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# Identity Detection of *Streptococcus Mutans* in Saliva Using PCR Technique

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# Identity Detection of *Streptococcus Mutans* in Saliva Using PCR Technique

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## Abstract

The purpose of this paper was to detect *Streptococcus mutans* in saliva in a group of students of the Faculty of Natural Sciences through the PCR technique. The oral cavity is a dynamic environment that undergoes large and rapid fluctuations in pH, nutrient source and availability, oxygen tension, temperature, and osmolality. The ability of *Streptococcus mutans* to survive in such an environment is attributed to its ability to produce acids as well as glucans from carbohydrates, implicating it in the etiology and progression of dental caries. Detection of *Streptococcus mutans* using rapid, sensitive and specific methods is very important for early diagnosis and effective assessment of an individual's risk for dental caries. In this study, 30 students, aged 18-20, participated voluntarily, where the average age is 19.07 years, with a standard deviation of 0.57. From analyzing the saliva samples, we found that 86.7% (26/30), SE =0.033, 95%CI (83.7%-89.7%), of them were positive for *S. mutans*. From the analysis of the $\chi 2$ test to see the relationship between the variables taken in the study and the presence of *S. mutans*, we did not find reliable relationships. PCR, as a molecular biology method, proved to be quite efficient, sensitive and rapid for the detection of *Streptococcus mutans* from saliva.

## Introduction

In 1924, J. Clarke isolated an organism from carious lesions and named it *Streptococcus mutans* because he thought that the oval-shaped cells observed were mutant forms of streptococcus (Clarke JK. 1924). However, it was in the late 1950s that *S. mutans* gained widespread attention within the scientific community and, by the mid-1960s, clinical laboratory and animal-based studies described *S. mutans* as an important etiological agent in dental caries (Loesche WJ. 1986).

There are more than 1,000 bacterial species that exist within the oral cavity, where *Streptococcus mutans* (SM) result most associated with dental caries. In humans, *S. mutans* has been implicated in the etiology and progression of dental caries by producing extracellular polysaccharides, which facilitate microbial adhesion and plaque or biofilm formation on the tooth surface. *S. mutans* metabolizes many types of sugars to produce acid, mainly lactic acid that demineralizes tooth structure leading to tooth decay (Wade WG. 2012).

Research into the identification and detection of *S. mutans* through DNA-based methods, including the PCR technique, has resulted in the development of several DNA-based probes and primers. Most primers are designed

to target specific sequences of genes associated with virulence, such as glucosyltransferases or dextranases, or highly conserved sequences of bacterial 16S rRNA genes (Oho T., *et al*., 2000). Acquiring knowledge and attitudes about dental health as well as the prevention, control and treatment of dental problems is very important not only for students but for the entire population.

## Material and Methods

A total of 30 students, 24 women and 6 men of the Department of Biology, Faculty of Natural Sciences, University of Tirana, participated in this study voluntarily, in the period March-April 2022. After obtaining consent, the participants who met the inclusion criteria were asked to donate a saliva sample and to complete a questionnaire on their demographic data as well as questions on their dental hygiene while maintaining anonymity.

The analysis of the samples was carried out in the Laboratory of Molecular Biology, Department of Biology, FNS, UT. Saliva samples were collected between 9 and 10 a.m. from individuals who had brushed their teeth after breakfast and had not consumed any food or drink for at least one hour before the examination. Saliva samples were collected using dry sterile swabs, applying gentle pressure to the space between the cheek and teeth on both sides. After taking the samples, it was immediately transferred to the Molecular Biology laboratory, FNS, UT, to extract DNA within the day. Bacterial DNA was extracted using the NucleoSpin® Tissue kit (Macherey–Nagel) according to the instructions described in the manual.

PCR analysis of saliva samples was performed with the ProFlex™ 96-well PCR System, using primers GtfB-F (5´-AGC CAT GCG CAA TCA ACA GGT T-3´) and GtfB-R (5 ´-CGC AAC GCG AAC ATC TTG ATC AG-3´), which were designed to amplify a 517 bp fragment of the S. mutans gtfB gene. The PCR reaction was performed in 20 µL volume containing 2 µL DNA, 4 µL dNTP, 1 µL of each primer, 1 µL Taq DNA Polymerase, and double-distilled water. The reaction conditions were as follows: 95°C for 1 minute (preheating and enzyme activation), followed by 30 cycles of 95°C for 10 seconds (denaturation), 58°C for 10 seconds (annealing of primers), 72°C for 10 seconds (extension), and a final extension at 72°C for 5 minutes (Lee YJ., *et al*., 2019). PCR products were analyzed by 1% agarose gel electrophoresis.

SPSS (Statistical Package for Social Sciences) version 16 for Windows was used for data processing. After processing the data for all students, the variables for descriptive statistics were obtained from the questionnaire and are age, gender, age, smoking, dental, frequency of dental visit, frequency of tooth brushing, as well as the way of transmission of dental caries. To see the relationship of the presence of *S. mutans* infection with the variables taken in the study, was used $\chi 2$ test (we accept as significant result for p-value < 0.05).

## Results

In this study, 30 students, aged 18-20, participated voluntarily, where the average age is 19.07 years, with a standard deviation of 0.57. From analyzing the saliva samples, we found that 86.7% (26/30), SE =0.033, 95%CI (83.7%-89.7%), of them were positive for *S. mutans*. The number of positive cases for *S. mutans* is higher among

students aged 19 with 17 positive cases out of 26 cases in total.

Table 1 shows the distribution of positive cases with *S. mutans* according to sociodemographic and dental care characteristics, smoking and knowledge about caries transmission.

Table 1: Distribution of Positive Cases with *S. mutans*

| Variable | N (%) | Positive case (%) | P |
|---|---|---|---|
| **Age in years** | | | |
| 18 | 4 (13.3) | 3 (75.0) | 0.486 |
| 19 | 20 (66.7) | 17 (85.0) | ($\chi$2=1.442) |
| 20 | 6 (20.0) | 6 (100) | |
| **Gender** | | | |
| Female | 24 (80.0) | 21 (87.5) | 0.788 |
| Male | 6 (20.0) | 5 (83.3) | ($\chi$2=0.072) |
| **Residence** | | | |
| Rural | 5 (16.7) | 5 (100) | 0.337 |
| Urban | 25 (83.3) | 21 (84.0) | ($\chi$2=0.923) |
| **Smoking** | | | 0.107 |
| Yes | 6 (20.0) | 4 (66.7) | ($\chi$2=2.596) |
| No | 24 (80.0) | 22 (91.7) | |
| **Dental visit** | | | |
| Yes | 29 (96.7) | 25 (86.2) | 0.690 |
| No | 1 (3.3) | 1 (100) | ($\chi$2=0.159) |
| **Frequency of dental visit** | | | |
| In an interval of less than 6 months | 1 (3.45) | 1 (100) | |
| Every 6 months | 10 (34.5) | 8 (80.0) | 0.584 |
| Once a year | 7 (24.1) | 7 (100) | ($\chi$2=1.947) |
| In an interval greater than one year | 11 (37.9) | 10 (90.9) | |
| **Frequency of tooth brushing** | | | |
| Once a day | 10 (33.3) | 10 (100) | |
| Twice a day | 19 (63.3) | 15 (78.9) | 0.263 |
| After every meal | 1 (3.33) | 1 (100) | ($\chi$2=2.672) |
| Never | 0 (0) | 0 (0) | |
| **Transmission of caries from one person to another** | | | |
| Yes | 3 (10.0) | 3 (100) | 0.159 |
| No | 17 (56.7) | 16 (94.1) | ($\chi$2=3.682) |
| I don’t know | 10 (33.3) | 7 (70.0) | |

The analysis showed that the association of sociodemographic characteristics such as age, gender and place of residence with the respective values (p=0.486), (p=0.788) and (p=0.337), with the presence of *S. mutans* isn’t significant. Also, we didn’t find any significant between dental care characteristics such as dental visit, the frequency of dental visit, and the frequency of tooth brushing, with the respective values (p=0.690), (p=0.584) and (p=0.263), with the presence of *S. mutans*.

In our study, 20% of the students stated that they are smokers and 66.7% of them tested positive for S. mutans,

while 80% of them stated that they did not smoke and tested positive in 22 cases. The analysis showed that smoking (p=0.107) has no significant relationship with the presence of S. mutans.

10% of students think that caries can be transmitted from one person to another, 56.7% of students think that it cannot be transmitted from one person to another and 33.3% of students do not know if it can be transmitted from one person to another. The presence of S. mutans is in 100% of students who think that caries is transmitted from one person to another, in 94.1% of students who think that it cannot be transmitted from one person to another and 70% of students who do not know if it can be transmitted from one person to another.

Figure 1 shows that there is a marked difference between male and female students in relation to the frequency of visits to the dentist, where we see that all males visit a ratio greater than one year, i.e., 100%. While the results obtained by women alternate more between every 6 months, once a year and at an interval greater than a year.

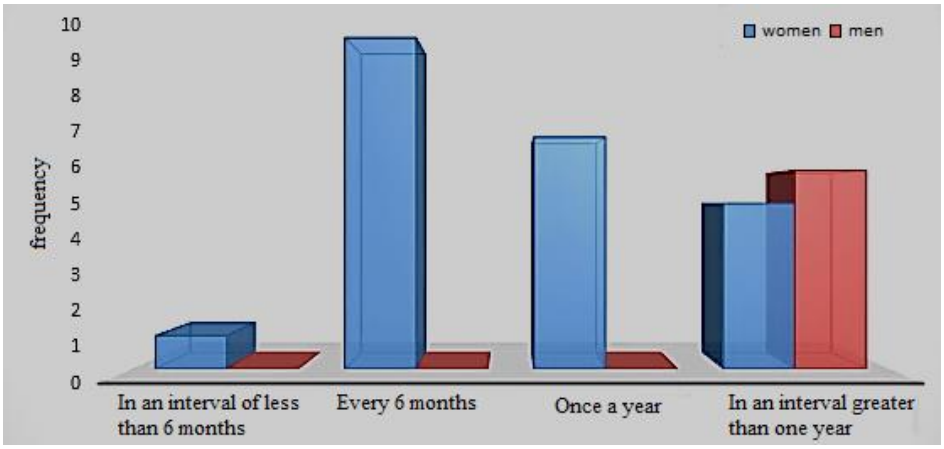


Figure 1. Distribution of Responses to the Frequency of Going to the Dentist according to Gender

Regarding tooth brushing, as shown in Figure 2, 66.7% of female students stated that they brush their teeth twice a day, while 50% of male students stated that they brush their teeth twice a day and 50% that they brush their teeth once a day.

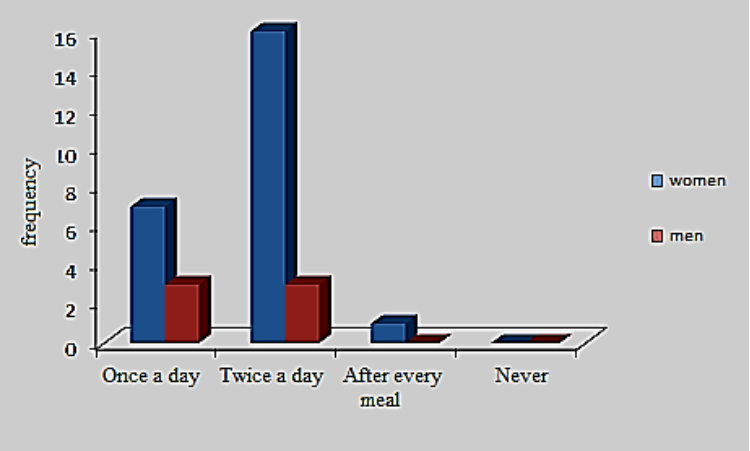


Figure 2. Distribution of Responses to the Frequency of Tooth Brushing by Gender

## Discussion and Conclusion

Compared to other works, the presence of *S. mutans* in adults in our sample is within the reported values, where the percentage of the presence of *S. mutans* in these works' ranges from 59.2% to 88.9% (Vieira AR *et al*., 2011; Lee YJ., et al., 2019; Pannu PK *et al.*, 2013). The microbiota of the oral cavity reaches a certain level of stability in adulthood; however, its condition can be easily disrupted by insufficient oral hygiene and tooth loss (Stuz˙ycka I. 2014; He XS & She WY, 2009).

Many studies have focused on the link between smoking and increased risk of dental caries. Chanea *et al*., 2014, in the study conducted found that the adherence of *S. mutans* increased significantly in the presence of nicotine. Also, the effects of nicotine and tobacco on oral microorganisms and the relationship with caries have been reported in other works, but more research is needed to confirm the relationship between smoking and the growth of cariogenic bacteria in the mouth (Ashkanane A *et al*., 2017; Alzayer YM *et al*., 2018; Jiang X *et al*., 2019).

Different studies support the hypothesis that women show greater care for oral hygiene than men, this difference is related to the fact that women usually care more about their body and appearance and therefore, they may be more worried about adoption of behaviors and habits, which promote their dental health (Mamai-Homata E., *et al*., 2016; Kateeb E, 2010).

Although the transmissible nature of dental caries is explained in the literature, little is known about whether information about this issue is correctly provided to the population. Knowledge of the transmission of caries from one person to another is at a very low level in our sample of students, where only 10% of them answered correctly, compared to other studies of this nature where higher percentages ranging from 25.2% to 29% (Díaz-Reissner CV *et al*., 2016; Soares Ferreira JM *et al*., 2005).

Detection of *Streptococcus mutans* using rapid, sensitive and specific methods is very important for early diagnosis and effective assessment of an individual's risk for dental caries. In the sample of 30 analyzed samples, 86.7% of them were positive for *S. mutans*, value similar with other works. From the analysis of the $\chi 2$ test to see the relationship between the variables taken in the study and the presence of *S. mutans*, we did not find a significant relationship. PCR as a method of molecular biology proved to be quite efficient, sensitive and fast for the detection of *Streptococcus mutans* from saliva.

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## References

Alzayer, Y. M., Gomez, G. F., Eckert, G. J., Levon, J. A., Gregory, R. L. (2018). The Impact of Nicotine and Cigarette Smoke Condensate on Metabolic Activity and Biofilm Formation of\r, Candida albicans\r, on

Acrylic Denture Material. *Journal of Prosthodontics.*

Ashkanane, A., Gomez, G. F., Levon, J., Windsor, L. J., Eckert, G. J., Gregory, R. L. (2017). Nicotine Upregulates Coaggregation of Candida albicans and Streptococcus mutans. *Journal of Prosthodontics Official Journal of the American College of Prosthodontists. (Suppl 1).*

Chanea, K., Palmire, A. (2014). *Smoking and Candy on Oral Bacteria, Streptococcus mutans,* Adherence. California State Polytechnic University Pomona.

Clarke, J. K. (1924). On the bacterial factor in the aetiology of dental caries. *British journal of experimental pathology, 5(*3), 141.

Díaz-Reissner, C.V., Pérez-Bajarano, N. M., Sanabria-Vazquez, D. A., Ferreira Gaoun, M. I., Cueto Gonzalez, N. C., Urquhart-Bareto, D. A., Arce-Mena, M. M., Ledesma-Amarrila, L. E. (2016). Nivel de conocimiento sobre prevención de caries dental en universitarios. *CES Odontología, 29*(1), 14-21.

He, X. S.; Shi, W. Y. (2009). Oral microbiology: Past, present and future. Int. J. Oral Sci. 1, 47–58.

Jiang, X., Jiang, X., Wang, Y., Huang, R. (2019). Correlation between tobacco smoking and dental caries: A systematic review and meta-analysis. *Tobacco Induced Diseases*. 17. 10.

Kateeb, E. (2010). Gender-specific oral health attitudes and behavior among dental students in Palestine. *East Mediterr Health J. 16*:329–33.

Lee, Y. J., Kim, M., Kim, J., Kim, J. (2019). Detection of Streptococcus mutans in human saliva and plaque using selective media, polymerase chain reaction, and monoclonal antibodies. *Oral Biol Res 43*:121-129.

Loesche, W. J. (1986). Role of Streptococcus mutans in human dental decay. *Microbiol Rev 50*:353–380.

Mamai-Homata, E., Koletsi-Kounari, H., & Margaritis, V. (2016). Gender differences in oral health status and behavior of Greek dental students: A meta-analysis of 1981, 2000, and 2010 data. *Journal of International Society of Preventive & Community Dentistry, 6*(1), 60–68.

Oho, T., Yamashita, Y., Shimazaki, Y., Kushiyama, M., Koga, T. (2000). Simple and rapid detectionof Streptococcus mutans and Streptococcus sobrinus in human saliva by polymerase chain reaction. *Oral Microbiol Immunol 15*(4):258-62.

Pannu, P. K., Gambhir, R. S., & Sujlana, A. (2013). Correlation between the salivary Streptococcus mutans levels and dental caries experience in adult population of Chandigarh, India. *European Journal of Dentistry, 7,* 191 - 195.

Soares Ferreira, J. M., de Lima Targino, A. C., Soares Forte, F. D., Corréia Sampaio, F. (2005). Conhecimento de alunos concluintes de Pedagogia sobre saúde bucal. *Interface - Comun Saúde Educ. 9*(17):381-388.

Stuz˙ycka, I. (2014) The oral mikrobiome in dental caries. Pol. J. Microbiol. 63, 127–135.

Vieira, A. R., Deeley, K. B., Callahan, N. F., Noel, J.B., Anjomshoaa, I., Carricato, W. M., Schulhof, L. P., Desensi, R. S., Gandhi, P., Resick, J. M., Brandon, C. A., Rozhon, C., Patir, A., Yildirim, M., Poletta, F. A., Mereb, J. C., Letra, A., Menezes, R., Wendell, S., Lopez-Camelo, J. S., Castilla, E. E., Orioli, I. M., Seymen, F., Weyant, R. J., Crout, R., McNeil, D.W., Modesto, A., Marazita, M. L. (2011). *Detection of Streptococcus mutans Genomic DNA in Human DNA Samples Extracted from Saliva and Blood.* ISRN Dent. 2011:543561.

Wade, W. G. (2013). The oral microbiome in health and disease. *Pharmacol Res 69*:137-143.

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