positive aerobic and facultative anaerobic bacteria. A lot of genera represented the main part of the total microbiota, independent of the presence or absence of caries.

We were able to pinpoint several caries-related genera included *S. mutans*, *E. faecium*, *A. viridans*, *A. meyeri*, *L. acidophilus* and *E. limosum*. When *A. viridans* was isolated for the first time from dental plaque, this bacterium was also isolated from supragingival and subgingival plaques of Algerian adults [34].

The present findings support the ecological plaque hypothesis, in that caries is the result of an imbalance in the total micro-flora due to ecological stress, resulting in a growth advantage of some “oral pathogens” or disease-related micro-organisms [57].

A strong association was found between carbohydrate consumption and the presence of dental caries among 20–35 year-old Algerian adults. Tooth brushing and oral hygiene practices were inversely associated with dental caries.

Cultured microorganisms are required for antibiotic resistance, capacity for biofilm formation and molecular methods.

Future research should focus on the structure as well as the behavior and function of plaque bacterial communities. It will also be important to control the oral micro-flora for systemic reasons since strong links are being established between oral pathologies and other diseases, such as cardiovascular disease, certain types of cancer, and pulmonary infections [58].

In conclusion, the microbial diversity found in the present study should, therefore, be considered in the treatment strategy of caries in adult patients.

## Conflict of interest statement

We declare that we have no conflict of interest.

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