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Original Article

# Occurrence of some Oral Potentially Pathogenic Microorganisms and their associated Risk Factors

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## Abstract

**Background**: The human mouth harbors one of the most diverse microbiomes in the human body. Multiple factors might affect the composition of oral flora, such as oral hygienic practices. Disturbance in the composition of the oral flora might lead to local as well as systemic diseases.
**Objective(s):** To estimate the occurrence of some oral potentially pathogenic microorganisms and their associated risk factors, as well as their association with dental caries.
**Methods**: Saliva samples were collected from 130 participants affiliated to the High Institute of Public Health (HIPH) including university teaching staff/ postgraduate students, administrative employees and workers. A questionnaire sheet was completed for everyone. It included demographic data, clinical data as well as oral hygienic practices. The DMFT index was recorded as an index for oral caries. All 130 saliva samples were examined for the presence of *Enterobacteriaceae*, *Streptococcus mutans* (*S. mutans*) and *Candida* spp. Demographic data, dental hygiene practices, dental complaints as well as the DMFT index were all studied in relation to the studied microbial agents.
**Results**: The most prevalent microorganism among participants was *S. mutans* (64.6% of isolates) followed by *Candida* spp (20.8%) then *Enterobacteriaceae* spp (10%). Isolates of *Enterobacteriaceae* spp were distributed as follows: *Citrobacter koseri* (4.6%), followed by *Klebsiella pneumoniae* (3.8%), then *Klebsiella oxytoca*. *S. mutans* was the only microorganism that was significantly affected by the type of snacks taken between meals (p=0.001). Among those who had sugary snacks between meals, *S. mutans* was isolated in 81.3% of them. *Candida* spp was present in 27.3% of married participants, and this finding was statistically significant. The intake of drinks between meals was also significantly associated with positive *Candida* cultures (31.1% of participants who took drinks in between meals had positive *Candida* cultures). *Enterobacteriaceae* were significantly less frequently isolated among participants with secondary and university education (5% and 6.5% respectively) compared to uneducated and primarily educated participants (13% and 40% respectively). None of the studied microorganisms were associated with any specific dental hygienic practice, or specific dental symptoms and signs. The only organism isolated that was significantly associated with DMFT index was *S. mutans* (p = 0.001). None of the microorganisms were found to be significantly associated with the presence of any of the other two microorganisms.
**Conclusions**: *S. mutans* was the most prevalent microorganism among participants and was significantly associated with higher DMFT index. The presence of potentially pathogenic oral microorganisms was influenced mainly by the educational status as well as dietary habits (the intake of sugars and drinks between meals) rather than specific oral hygienic practices.

***Keywords*:** Oral hygiene, *S. mutans*, DMFT index, Oral flora

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## INTRODUCTION

Oral structures are harbored with various microorganisms, which are collectively referred to as the “oral microbiome”. Distinct habitats for microbial colonization include the teeth, gingiva, tongue, cheeks, lips and hard and soft palates. These structures comprise heterogeneous ecological systems that support the growth of different microorganisms.[1, 2] The anatomic proximity of oral microorganisms to the systemic circulation may cause the systemic spread of bacteria and their products. Moreover, oral microorganisms might also contribute to dental caries, halitosis, gingivitis and periodontitis.[3] *Streptococcus mutans* (*S. mutans*) are

gram positive, facultative anaerobic bacteria which are a major component of the oral flora. They belong to a larger group called *mutans group streptococci* (MGS) that contains nine species which are morphologically and biochemically similar, with the most pathogenic and cariogenic species being *S. mutans*. It is considered one of the main initiators of dental plaque because of its ability to ferment multiple sugars into acids, leading to the erosion of enamel, initiating caries.[4]

Other possible oral microorganisms include members of the *Enterobacteriaceae* family, which might cause oral ulcerations in immunocompromised patients. Oral isolates of *Klebsiella spp* are a possible cause of aspiration pneumonia in comatosed patients.[5] *Candida spp* is part of the normal fungal oral flora in most people. However, it can act as an opportunistic pathogen and cause oral candidiasis in immunocompromised hosts. Moreover, high salivary *Candida spp*. carriage has been associated with severe caries as well as oral S. *mutans*.[6] The oral flora is affected by multiple factors such as the individual's age, sex, pregnancy, dietary habits, oral hygiene, presence of artificial teeth and dentures, presence of any systemic disorders and antibiotic therapy. Oral hygiene practices include the frequency of dentist visits and frequency of tooth brushing and flossing. Poor oral hygiene is the main factor for dental caries, gingivitis, periodontitis as well as several extra-oral diseases.[1, 7]

The decayed, missing, and filled teeth (DMFT) index is one of the most common indices in oral epidemiology . It has been recommended by the World Health Organization (WHO) to assess dental caries and hygiene. It is based on clinical examination of teeth using a probe and mirror and counting the number of decayed, missing and filled teeth. The DMFT can be arranged from 0 to 28 or to 32 depending on whether the third molars are included in the scoring.[8]

This study aimed at estimation of the occurrence of oral potentially pathogenic microorganisms and their associated risk factors, as well as their association with dental caries.

## METHODS

This cross-sectional study included 130 participants working at HIPH. The sample size was calculated based on an internal pilot experiment on 20 participants, which yielded a 10% prevalence rate of salivary *Enterobacteriaceae*. Participants were randomly selected from university teaching staff, postgraduate students, administrative staff and workers. Persons who had eaten, smoked or used dental wash within the last 2 hours, and individuals who were on antibiotic therapy during the last 2 weeks were excluded from the study. Subjects with systemic diseases (such as those on chemotherapy, diabetics, renal failure patients...etc) as well as pregnant females were also excluded.

A pre-formed questionnaire was filled for each participant concerning demographic data as: age, sex, marital status, smoking status, profession, educational status and snack type between main meals. Data on dental hygiene practices were also included in the questionnaire such as: the frequency of tooth brushing/ flossing per day, the frequency of changing the tooth brush per year, the frequency of dentist visits per year. The following dental signs/ symptoms were also reported (if any): dental pain or caries, halitosis, existence of any artificial teeth or dentures.

Clinical examination was performed using a dental probe and mirror to assess the number of decayed (D), missing (M) and filled (F) teeth. However, this was performed for 90 participants only. All 130 saliva samples were examined for the presence of: *Enterobacteriaceae*, *S. mutans* and *Candida* spp. Dental hygiene practices, dental complaints as well as the DMF index were all studied in relation to the studied microbial agents.

One un-stimulated saliva sample (0.7 to 1.0 ml) was collected from each participant and each was placed in a disposable and sterile capped plastic container and transferred to the laboratory for examination within 1 – 2 hours. Each sample was vortexed for 1 minute, Gram stained and then a loopful was used to culture the following agar plates:

a) Tryptone-yeast extract -cysteine with sucrose and bacitracin (TYCSB) agar (Bio Merieux, Sydney, Australia). This media is selective for *S. mutans*.
b) MacConkey agar plate for the isolation of *Enterobacteriaceae*.
c) Sabouraud dextrose agar (SDA), supplemented with chloramphenicol (0.01 g/l) for the isolation of *Candida spp.*

TYCSB agar plates were incubated anaerobically using anaerobic gas packs in an anaerobic jar (atmosphere with 10% hydrogen, 10% $CO_2$ and 80% $NO_2$) at 37°C for 24- 48 hours. MacConkey and SDA plates were incubated aerobically at 37° C and 25°C respectively for 24- 48 hours.

Colonies of *S. mutans* on TYCSB were whitish, with a rough surface and crystalline in appearance. Gram staining revealed *S. mutans* to be gram positive cocci in chains. Typical colonies of *S. mutans* on TYCSB agar were sub-cultured on blood agar anaerobically and incubated at 37° C for 24 hours. *S. mutans* colonies were convex and showed variable hemolytic activities. Isolates were further identified by being: negative for catalase and starch hydrolysis, and positive for growth in mannitol broth.[9, 10] *Enterobacteriaceae* colonies on MacConkey agar appeared as either pinkish or pale and were gram negative bacilli by microscopy. Colonies were identified using: oxidase, triple sugar iron (TSI) agar,

urease, indole, methyl red, Voges-Prosakaur and citrate (IMViC) tests. All isolates were identified according to their biochemical test results. [9]

*Candida* spp colonies on SDA appeared as bacterial like smooth, creamy white colonies with a characteristic yeast odor. *Candida* spp. isolates were gram positive oval cells with occasional budding/ pseudohyphae.

**Statistical Analysis**

Statistical analyses were carried out using the statistical package for social sciences (SPSS) version 20[11]. Comparison between different groups regarding categorical variables was tested using Chi-square test. Mann Whitney (Z-test) was used for abnormally distributed data. Statistical significance was set at 5% ($p < 0.05$).

**Ethical Considerations**

The research protocol was approved by the Institutional Review Board and the Ethics Committee of the High Institute of the Public Health. The aim and concerns of the research was disclosed to the participants. After clarifying the procedures of the study, a verbal consent from every participant was obtained. The research steps were done in compliance with the international guidelines for research ethics and that of Helsinki Declaration. Anonymity and confidentiality of any given information was assured.

## RESULTS

Out of the 130 participants in the study, females comprised 68.5%. The mean age of participants was 39 years. About 60% of participants were married and 80% were non-smokers. Most of the participants (65.4%) did not take drinks between meals (neither sugary nor caffeinated drinks). On the other hand, 66% had snacks between main meals, mainly in the form of fruits and vegetables (51%).

Half of the participants regularly brushed their teeth at least once daily, while 25% did not brush their teeth on daily basis. Dental flossing was not a common practice among participants (90% did not floss). Sixty-six participants changed their toothbrush only once per year. Half of the participants visited the dentist twice per year, and half of them had no dental complaints of pain or halitosis. Upon dental examination, only 15.6% of participants had decayed teeth, while 22.2% had missing teeth and 28% had filled teeth. The mean DMFT index was 3.52 ± 6.19 and the median was 1.

The most prevalent organism among participants was *S. mutans*, followed by *Candida* spp and the least common was the *Enterobacteriaceae* group (64.6%, 20.8% and 10% respectively of participants had a positive culture). The isolated *Enterobacteriaceae* were: *Citrobacter koseri* (4.6%), followed by *Klebsiella pneumoniae* (3.8 %), then *Klebsiella oxytoca* (1.5%) (Table 1).

It was found that age, sex and smoking status all were insignificant risk factors for the presence of the studied microorganisms in the saliva. *Enterobacteriaceae* were significantly less frequently isolated among university educated participants as well as those with secondary education (6.5% and 5% respectively) when compared with uneducated and primary educated participants (13% and 40% respectively). This finding was statistically significant, ($p= 0.018$ (Table 2).

**Table 1: Isolated micro-organisms from the saliva of High Institute of Public Health participants, Alexandria**

| Organisms | Participants | |
|---|---|---|
| | No. | % |
| ***Streptococcus mutans*** | | |
| negative | 46 | 35.4 |
| positive | 84 | 64.6 |
| ***Enterobacteriaceae*** | | |
| negative | 117 | 90.0 |
| *Klebsiella oxytoca* | 2 | 1.5 |
| *Citrobacter koseri* | 6 | 4.6 |
| *Klebsiella pneumoniae* | 5 | 3.8 |
| ***Candida spp*** | | |
| negative | 103 | 79.2 |
| positive | 27 | 20.8 |

Marriage was significantly associated with positive *Candida* spp cultures (27.3% of married participants had positive cultures, while only 11.3% of unmarried ones had positive cultures of *Candida*, ($p=0.028$). *Candida* spp was also significantly associated with the intake of drinks between meals, where 31.1% of participants who took drinks in between meals had positive *Candida* cultures from their saliva in contrast to only 15.3% among those who did not take drinks between meals, $p =0.034$ (table 3). *S. mutans* was the only microorganism that was significantly affected by the type of snacks taken between meals ($p=0.001$).

Among those who had sugary snacks between meals, *S. mutans* was isolated in 81.3% of them, and 83.3 % among those who had mixed types of snacks, and 75 % among those who had fruits and vegetables. On the contrary, the intake of sandwiches was not associated with the presence of *S. mutans* (only 21.4% of those participants had salivary *S. mutans* (table 3). None of *S. mutans*, *Enterobacteriaceae* and *Candida* spp were associated with the presence of artificial teeth/ dentures, dental pain, halitosis, frequency of tooth brushing/ flossing, changing of tooth brush per year or frequency of dentist visits (data not shown). Dental hygienic practices were thus insignificantly associated with any of the studied microorganisms.

The isolation of *S. mutans* was not significantly associated with the isolation of *Enterobateriaceae* or *Candida* spp. Similarly, *Candida* spp and *Enterobateriaceae* were not significantly associated (data not shown). The only microorganism that was significantly associated with DMFT index was *S. mutans* (p = 0.001). The mean DMFT index among those with *S. mutans* was 4.7 ± 6.7 (Table 4)

**Table 2: Relationship between the isolated micro-organisms and demographic data among HIPH participants, Alexandria**

| Demographic data | Row total (n=130) | *S. mutans* | | | | *Enterobacteriaceae* | | | | *Candida* spp | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Absent (n = 46) | | Present (n = 84) | | Absent (n = 117) | | Present (n = 13) | | Absent (n = 103) | | Present (n = 27) | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Sex** | | | | | | | | | | | | | |
| Male | 41 | 13 | 31.7 | 28 | 68.3 | 36 | 87.8 | 5 | 12.2% | 36 | 87.8 | 5 | 12.2 |
| Female | 89 | 33 | 37.1 | 56 | 62.9 | 81 | 91.0 | 8 | 9.0% | 67 | 75.3 | 22 | 24.7 |
| $\chi^2$(p) | | 0.354(0.552) | | | | 0.321($^{FE}$p= 0.547) | | | | 2.675(0.102) | | | |
| **Age (years)** | | | | | | | | | | | | | |
| 20 – 30 | 41 | 17 | 41.5 | 24 | 58.5 | 38 | 92.7 | 3 | 7.3% | 34 | 82.9 | 7 | 17.1 |
| 31 – 40 | 39 | 12 | 30.8 | 27 | 69.2 | 33 | 84.6 | 6 | 15.4% | 31 | 79.5 | 8 | 20.5 |
| 41 – 50 | 27 | 12 | 44.4 | 15 | 55.6 | 26 | 96.3 | 1 | 3.7% | 19 | 70.4 | 8 | 29.6 |
| >50 | 23 | 5 | 21.7 | 18 | 78.3 | 20 | 86.9 | 3 | 13.1% | 19 | 82.6 | 4 | 17.4 |
| $\chi^2$(p) | | 3.868(0.276) | | | | 2.886($^{MC}$p= 0.412) | | | | 1.790(0.617) | | | |
| **Marital status** | | | | | | | | | | | | | |
| Not married | 53 | 23 | 43.4 | 30 | 56.6 | 50 | 94.3 | 3 | 5.7% | 47 | 88.7 | 6 | 11.3 |
| Married | 77 | 23 | 29.9 | 54 | 70.1 | 67 | 87.0 | 10 | 13.0% | 56 | 72.7 | 21 | 27.3 |
| $\chi^2$(p) | | 2.512(0.113) | | | | 1.872(0.171) | | | | 4.854*(0.028) | | | |
| **Smoking status** | | | | | | | | | | | | | |
| No | 108 | 38 | 35.2 | 70 | 64.8 | 98 | 90.8 | 10 | 9.2% | 88 | 81.5 | 20 | 18.5 |
| Yes | 22 | 8 | 36.4 | 14 | 63.6 | 19 | 86.4 | 3 | 13.6% | 15 | 68.2 | 7 | 31.8 |
| $\chi^2$(p) | | 0.011(0.916) | | | | 0.389(0.461) | | | | 1.965(0.246) | | | |
| **Profession** | | | | | | | | | | | | | |
| Workers | 44 | 19 | 43.1 | 25 | 56.9 | 37 | 84.1 | 7 | 15.9% | 34 | 77.3 | 10 | 22.7 |
| Administrative staff | 43 | 9 | 20.9 | 34 | 79.1 | 41 | 95.3 | 2 | 4.7% | 35 | 81.4 | 8 | 18.6 |
| University students/ staff members | 43 | 18 | 41.9 | 25 | 58.1 | 39 | 90.7 | 4 | 9.3% | 34 | 79.1 | 9 | 20.9 |
| $\chi^2$(p) | | 5.888(0.053) | | | | 2.931($^{MC}$p= 0.230) | | | | 0.226(0.893) | | | |
| **Education status** | | | | | | | | | | | | | |
| Uneducated | 23 | 9 | 39.1 | 14 | 60.9 | 20 | 87.0 | 3 | 13.0% | 17 | 73.9 | 6 | 26.1 |
| Primary education | 10 | 4 | 40.0 | 6 | 60.0 | 6 | 60.0 | 4 | 40.0% | 9 | 90.0 | 1 | 10.0 |
| Secondary education | 20 | 9 | 45.0 | 11 | 55.0 | 19 | 95.0 | 1 | 5.0% | 18 | 90.0 | 2 | 10.0 |
| University education | 77 | 24 | 31.2 | 53 | 68.8 | 72 | 93.5 | 5 | 6.5% | 59 | 76.6 | 18 | 23.4 |
| $\chi^2$(p) | | 1.642(0.650) | | | | 8.769*(0.018) | | | | 2.497(0.478) | | | |

MC: Monte Carlo for Chi square test
FE: Fisher Exact for Chi square test
* Statistically significant at $p \leq 0.05$
Percentages were calculated from row total

**Table 3: Relationship between the isolated micro-organisms and some dietary habits among HIPH participants, Alexandria**

| Dietary habits | Row total (n=130) | *S. mutans* absent (n = 46) | | *S. mutans* present (n = 84) | | *Enterobacteriaceae* absent (n = 117) | | *Enterobacteriaceae* present (n = 13) | | *Candida spp* absent (n = 103) | | *Candida spp* present (n = 27) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| **Intake of drinks between meals** | | | | | | | | | | | | | |
| No | 85 | 31 | 36.5 | 54 | 63.5 | 76 | 89.4 | 9 | 10.6% | 72 | 84.7 | 13 | 15.3 |
| Yes | 45 | 15 | 33.3 | 30 | 66.7 | 41 | 91.1 | 4 | 8.9% | 31 | 68.9 | 14 | 31.1 |
| **$\chi^2$(p)** | | 0.127(0.722) | | | | 0.094($^{FE}$p= 1.000) | | | | 4.473*(0.034) | | | |
| **Type of drinks** | | | | | | | | | | | | | |
| Not sugary (caffeinated) drinks | 35 | 13 | 37.1 | 22 | 62.9 | 32 | 91.4 | 3 | 8.6% | 23 | 65.7 | 12 | 34.3 |
| Sugary drinks (juices) | 8 | 1 | 12.5 | 7 | 87.5 | 7 | 87.5 | 1 | 12.5% | 7 | 87.5 | 1 | 12.5 |
| Both sugary and non-sugary drinks | 2 | 1 | 50.0% | 1 | 50.0 | 2 | 100.0 | 0 | 0.0% | 1 | 50.0 | 1 | 50.0 |
| **$\chi^2$(p)** | | 2.173 (0.315) | | | | 0.921 (1.000) | | | | 1.935 (0.464) | | | |
| **Snacks between main meals** | | | | | | | | | | | | | |
| No | 44 | 19 | 43.2 | 25 | 56.8 | 39 | 88.6% | 5 | 11.4% | 36 | 81.8 | 8 | 18.2 |
| Yes | 86 | 27 | 31.4 | 59 | 68.6 | 78 | 90.7% | 8 | 9.3% | 67 | 77.9 | 19 | 22.1 |
| **$\chi^2$(p)** | | 1.769(0.184) | | | | 0.137($^{FE}$p= 0.761) | | | | 0.271(0.603) | | | |
| **Type of snacks between meals** | | | | | | | | | | | | | |
| Sugars only | 16 | 3 | 18.8 | 13 | 81.3 | 14 | 87.5 | 2 | 12.5% | 11 | 68.8 | 5 | 31.3 |
| Sandwiches only | 14 | 11 | 78.6 | 3 | 21.4 | 13 | 92.8 | 1 | 7.1% | 10 | 71.4 | 4 | 28.6 |
| Fruits and vegetables only | 44 | 11 | 25.0 | 33 | 75.0 | 41 | 93.1 | 3 | 6.8% | 37 | 84.1 | 7 | 15.9 |
| More than one type of snack | 12 | 2 | 16.7 | 10 | 83.3 | 10 | 83.3 | 2 | 16.7% | 9 | 75.0 | 3 | 25.0 |
| **$\chi^2$(p)** | | 15.775* (0.001) | | | | 1.852 (0.624) | | | | 2.535 (0.483) | | | |

FE: Fisher Exact for Chi square test * Statistically significant at $p \leq 0.05$ Percentages were calculated from row total

**Table (4): Relationship between DMFT index and the isolated microorganisms among 90 participants in HIPH, Alexandria**

| Microorganisms | Total n =90 | Decayed teeth Median (Min. – Max.) | Decayed teeth Mean ± SD. | Missing teeth Median (Min. – Max.) | Missing teeth Mean ± SD. | Filled teeth Median (Min. – Max.) | Filled teeth Mean ± SD. | DMFT index Median (Min. – Max.) | DMFT index Mean ± SD. |
|---|---|---|---|---|---|---|---|---|---|
| ***S. mutans*** | | | | | | | | | |
| Negative | 46 | 0(0 – 5) | 0.2 ± 0.9 | 0(0 – 32) | 1.4 ± 5.3 | 0(0 – 8) | 0.8 ± 1.8 | 0(0 – 32) | 2.4 ± 5.5 |
| Positive | 44 | 0(0 – 10) | 0.9 ± 2.1 | 0(0 – 32) | 2.1 ± 6.7 | 0(0 – 10) | 1.7 ± 2.6 | 4(0 – 32) | 4.7 ± 6.7 |
| **MW(p)** | | 1.408(0.159) | | 1.470(0.141) | | 1.928(0.054) | | 3.197*(0.001) | |
| ***Enterobacteriaceae*** | | | | | | | | | |
| Negative | 81 | 0(0 – 10) | 0.5 ± 1.7 | 0(0 – 32) | 1.9 ± 6.3 | 0(0 – 10) | 1.2 ± 2.2 | 1(0 – 32) | 3.6 ± 6.5 |
| Positive | 9 | 0(0 – 4) | 0.7 ± 1.4 | 0(0 – 1) | 0.2 ± 0.4 | 0(0 – 7) | 1.7 ± 2.7 | 2(0 – 7) | 2.6 ± 2.6 |
| **MW(p)** | | 0.565(0.572) | | 0.240(0.810) | | 0.511(0.609) | | 0.328(0.743) | |
| ***Candida spp*** | | | | | | | | | |
| Negative | 74 | 0(0 – 10) | 0.6 ± 1.6 | 0(0 – 0.3) | 1.9 ± 6.5 | 0(0 – 10) | 1.3 ± 2.3 | 1(0 – 32) | 3.8 ± 6.7 |
| Positive | 16 | 0(0 – 7) | 0.6 ± 1.8 | 0(0 – 7) | 0.9 ± 1.9 | 0(0 – 7) | 1.0 ± 2.0 | 1(0 – 8) | 2.4 ± 2.9 |
| **MW(p)** | | 0.318(0.751) | | 0.755(0.451) | | 0.388(0.698) | | 0.229(0.819) | |

* Statistically significant at $p \leq 0.01$

## DISCUSSION

The role of the microbiome in dental diseases has been gaining rapid attention. Foci of infection in the oral cavity such as chronic periodontitis or chronic periapical abscesses may lead to subacute bacterial endocarditis and glomerulonephritis.[3] A recent study linked *Candida*-related oral mucosal lesions with a 70% excess risk of developing pancreatic cancer. [12] The present work was carried out to investigate the association of some oral microorganisms with some risk factors including dental hygiene practices. The association of the DMFT index with the studied microorganisms was also investigated.

In the present study, the most prevalent microorganism among participants was *S. mutans* (64.6% of isolates). It was significantly associated with DMFT index. The median DMFT index among those with *S. mutans* was 4, while patients who had no growth of *S. mutans* had a median DMFT = zero. This significant association highlights the cariogenic role of *S. mutans*. Similarly, Gábris et al. compared *S. mutans* with the DMFT index and found a strong association between them. [13] On the contrary, a Turkish study reported lack of association between DMFT values and salivary *S. mutans*. Their high rates of *S. mutans* and dental caries were attributed to low educational level of the local people living in the study area, bad economic conditions, lack of health services, dietary habits rich in carbohydrates and insufficient tooth brushing habits. [14] In the present study, *S. mutans* was associated with dietary intake of sugars, fruits and vegetables rather than frequency of tooth brushing, flossing or dental visits. Svanberg and Loesche stated that dietary sucrose promotes the attachment of *S. mutans* to teeth, and eventually increases the risk of caries.[15] It is therefore recommended to brush the teeth after eating sugars and fruits. Bagramian *et al.* reported that *S. mutans* causes halitosis, foul tastes and gingivitis.[16] On the contrary, a study in 2015 found no association between halitosis and the presence of *S. mutans* in saliva. [17] Similarly, the present study showed no association between *S. mutans* in saliva and halitosis.

A study performed in Brazil (2015) found higher numbers of oral *Enterobacteriaceae* among their participants (18.7%) in comparison to 10% colonization in the present study. Their most common isolated genus of *Enterobacteriaceae* was *Enterobacter*.[18] In contrast, in the present study, the most common isolated *Enterobacteriaceae* were *Citrobacter koseri* (4.6%), *Klebsiella pneumoniae* (3.8 %) and *Klebsiella oxytoca* (1.5 %). Ha¨gg *et al* (2014) reported a significant increase in the prevalence of *Enterobacteriaceae* after insertion of fixed orthodontic appliances due to their adhesive ability on dentures and artificial teeth.[19] However, this relationship was not present in the present study. *Enterobacteriaceae* were significantly less frequently isolated among university and secondary educated participants, compared to other less educated categories. This might be attributed to better oral hygiene practices performed by educated participants rather than the uneducated ones. Similarly, Reisine and Psoter in their study found that individuals with low educational levels had lower oral hygiene practices and poor DMFT indices. Families had a negative attitude and behavior towards the importance of oral hygienic practices. They only sought medical treatment after oral pathological processes had reached an advanced stage and preferred tooth extraction rather than conservative treatments which are generally more expensive.[20] On the contrary to this study finding, Bakdash and Proshek noted that many dental students did not demonstrate effective oral hygiene practices despite attending an educational program on preventive dentistry.[21]

In the present study, *Candida* spp. was present in the saliva of 20.8% of participants. This was similar to a study that showed the mean *Candida* carriage rate in individuals with no known underlying disease to be 24%.[22] In the present study, *Candida* spp was significantly associated with the intake of drinks between meals. The virulence determinants of *Candida* spp. such as their ability to form biofilms, their acidogenic tendency, their fermentation and assimilation of sugars contribute all to their latent cariogenic ability. Accordingly, oral candidal counts have been used by some studies as a caries risk indicator. On the contrary, other studies suggest that *Candida* is not a true cariogen. [22, 23] In the present study, no association between *Candida* spp. and the DMFT index (as a measure of dental caries) was found. In the current study, smoking was not associated with any of the microorganisms. This might be attributed to the small number of males included in the study, and lack of smoking among the female participants. Sheth *et al* (2017) found that a low concentration (1 mg/ ml) of nicotine promoted the growth of *S. mutans* and *C. albicans* in cultures and encouraged their attachment to each other in biofilms. On the contrary, a high concentration (4mg/ ml) of nicotine suppressed *C. albicans* in cultures. [24]

Poor dental hygienic practices of some participants in the study were evident by the low frequency of flossing (only 10%) and 25% did not brush their teeth on daily basis. About half of the participants changed their toothbrush only once per year. It is recommended that toothbrushes be changed at least four times a year.[25] Contamination of toothbrushes with *Enterobacteriaceae* and *Pseudomonas aeruginosa* is attributed to the incorrect keeping of the toothbrushes close to toilets where aerosols from the latter can easily reach them. Contaminated toothbrushes help to translocate microorganisms to the mouth, especially when toothbrushes are not changed frequently, allowing the proliferation of bacteria.[25,26]

## CONCLUSION AND RECOMMENDATIONS

The most prevalent microorganism among participants was *S. mutans*, followed by *Candida* spp. *S. mutans* was significantly associated with the intake of sugars, fruits and vegetable snacks between meals. Also, *Enterobacteriaceae* were significantly less frequently isolated among university and secondary educated participants compared to other less educated categories. The only isolated microorganism that was significantly associated with DMFT index was *S. mutans* (p = 0.001) with a median DMFT index of 4 among those with *S. mutans*.

It is recommended to reduce the intake of fruits and sugary juices between meals to limit the cariogenic effect of *S. mutans*. It is also recommended to improve the awareness of less educated people on the importance of adopting dental hygiene practices.

***Conflict of Interest:*** None to declare.

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