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Low prevalence of *Streptococcus mutans* serotypes and poor eating habits in undergraduate dental students: *Streptococcus mutans* serotypes and caries in dental students

Baixa prevalência de sorotipos de *Streptococcus mutans* e maus hábitos alimentares em estudantes universitários de odontologia: sorotipos de *Streptococcus mutans* e cárie em estudantes de odontologia

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ABSTRACT

The prevalence of caries in southeastern Mexico in individuals aged 20 to 24 is 83.5%. Caries development is multifactorial, including a cariogenic diet, poor oral hygiene, and limited health education. This preliminary longitudinal, single-blind study, utilizing non-probability sampling, aimed to evaluate oral hygiene, *Streptococcus mutans* prevalence, and caries frequency in undergraduate dental students. Sixty-seven students in their first semester and fifty-four in their second semester follow-up participated. Self-administered questionnaires gathered data on oral hygiene habits. Additionally, caries diagnosis using DMFT, supragingival plaque culture from tooth 46, and PCR with specific primers for *S. mutans* and its serotypes were performed. Over 74% of participants reported a high consumption of sugary beverages, and the Bass brushing technique was the most known. The overall caries prevalence was 54%. The DMFT index was 3.34 and 3.30 in the first and second semesters, respectively. Colony growth on Mitis-Salivarius agar was significantly higher in the first semester ($Z=-4.8$, $p<0.0001$). Culture-based prevalence of *S. mutans* was 85% and 44% in the first and second semesters, respectively. In contrast, PCR confirmation showed prevalence rates of 41% and 37% for the same periods, serotype c accounting for 80%. Undergraduate dental students exhibited high sugar consumption. The prevalence of caries and *S. mutans* was lower than that reported for the general population, and no significant association between *S. mutans* and caries was observed.

Keywords: Oral care. Serotypes. *Streptococcus mutans*.

RESUMO

A prevalência de cárie no sudeste do México entre pessoas de 20-24 anos é de 83,5%. Seu desenvolvimento é multifatorial, podendo incluir dieta cariogênica, higiene oral precária e educação em saúde limitada. O presente estudo preliminar longitudinal, simples-cego e de amostragem não probabilística buscou avaliar a higiene oral, a prevalência de *Streptococcus mutans* e a frequência de cáries em estudantes de odontologia. Participaram 67 estudantes no primeiro semestre e 54 no acompanhamento no segundo semestre. Questionários autoaplicáveis obtiveram informações sobre hábitos de higiene oral. Realizou-se CPOD para detectar cárie, cultura de placa supragengival do dente hígido 46 e PCR com primers específicos para *S. mutans* e seus sorotipos. O alto consumo de bebidas açucaradas excedeu 74%, a técnica de escovação de Bass foi a mais conhecida e a prevalência geral de cárie foi de 54%; o índice CPOD foi de 3,34 e 3,30 no primeiro e segundo semestres, respectivamente. O número de crescimento de colônias em ágar Mitis Salivarius foi maior no primeiro semestre ($Z=-4,8$, $p<0,0001$). A prevalência de *S. mutans* baseada em cultura foi de 85% e 44% no primeiro e segundo semestres, respectivamente. Em contraste, a confirmação por PCR mostrou taxas de prevalência de 41% e 37% para os mesmos períodos, com o sorotipo c representando 80%. Os estudantes de odontologia exibiram alto consumo de açúcar. A prevalência de cárie e *S. mutans* foi menor que a relatada para a população geral e nenhuma associação clara entre *S. mutans* e cárie foi observada.

Palavras-chave: Cuidados bucais. Sorotipos. *Streptococcus mutans*.

INTRODUCTION

Oral diseases are prevalent in Mexico, and the presence of dental debris and/or calculus is a significant indicator of poor oral hygiene, predisposing individuals to caries and other oral conditions. According to the General Direction of Epidemiology of the Mexican Ministry of Health in 2021, 40% of individuals aged 20 to 24 demonstrated acceptable oral hygiene based on the Simplified Oral Hygiene Index (SOHI). Conversely, 60% exhibited dental debris and/or calculus. Moreover, the caries prevalence in this age group was 83.5% (INEGI, 2020).

Caries development is a chronic, multifactorial disease influenced by factors such as cariogenic diet, poor oral hygiene, limited health education, host susceptibility, and biofilm microbiota composition (Duque-de-Estrada-Riverón, Pérez-Quiñonez & Hidalgo-Gato-Fuentes, 2006; García-Cortés et al., 2014; Petersen et al., 2005). A Brazilian study demonstrates that higher mean income was associated with the lowest risk of dental caries, while lower maternal education and poor self-perception of oral health were associated with an increased risk of dental caries (Ruffo, Toledo & Machado, 2022). Economic crises affect unemployed individuals, who are more likely to experience dental caries and missing teeth. Working-class subjects are at higher risk of tooth extractions, whereas individuals with an intermediate level of education had a higher probability of tooth mobility (Méndez, Román-Montero, Miguel, Rojo & López, 2022).

Health education and oral hygiene have been extensively studied across diverse populations, including dental students, who often exhibit poor oral hygiene with gingival inflammation ranging from 30% to 66% (Aguilar-Díaz et al., 2021; Kawamura, Spadafora, Kim & Komabayashi, 2002, Qian et al., 2022).

The transition from a healthy dental/oral condition to a diseased condition occurs when dental biofilm changes in composition to an acid-producing and acid-tolerant microbial community. Metatranscriptomic studies have revealed tissue specific microbiota. For instance, in tooth enamel, carious lesions show the presence of *Streptococci*, *Rothia*, *Leptotrichia*, and *Veillonella* that are found at higher levels, whereas *Lactobacillus*, *Shlegelella*, *Pseudoramibacter*, and *Atopobium* are more typically associated with dentin lesions. In enamel caries,

Streptococci accounted for 40% of the total community (Simón-Soro, Guillén-Navarro & Mira, 2014).

Studies have identified a relative abundance of *Lactobacillus*, *Actinomyces*, *Prevotella*, and *Mitsuokella* in carious teeth (Marsh, 2018). While *Streptococcus mutans* is implicated in caries development and cariogenic biofilm formation, it constitutes only 0.02–0.73% of the total bacterial community (Marsh, 2018; Simón-Soro et al., 2014). Despite its low relative abundance, *S. mutans* remains a key etiological agent in human dental caries due to its ability to form adherent biofilms and produce lactic acid, which erodes tooth enamel.

S. mutans actively produces glycosyltransferase extracellular enzymes, which are crucial for the synthesis of insoluble extracellular polysaccharides. These polysaccharides play a vital role in biofilm formation, facilitating adherence to the tooth surface (Beighton, 2005; Gómez-García, López-Vidal, Pinto-Cardoso & Aguirre-García, 2022; Kristoffersson et al., 1985; Loesche, 1986; Mattos-Graner, Klein & Smith, 2014; Wu et al., 2022).

S. mutans is classified into four clinical serotypes (*c*, *e*, *f*, and *k*) based on the presence of rhamnose-glucose polysaccharide on its cell wall (Qin et al., 2003; Shibata et al., 2003). Serotypes *c* and *e* account for 90% of clinical cases, with serotype *c* being the most prevalent (Zheng et al., 2023). Serotypes *f* and *k* are more invasive to human endothelial cells. Furthermore, serotype *k* may interfere with platelet aggregation, potentially leading to haemorrhagic strokes (Abranches et al., 2011).

The distribution of *S. mutans* serotypes varies based on race, population, age, and cultural background. For instance, a study of African-American children in rural Alabama reported a 98% prevalence of serotype *c*, which was associated with higher caries scores in older children. Serotype *k* was statistically more prevalent in females (Momeni et al., 2019). In contrast, a study of Iranian and Afghani children found a 47.5% prevalence of serotype *c* and an 8.1% prevalence of serotype *k* (Elyassi, Babaeekhou & Ghane, 2022).

Studies across diverse populations have consistently shown variations in *S. mutans* serotype distribution. In Colombian children, serotype c was the most prevalent at 73%, followed by serotype *f* at 16.3% and serotype *e* at 10.5% (Rincón-Rodríguez, Parada-Sanchez, Bedoya-Correa & Arboleda-Toro, 2019). Similarly, in a young Galician population, serotype *c* predominated at 86%, with serotypes *e*, *k*, and *f* accounting for 8%, 3%, and 2%, respectively (Rosero, Prado, Guirao & Santos, 2020).

Among school children in Córdoba, Argentina, serotype *c* was again the most prevalent at 53.2%, followed by serotypes *e*, *f*, and *k* at 31.9%, 8.5%, and 6.4%, respectively (Carletto-Körber, González-Ittig, Jimenez & Cornejo, 2015). In Japanese children, serotype *c* represented 70-80% of isolates, serotype *e* 20%, and serotypes *f* and *k* less than 5% each (Nakano & Ooshima, 2009). Finally, in diabetic Italian adults, 82% of isolates were serotype *c*, with no serotype *k* detected (Angelis et al., 2016).

Existing data strongly suggest that bacterial diversity and prevalence are significantly influenced by race, age, diet, geography, and cultural background. To date, no studies have been conducted in southeastern Mexico, where Mestizo and Mayan populations predominate. The genetic background of HLA diversity in human populations is relevant because HLA loci can play a role in the immune response to infectious pathogens and inflammation. Recent studies have indicated that the Maya population from Yucatan and Chiapas, Mexico, can be clustered in a specific clade (Barquera et al., 2020; Moreno-Estrada, 2014). This study aimed to determine oral care habits and the prevalence of *S. mutans* and its serotypes in dental students in Campeche, Mexico.

MATERIALS AND METHODS

Sample and study design

This study was conducted at the Faculty of Dentistry of the Autonomous University of Campeche (UACAM), Mexico. Of the 145 first-semester students, 67 agreed to participate, and 54 completed the second-semester follow-up. All volunteers received detailed information about the study before enrollment. Written informed consent was obtained from each participant, outlining the research objectives, professional benefits, and guaranteeing confidentiality. Inclusion criteria included first-semester dental students (baseline) and those continuing into the second-semester follow-up. Exclusion criteria were: autoimmune diseases, immunodeficiency, ongoing dental or antibiotic therapy, pregnancy, and severe periodontal disease.

A non-probability sampling approach was used to select participants. All procedures adhered to the ethical standards of the 1964 Declaration of Helsinki. The research protocol was approved by the UACAM Research and Ethics Committee (registration numbers UACAM-080/UAC/2023 and CBI-08-2023-12). This study followed the STROBE guidelines (Elm et al., 2007).

Self-administered questionnaires collected data on the participants' social, economic, behavioural, and demographic backgrounds. The questionnaires included questions about dietary patterns (e.g., meal descriptions, consumption frequency of sugary drinks and snacks) and oral hygiene habits (e.g., toothbrushing techniques: Bass, modified Bass, Charter, Stillman; frequency of dental flossing and dental visits), as previously described (Lara-Capi et al., 2018).

Caries diagnosis: all participants underwent oral examinations under natural light using a No. five flat mirror. Two qualified dentists independently examined and confirmed the presence of dental caries based on the DMFT index.

Isolation of streptococci from clinical samples

Supragingival plaque was collected from the buccal surface of a healthy tooth number 46 using a sterile curette, scraping the enamel surface, and transferred to a vial containing 500 µl of thioglycolate broth. A 25 µl sample of the inoculated broth was then plated onto Mitis-Salivarius agar (MSA) supplemented with 20% sucrose and bacitracin for the isolation and identification of *S. mutans*.

Mitis-Salivarius agar (MSA) was prepared by dissolving 90 g of MSA and 150 g of sucrose in 1000 ml of distilled water. After autoclaving and cooling to 50 °C, 1000 µl of 1% tellurite solution and 1000 µl of 3 mg/ml bacitracin were added. Agar plates were poured and allowed to solidify overnight at room temperature, followed by a 24-hour incubation at 37 °C to confirm sterility. From the supragingival plaque samples collected in vials containing 500 µl of thioglycolate broth, a 25 µl aliquot was plated onto MSA and incubated in 5% CO_2 at 37 °C for 48 hours.

S. mutans (ATCC 25175) was included as a positive control in each experiment. Following incubation, colonies exhibiting *S. mutans*-like morphology (e.g., frosted glass appearance, irregular margins, black/blue coloration, rooted growth, and star-shaped formations) were enumerated. Colonies with dissimilar morphologies were also counted.

Bacteria Identification

Five colonies with *S. mutans* morphology from each positive plate were selected and transferred to 5 ml of BHI media for 48 hours. After incubation, 2 ml of bacterial suspension was centrifuged and pelleted. The pellet was transferred and

resuspended into another vial with 300 µl lysis solution. The samples were kept frozen at -20 °C until use.

Vials containing lysis solution were incubated with lysozyme for one hour at 37 °C. After adding 80 µl of nuclei lysis solution (Promega), samples were incubated at 80 °C for five minutes, followed by the addition of 60 µl of protein precipitation solution and incubation at 4 °C for five minutes (Promega). Proteins and cell debris were removed by centrifugation at 16,000 × g for three minutes, and the supernatant was transferred to a new vial for DNA precipitation with isopropyl alcohol. The DNA pellet was collected by centrifugation at 16,000 × g for 20 minutes. After washing the DNA with 70% ethanol, the pellet was dissolved and resuspended in 20 µl of DNA solution (Promega) and stored at -20 °C until use.

All samples were checked for the presence of DNA using ribosomal-specific primers. The primers U16S-3-F (5'-TCC TAC GGG AGG CAG CAG T-3') and U16S-4-R (5'-GGA CTA CCA GGG TAT CTA ATCCTG TT-3') were used, which amplify a 466 bp product. PCR conditions were: 95 °C for five minutes (denaturation), followed by 35 cycles of 95 °C for 15 seconds, 55 °C for 45 seconds (annealing), and 72 °C for 45 seconds (extension), with a final extension at 72 °C for ten minutes (Zeng et al., 2020).

Streptococcus mutans identification

Ribosomal-positive samples were further analysed for the presence of *S. mutans* using two sets of specific primers targeting the htrA locus and an intergenic region. The primers Sm479F (5'-TCGCGAAAAAGATAAACAAACA-3') and Sm479R (5'-G CCCCTTCACAGTTGGTTAG-3') were used, which amplify a 479 bp product. PCR conditions were: 95 °C for five minutes (denaturation), followed by 35 cycles of 95 °C for 15 seconds, 55 °C for 30 seconds (annealing), and 72 °C for one minute (extension), with a final extension at 72 °C for ten minutes (Chen et al., 2007).

The second PCR for *S. mutans* was run with primers specific for and designed from the *gtfB* gene. Primers mut3368-F (GCC TAC AGC TCA GAG ATG CTA TTCT) and Smut3481-R (GCC ATA CAC CAC TCA TGA ATT GA) produce an amplicon of 114 bp. The PCR reaction conditions: DNA denaturation at 94 °C for five minutes, followed by 25 cycles of 94 °C for 15 seconds, 55 °C for 30 seconds, and 72 °C for one minute (Yoshida et al., 2003).

Streptococcus mutans serotypes identification

The following primer pairs were used to amplify specific *S. mutans* serotypes: SC-F/SC-R (serotype *c*), producing a 727 bp amplicon; SE-F/SE-R (serotype *e*), producing a 517 bp amplicon; SF-F/SF-R (serotype *f*), producing a 316 bp amplicon; and CEFK-F/CEFK-R (serotype *k*), producing a 294 bp amplicon (Qin, 2003; Shibata, 2003; Zheng, 2023).

The PCR mixture consisted of 0.2 mM of each deoxyribonucleotide triphosphate, 2 mM $MgCl_2$, 10 mM Tris-HCl buffer (pH 8.3), 50 mM KCl, 50 pMol of each primer, 2 µL template DNA, 2.5 U Taq DNA polymerase. The mixture was subjected to initial DNA denaturation at 96 °C for two minutes, and a total of 25 PCR cycles, each cycle consisted of 15 seconds at 96 °C, 30 seconds of annealing at 61 °C and one minute at 72 °C.

PCR products were analysed by agarose gel electrophoresis. Amplicons larger than 300 bp were resolved on 1.5% agarose gels, while amplicons smaller than 200 bp were resolved on 3% agarose gels. A 15 µL sample was loaded into each well and electrophoresis was performed at 80 mA and 100 V for 30-45 minutes. Each gel included a blank and *S. mutans* ATCC 25175 as a positive control. Gels were stained with ethidium bromide, destained, and visualized under UV light.

Statistical analysis

Descriptive data are presented as percentages and frequencies. Non-parametric comparisons were made using the chi-squared test for categorical variables (significance level $p<0.05$). Bivariate analysis such as Wilcoxon for ordinal and quantitative paired data; data were analyzed with the SPSS program (SPSS version 15) and GraphPad Prism (version 10.2).

RESULTS AND DISCUSSION

Sixty-seven first-year students initially participated, with 54 completing the follow-up. The median age was 18.1 years (SD ± 1.09) and 18.7 years (SD ± 1.13) for the initial and follow-up groups, respectively. In the first semester, 40 (60%) participants were female and 27 (40%) were male. In the follow-up, 67% were female and 33% were male.

Dental and medical students at our public university have traditionally come from middle to high-income backgrounds, and only 10% of them studying and working concurrently. Despite this population generally experiencing a "better" lifestyle, their health and dietary habits remain influenced by the surrounding culture. In our sample studied, 12% recognized themselves as Maya and the rest of students were Mestizos. Within the study area, individuals of Maya descent continue to be a significant demographic, reaching up to 60% of native speakers in certain regions, yet constitute 10% overall.

In the Yucatan Peninsula, Mexico, the two main population groups are Mestizo and Maya descendants. The genetic background of HLA diversity in human populations is relevant because HLA loci can play a role in the immune response to infectious pathogens and inflammation. Recent studies have indicated that the Maya population from Yucatan and Chiapas, Mexico, can be clustered in a specific clade (Barquera et al., 2020; Moreno-Estrada, 2014). Notably, during the recent COVID-19 pandemic, protective HLA alleles against severe COVID-19 were reported in the Maya population (Hernández-Doño et al., 2022). On the other hand, patients possessing autochthonous Amerindian alleles from Chiapas, Mexico, demonstrated a propensity for elevated rates of Systemic Lupus Erythematosus (García-Silva et al., 2021). Therefore, our study is important in light of the fact that the prevalence of *S. mutans* and its serotypes in Mestizo and Maya from Yucatan, Mexico, has not yet been documented.

Over 74% of students reported having a strong habit of drinking sugary beverages in the first semester, which decreased to 58% in the follow-up. Unfortunately, Mexico has the highest Coca-Cola™ consumption globally. In our study population, 43.9% reported drinking this type of sugary beverage, followed by black tea (19.5%), a regional drink. Less than 10% drink sugary beverages in the morning, but nearly 70% of students reported consuming them at night. This habit may influence microorganism growth in individuals with poor oral hygiene and the progression of caries.

Then, a higher number of *S. mutans* colonies grew on Mitis-Salivarius agar in first-semester students compared to the assessment six months later. The Wilcoxon test demonstrated a statistically significant difference ($p<0.0001$). Likewise, the timing of their sugary beverage intake showed no significant difference ($p = 0.87$). Despite a decrease in sugary beverage consumption during the second semester, no statistically significant difference was observed (Wilcoxon test, $p = 0.1$). Consequently, the data suggests that these habits did not substantially change overall.

In contrast, more than 60% of dental students seldom

seek professional dental care, while less than 5% reported frequent utilization of dental services. When questioned about brushing techniques, the Bass technique was identified as the most prevalent method known and practiced, followed by the Stillman technique. When comparing the oral health habits mentioned above, no statistically significant difference was observed in seeking dental care or brushing technique ($p = 0.86$).

Regarding brushing frequency, 50% of first-semester students reported brushing after meals. Nevertheless, this practice declined considerably to 5.5% in the second semester, though this change was not statistically significant ($p = 0.15$). The observed decrease in the growth of *S. mutans*-like colonies in the second semester likely resulted from these habit changes, even though it did not reach statistical significance.

The prevalence of caries was 54% among the dental students examined (Table 1). The DMFT index for first-semester students was 3.34 and 3.30 for second-semester ones, which is considered moderate. Statistical analysis indicated no significant association between caries occurrence and the frequency of sugary beverage intake, candy consumption, or utilization of dental services.

When comparing dietary habits between first and second-semester students, a significant increase in candy consumption was observed exclusively in the second-semester group (Wilcoxon test: $Z = -5.6$, $p < 0.05$). The absence of significant differences might be due to the sample size, the duration of the observation, and the specific population studied, which demonstrated to have some oral health awareness.

Individuals with inadequate oral hygiene are susceptible to developing oral diseases. Nevertheless, enhanced knowledge and heightened awareness regarding the risks of poor oral hygiene can potentially mitigate oral health issues. Among the first and second-semester dental students studied, the prevalence of dental caries was 54%, which is significantly lower than the national average of 83% reported in Mexico (INEGI, 2020). This difference may be because our sample belongs to a very specific group such as dental students.

Several studies have explored the knowledge, practices, and perceptions of oral health among undergraduate dental students in Mexico, exemplified by a study published in 2021 (Aguilar-Díaz et al., 2021). Specifically, Aguilar-Díaz et al. (2021) found that second-year students exhibited a higher frequency of preventive practices, which subsequently declined as students advanced in their academic program. However, in our study we did not observe statistical differences in this issue.

The presence of *S. mutans*-like colonies, characterized by a frosted glass appearance, irregular margins, dark blue coloration, rooted growth into the agar, and a star-shaped morphology (Figure 1), was assessed on mitis-salivarius-bacitracin (MSB) agar.

In the initial cohort of first-year students, 85% (57/67) exhibited growth. Six months later, in the follow-up group, the detection rate decreased significantly to 44% (24/54). Additionally, the total colony count on MSB agar was substantially greater in the first-year student group compared to the follow-up group. This difference was statistically significant, as determined by the Wilcoxon test ($Z = -4.8$, $p < 0.0001$). Even with the understanding that not all colonies were necessarily *S. mutans*, it was clear that by the second semester, the students showed less bacterial development akin to *S. mutans.*

Notably, the prevalence of *S. mutans*-like colonies was observed to be 85% among first-semester dental students, subsequently decreasing to 44% six months later. Although mitis-salivarius-bacitracin (MSB) agar is widely employed in both clinical and epidemiological investigations, it is not selective for *S. mutans*. Consequently, other bacterial species, such as *S. sobrinus*, *S. anginosus*, *Phytobacter*, *S. epidermidis*, and *E. kobei*, are capable of growth on MSB agar.

Table 1
Demographic oral hygiene and eating habits data in undergraduate dental students.

Variable	Category	First semester (67 students)	Second semester (54 students)
Age	(Years, X+/-SD)	18.17 +/- 1.09	18.78 +/- 1.13
Sex	Female	40 (60%)	36 (67%)
	Male	27 (40%)	18 (33%)
Frequency of sugary drink intake	Scarcely	17 (25%)	22 (40.7%)
	Highly	39 (58%)	23 (42.5%)
	Frequently	11 (16%)	9 (16.6%)
Frequency of candy intake	Scarcely	45 (67%)	37 (68.5%)
	Highly	20 (29.8%)	14 (25.9%)
	Frequently	2 (3%)	3 (5.5%)
Day-time intake	Morning	9 (13%)	4 (7.4%)
	Afternoon	16 (23.8%)	13 (24.0%)
	Night	42 (62.6%)	37 (68.5%)
Dental assistance	No	8 (13.4%)	6 (11.1%)
	Seldom	34 (50.7%)	22 (40.7%)
	Regular	21 (31%)	23 (40.7%)
	Frequent	5 (7.4%)	3 (5.5%)
Brushing technique	Bass	43 (64%)	34 (62.9%)
	Charters	6 (9%)	4 (7.4%)
	Bass modified	7 (10%)	7 (12.9%)
	Stillman	11 (16%)	9 (16.6%)
Frequency of brushing	Two times a day	24 (35%)	22 (40.7%)
	After meals	35 (52%)	3 (5.5%)
	Three times a day	8 (11.9%)	23 (42.5%)
	None	-	6 (11.1%)
Caries	Yes	54%	54%

Source: The authors.

Consequently, it is imperative to exercise caution during the quantification of colony-forming units (CFU) to avoid the potential overestimation of *S. mutans* populations, given that not all colonies developing on MSB agar are attributable to *S. mutans* and the use of molecular means such as PCR is helpful (Zeng et al., 2020).

Furthermore, research has indicated that approximately 57% of CFUs observed on MSB agar may correspond to non-*S. mutans* streptococcal species (Shibata et al., 2003). Our study confirmed *S. mutans*-like colonies via PCR. A comparison of the two procedures revealed 100% sensitivity, 73% specificity, a positive predictive value of 56%, and a negative predictive value of 100%.

Contrary to expectations, there was no statistically significant correlation or difference between the presence of caries and the presence of *S. mutans*. However, the paradigm of caries etiology has shifted: it is no longer attributed to a single pathogen (like *S. mutans*), but is now understood as a consequence of ecological dysbiosis in the oral microbiome.

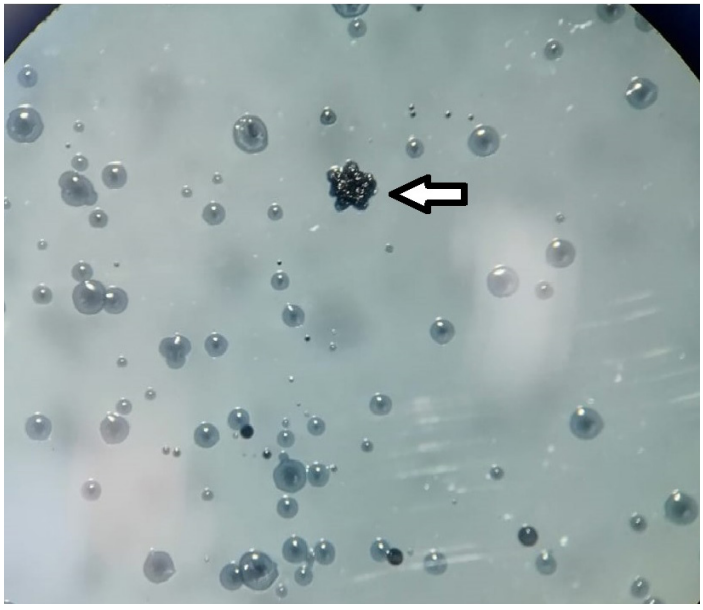
Frequent sugar intake drives the selection of acidogenic and acidophilic species, resulting in decreased microbial diversity and in an acidic environment that promotes demineralization. Consequently, prevention and treatment strategies are evolving to focus on restoring oral homeostasis, moving away from complete microbial elimination via antibiotics or targeting specific pathogens. This involves fostering a healthy microbiota and modulating the oral environment to enhance remineralization

and control pathogenic growth (Ballón-Salcedo, Cacya-Apaza, & Valdivia-Silva, 2021).

Bacterial colonies were subjected to PCR analysis using primer sets targeting the *S. mutans gtfB* gene and the *htrA* locus. In the initial of first-semester dental students, the *gtfB* gene was detected in 40.2% (27/67) of samples, resulting in a 114 bp amplicon. The *htrA* locus was identified in 35.8% (24/67) of samples, generating a 479 bp amplicon. Six months later, the prevalence decreased significantly, with the *gtfB* gene detected in 22.2% (12/54) and the *htrA* locus in 18.5% (10/54) of samples (Figure 2).

Figure 1

Representative images of *Streptococcus mutans*-like colony growth on mitis-salivarius-bacitracin (MSB) agar from supragingival plaque from healthy teeth.



Source: The authors.

Note. Colonies with an aspect of frosted glass, irregular margin, black blue, rooted into the agar, and star-shaped were considered as *S. mutans*.

Consequently, molecular diagnostic tools, such as PCR employing primers specific for *S. mutans* genes, represent a more accurate and reliable approach compared to MSB agar, which exhibits a positive predictive value of 56%. Utilizing specific PCR assays, our investigation demonstrated an *S. mutans* prevalence of 41% in first-semester students, which decreased to 37% after a six-month interval. In a comparative context, a recent study reported a 66.7% prevalence of *S. mutans* in Indian adults aged 18-35 years old with dental caries and a 42.7% prevalence in individuals without dental caries (Praveen et al., 2024).

The observed prevalence of *S. mutans* in our sample is lower than what has been previously documented in other populations. A potential explanation for this discrepancy lies in the differing genetic backgrounds and/or oral health, social, and economic variables when compared to dental students.

The total percentage of positive PCR tests was 41.7% (28 out of 67) for first-semester students and 24% (13 out of 54) for second-semester students. PCR-based identification of *S. mutans* using primers targeting the *gtfB* gene exhibited greater sensitivity compared to primers targeting the *htrA* locus.

Our investigation revealed an overall *S. mutans* prevalence of 39% and a dental caries prevalence of 54% among Mexican undergraduate dental students. However, a significant observation was that only 21% of students diagnosed with caries also exhibited the presence of *S. mutans*.

This data suggests that the prevalence of *S. mutans* is heavily dependent on the specific population under study. In contrast, data from the General Direction of Epidemiology of the Mexican Ministry of Health (INEGI, 2020) indicates a dental caries prevalence of 83.5% in the same age demographic.

These contrasting findings reinforce the notion that *S. mutans* prevalence is significantly affected by the level of background oral health education.

Following the isolation of colonies that exhibited positive results for either the *S. mutans gtfB* gene or *htrA* locus, PCR amplification was performed for serotype identification.

Serotype *c* was found to be the predominant serotype, accounting for 75% of isolates in first-semester students and 85% in second-semester students, generating a 727 bp amplicon. Serotype *e* was identified in 25% and 15% of isolates, respectively, resulting in a 517 bp amplicon.

Notably, serotypes *f* and *k* were not detected among the studied population (Figure 3). In our study, serotype *c* was identified as the predominant serotype, with prevalence rates ranging from 75% to 85%. Serotype *e*, conversely, exhibited prevalence rates between 15% and 25%.

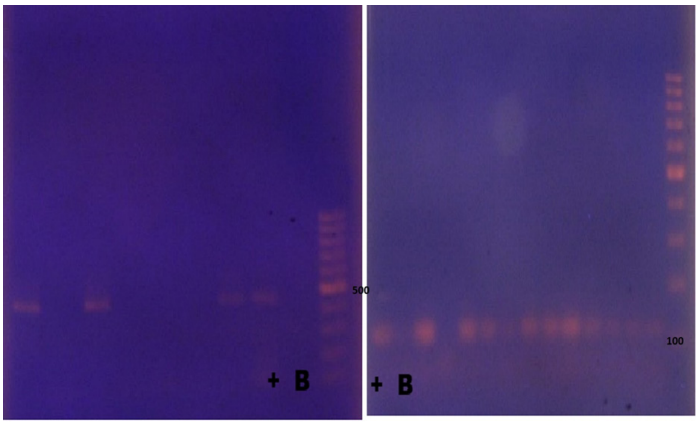
Our observed data closely resemble findings reported in Japanese populations, where serotype *c* prevalence ranges from 70% to 80% (Nakano & Ooshima, 2009), and in Galician populations, with a reported serotype c prevalence of 86% (Rosero et al., 2020). However, our results diverge from those observed in Iranian and Argentinian populations, where serotype *c* prevalence was reported as 47.5% and 53.2%, respectively (Carletto-Körber et al., 2015; Elyassi et al., 2022).

These findings collectively support the premise that the prevalence of *S. mutans* serotypes is significantly influenced by factors such as race, age, geographical location, and cultural background.

Despite the absence of conclusive evidence regarding the clinical relevance of *S. mutans* serotypes, existing research indicates that serotype variation or genetic background may play a role in modulating biofilm formation, specifically concerning aciduric properties and starvation responses (Bedoya-Correa, Rincón-Rodríguez & Parada-Sanchez, 2021).

Figure 2

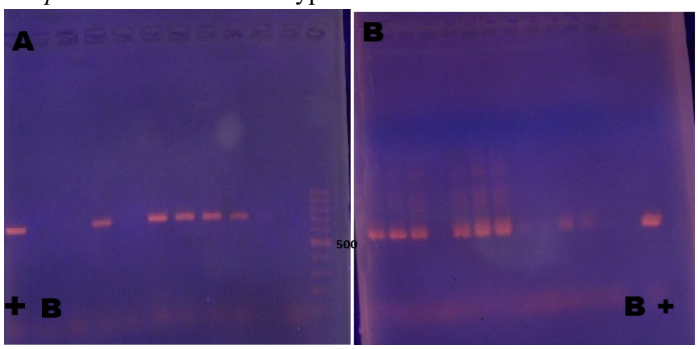
Representative images of PCR amplification products from *Streptococcus mutans*-like colony growth on mitis-salivarius-bacitracin (MSB) agar.



Source: The authors.

Note. In the left image, amplicon 479 bp using set of primers targeting *htrA* locus; in the right image, amplicon 114 bp using primers specific for *gtfB* gene. Blank: B and + Positive control.

Figure 3
Representative images of PCR amplification products for *Streptococcus mutans* serotypes.



Source: The authors.
Notes. The left panel corresponds to serotype *c* which produces an amplicon of 727 bp; the right panel corresponds to serotype *e* which produces an amplicon of 517 bp. Blank: B and + Positive control.

CONCLUSION

This preliminary study was carried out in a sample population with Maya and Mestizo background in undergraduate dental students from southeastern Mexico who demonstrated suboptimal dietary habits, characterized by elevated sugar consumption. PCR confirmed half of *S. mutans*-like colonies for overall prevalence of 39%. The observed caries prevalence of 54% was notably lower than the 83% reported for the general population. Furthermore, no discernible correlation was established between *S. mutans* presence and caries occurrence, probably due to the size of the sample studied.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: F. L. P., V. M. *Data curation*: J. C. C. *Formal analysis*: V. M. *Investigation*: F. L. P., S. V. E. *Methodology*: S. V. E., V. M. *Project administration*: V. M. *Resources*: J. C. C. *Supervision*: V. M. *Validation*: J. C. C., V. M. *Writing the initial draft*: F. L. P. *Revision and editing of writing*: J. C. C.

PEER REVIEW

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