DOI: 10.7860/JCDR/2025/74884.21469

Original Article

Microbiology Section

# Frequency of *spaP* Genetic Determinants in *Streptococcus mutans* Isolated from Patients with Oral Premalignant Disorders: A Cross-sectional Study

K PREETHI[1], AS SMILINE GIRIJA[2], J VIJAYASHREE PRIYADHARSINI[3], KANNIKA PARAMESHWARI KANNAN[4]




## ABSTRACT

**Introduction:** The surface-associated Protein (*spaP*) of *Streptococcus mutans* is crucial for its virulence, aiding in adherence and biofilm formation. This study explores the prevalence of the *spaP* gene in clinical strains of *S. mutans* to understand its potential role in the progression of Oral Premalignant Disorders (OPMD).

**Aim:** To phenotypically characterise *S. mutans* from clinical samples of patients with OPMD and to assess the frequency of *spaP* genes in clinical strains of *S. mutans*.

**Materials and Methods:** This cross-sectional study was conducted at the Department of Microbiology, Saveetha Dental College and Hospitals, Chennai, India, from January 2023 to April 2023. A total of 60 saliva samples were randomly collected from three groups: Group 1 (OPMD, n=20), Group 2 (healthy individuals with dental caries, n=20), and Group 3 (healthy individuals without caries, n=20). The primary inclusion criteria were participants within these three groups, with exclusions for those with systemic diseases, ongoing infections, or recent antibiotic/antifungal use (within the last three months). Saliva samples were cultured on Mutans Sanguis Agar (MSA), and colonies were characterised through Gram staining and catalase testing. Genomic Deoxyribonucleic Acid (DNA) was extracted, and Polymerase Chain Reaction (PCR) was performed to detect the *spaP* gene. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) v25.0, with significance considered at $p<0.05$. Demographic parameters such as age, sex, and clinical status were analysed.

**Results:** The prevalence of *S. mutans* was found to be 45% (n=9) in OPMD patients, 40% (n=8) in healthy individuals with caries, and 15% (n=3) in healthy individuals without caries. The frequency of the *spaP* gene was found to be 88% (n=8) in OPMD patients and 75% (n=6) in healthy individuals with caries.

**Conclusion:** The comparative evaluation of the prevalence of *S. mutans* and the frequency of the *spaP* gene among the strains suggests a significant role of the *spaP* gene in association with both caries and OPMD conditions.


**Keywords:** Oral health, Oral leukoplakia, Surface-associated protein, Virulence

## INTRODUCTION

OPMD is a term used to describe a group of conditions that have the potential to progress into oral cancer if left untreated. OPMD often refers to conditions marked by changes in oral tissue that possess an elevated risk of developing into cancer compared to normal oral mucosa [1,2]. The rates of transformation into malignancy differ among various OPMD subtypes, including Oral Leukoplakia (OL), Oral Erythroplakia (OE), Proliferative Verrucous Leukoplakia (PVL), Oral Submucous Fibrosis (OSMF), Oral Lichen Planus (OLP), and Actinic Chelitis (AC). Certain prognostic factors, including clinical presentation and the presence of epithelial characteristics, impact the probability of malignant transformation [3].

In the last ten years, there has been a notable increase in literature aimed at understanding the complexities of microbial behavior, particularly concerning *Streptococcus mutans*. There is a strong desire for in-depth research due to the realisation of its importance for oral health and possible connections to OPMD. The microbial composition of the oral cavity in patients with OPMD may undergo changes encompassing *Streptococcus* spp., *Candida* spp., *Prevotella* spp., *Fusobacterium* spp., etc. Metagenome sequencing findings from punch biopsies in a previous study offer intriguing insights into the oral microbiome of patients with OPMD [4]. Notably, the bacterial composition in healthy oral mucosa revealed distinct phyla such as *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Actinobacteria*, and *Bacteroides*.

In this context, *Streptococcus mutans* is a bacterium commonly found in the human oral cavity, considered one of the primary causative agents of dental caries and part of the functional oral microbiome [5]. The key factors contributing to the cariogenicity of *S. mutans* involve adhesion, acidogenicity, and acid tolerance. These factors collaboratively alter the physical and chemical properties of the biofilm, leading to ecological shifts marked by higher proportions of *S. mutans* and other acidogenic and aciduric species. Additionally, observations of elevated numbers of *S. mutans* genotypes have been noted in amplifying the risk of the disease [6].

Major virulent factors of *S. mutans* include the production of glucosyltransferases (*gtf* gene), fructosyltransferases (*ftf* gene), extracellular polysaccharides, increased surface adherence (*spaP* gene), acidogenicity, and acidurity. Among these virulent factors, *spaP* is a cell surface-associated antigen that plays a role in bacterial adherence and biofilm formation. The *spaP* protein in *S. mutans* is a member of the antigen I/II family of polypeptides produced by oral streptococci. These proteins are adhesins and mediate species-specific binding of cells to a variety of host and bacterial receptors. Antigen I/II family polypeptides bind collagen and mediate a morphological growth response of streptococci to collagen. The activities of these antigen I/II polypeptides are critical for the intratubular growth of streptococci and, thus, for the establishment of endodontic infections [7]. The *spaP* gene encodes the cell surface protein and is also responsible for the LPXTG – Leucine–Proline–any amino acid (X)–Threonine–Glycine major surface protein found in *S. mutans* [8,9].

Though the genetic and molecular components of *S. mutans* have been studied in detail [10], there is limited research on *spaP* and its correlation with OPMD and caries. Thus, the primary goal of this study is to characterize *S. mutans* from clinical samples taken from individuals who have OPMD and caries. The study is further designed to assess the frequency of the *spaP* gene among the clinical strains of *S. mutans*.

## MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Microbiology, Saveetha Dental College and Hospitals, Chennai, India, from January 2023 to April 2023. Institutional ethical clearance was obtained prior to the initiation of the study (Ref: SRB/SDC/UG-2138/23/MICRO/127; IHEC/SDC/UG-2138/23/MICRO/277), and informed consent was acquired from all participants.

This was a time-bound study, and all eligible subjects meeting the inclusion criteria during the study period were included. The total sample size of 60 (20 participants in each group) was based on the availability of subjects during the study period. Participants were randomly selected from three categories: Group 1 (OPMD, n=20), Group 2 (healthy individuals with dental caries, n=20), and Group 3 (healthy individuals without caries, n=20).

**Inclusion and Exclusion criteria:** The inclusion criteria were participants within these three groups i.e. patients with OPMD, healthy individuals with and without dental caries during the study period. Exclusion criteria included individuals with systemic diseases, ongoing infections, or those on antibiotics/antifungal medications within the last three months.

### Study Procedure

**Sampling and isolation of *S. mutans*:** The samples were immediately transported to the laboratory and inoculated onto Mutans Sanguis Agar (MSA) plates, which were then incubated at 37°C for 48 hours. After incubation, the colonies were characterised by Gram staining and catalase testing. Genomic DNA was extracted from the isolated *S. mutans* strains, and Polymerase Chain Reaction (PCR) was performed to detect the presence of the *spaP* gene.

**Molecular detection of *spaP* genes:** Following the guidelines from the manufacturer, genomic DNA was extracted from *S. mutans* cultures using the Qiagen DNA Extraction Kit (Qiagen, Germany) and stored at -20°C for future use [11]. For the amplification reaction, a 15 µL mixture was prepared with 5.6 µL of double-distilled water and 7.8 µL of 2× Master Mix (Takara, Japan). Specific primers for the *spaP* gene (see [Table/Fig-1]) were included, and PCR was conducted for 36 cycles in an Eppendorf Mastercycler from Germany. The resulting PCR amplicons were observed through 1% agarose gel electrophoresis and verified using a 100 bp DNA ladder.

| Target gene | Primer sequence | Annealing temperature (°C) | Amplicon size (bp) |
|---|---|---|---|
| *spaP* | F: GACTTTGGTAATGGTTATGCATCAA | 55 | 101 |
| | R: TTTGTATCAGCCGGATCAAGTG | | |

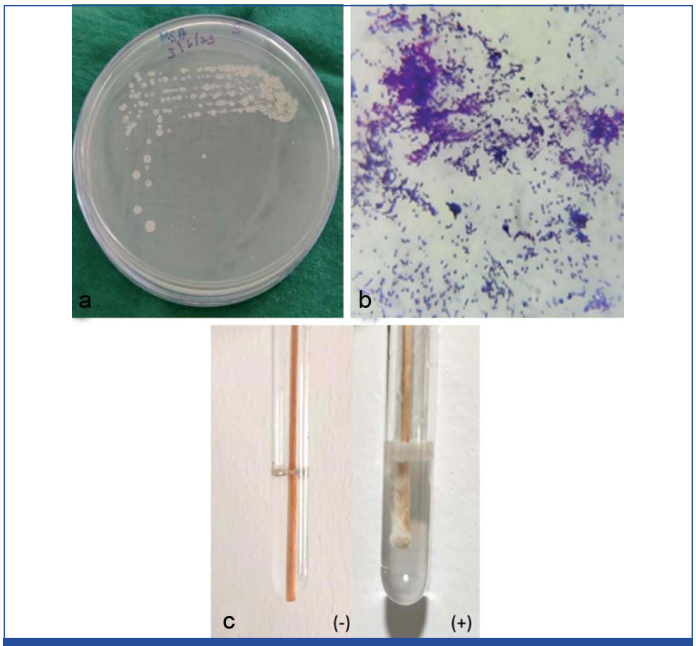
**[Table/Fig-1]:** Primer details and the PCR condition for the detection of *spaP* from *S. mutans*.

## STATISTICAL ANALYSIS

Data analysis was performed using SPSS software version 25.0 (IBM, USA). Descriptive statistics, including frequencies and percentages, were used to summarise categorical variables such as the prevalence of *S. mutans* and the *spaP* gene. Group-wise comparisons for prevalence rates were analysed using the Chi-square test. A p-value of <0.05 was considered statistically significant.

## RESULTS

**Identification of *S. mutans*:** The *S. mutans* colonies were observed as smooth, elevated, and sticky, giving them a unique frosted glass appearance on the MSA medium. Gram staining revealed gram-positive cocci arranged in short chains. The negative catalase test ruled out the presence of *Staphylococci*, confirming the identification as *Streptococci* spp [Table/Fig-2].

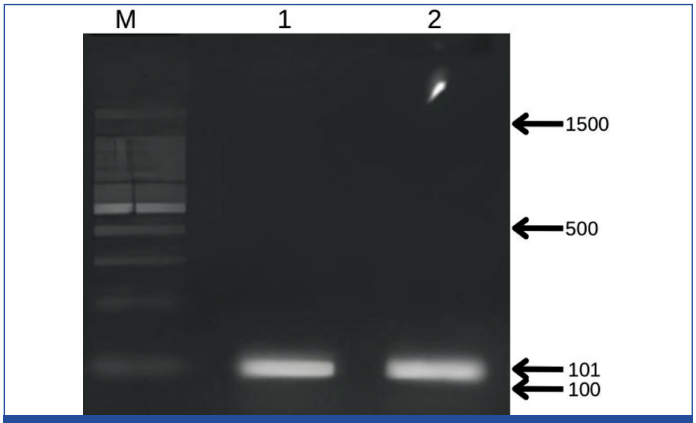



**[Table/Fig-2]:** a) Colony morphology of *S. mutans* on MSA Agar, displaying smooth, elevated, and sticky colonies; b) Gram staining showing gram-positive cocci arranged in short chains (100× magnification); c) Negative catalase test confirming the absence of catalase activity in *S. mutans*.

**Frequency of *S. mutans* and detection of *spaP* gene:** [Table/Fig-3,4] outlines the frequency of the *spaP* gene among clinical strains of *S. mutans* isolated from three distinct groups under study (N=60; n=20 in each group). The prevalence of both *S. mutans* and the *spaP* gene was significantly higher in Groups 1 and 2. The frequency of *S. mutans* was 45% (n=9) in OPMD patients, 40% (n=8) in healthy individuals with caries, and 15% (n=3) in healthy individuals without caries. The frequency of the *spaP* gene was 88% (n=8) in OPMD patients and 75% (n=6) in healthy individuals with caries. The *spaP* gene was not observed in Group 3, which consisted of

| GROUPS | *S. mutans* | *spaP* |
|---|---|---|
| Group 1: OPMD (n=20) | 9 (45%) | 8 (88%) |
| Group 2: Healthy individuals with caries (n=20) | 8 (40%) | 6 (75%) |
| Group 3: Healthy individuals without caries (n=20) | 3 (15%) | - |
| Chi-square value ($\chi^2$) | 6.32 | 10.24 |
| p-value | 0.042 | 0.006 |

**[Table/Fig-3]:** Frequency of *spaP* gene among the clinical strains of *S. mutans* isolated from the three different groups under study.




**[Table/Fig-4]:** Electrophoretogram showing the amplification of the *spaP* gene (amplicon size: 101 bp). Lane M: 100 bp DNA ladder, serving as a molecular weight marker with prominent bands at 100 bp, 500 bp, and 1,500 bp. Lane 1 and Lane 2: PCR products of the *spaP* gene, demonstrating successful amplification with clear bands at the expected size of 101 bp.

healthy individuals without caries. Chi-square analysis indicated a statistically significant association between the presence of the *spaP* gene and the study groups ($p<0.05$), with higher frequencies observed in strains from OPMD patients and healthy individuals with caries compared to those without caries.

## DISCUSSION

OPMD encompasses a spectrum of conditions in the oral cavity that exhibit an increased risk of malignant transformation [12]. Commonly encountered OPMDs, such as leukoplakia, erythroplakia, and OLP, are influenced by various host factors. Regular monitoring and early intervention are critical in managing these disorders, underscoring the importance of comprehensive oral health assessments and timely medical attention for individuals with precancerous lesions.

The human oral microbiome, particularly the diverse biofilm it forms, plays a crucial role in the progression of oral disorders. Among microbial species, oral streptococci, including *S. mutans*, significantly contribute to disease progression and are strongly associated with carious lesions [13,14]. The *spaP* gene of *S. mutans* encodes the surface-associated adhesin protein AgI/II, which is pivotal for the bacterium's adherence to tooth surfaces [15].

In the present study, we aimed to investigate the prevalence of *S. mutans* and the frequency of the *spaP* gene across three groups: individuals with OPMDs, individuals with dental caries, and healthy controls. This stratified approach provides valuable insights into the microbial factors influencing oral health and disease progression. Our findings revealed a notable correlation between the presence of *S. mutans* and OPMDs, with 45% of OPMD cases and 40% of caries cases testing positive for *S. mutans*. Previous studies have reported a prevalence of *S. mutans* in carious lesions ranging from 65% to 78% [16]. Interestingly, our results demonstrate that *S. mutans* can also be widely distributed in populations with low caries prevalence, suggesting that the bacterium's spread is not strictly dependent on caries-promoting behaviors [17].

A previous investigation into the presence of the *spaP* gene reported detection in 91.3% of patients with caries and only 8% of individuals without caries [18]. Our study expands this understanding by documenting the significant association of *S. mutans* and the *spaP* gene with OPMDs for the first time. Among the 20 individuals diagnosed with OPMDs (Group 1), *S. mutans* was isolated from nine individuals, and 88.9% of these isolates harbored the *spaP* gene. In contrast, 15% of healthy controls were positive for *S. mutans*, but none exhibited the *spaP* gene.

These findings highlight the strong correlation between *S. mutans* carrying the *spaP* gene and pathological conditions such as OPMDs and caries, while its prevalence is significantly lower in healthy individuals. This underscores the need for periodic monitoring of *S. mutans* and its genetic determinants in dental healthcare settings to facilitate early detection and targeted interventions. Moreover, with the increasing prevalence of drug-resistant *S. mutans* strains in dental setups [19,20], there is an urgent need to educate dentists about such trends to adopt targeted treatment regimens. Computational approaches to predict vaccine candidates for priority pathogens [21,22] suggest that *spaP* could serve as a novel target for designing a vaccine against caries. Future studies should focus on validating *spaP* as a vaccine target and exploring its potential to mitigate *S. mutans*-associated oral diseases, including OPMDs.

The study underscores the role of the *spaP* gene in *S. mutans* pathogenicity, particularly in patients with OPMD and dental caries. These findings suggest that the *spaP* gene could serve as a biomarker for early detection and risk stratification in dental practice. Clinically, *spaP* may be targeted for the development of diagnostic tools, vaccines, and antimicrobial treatments aimed at preventing caries and reducing OPMD progression. Periodic screening for the *spaP* gene in at-risk patients could enable personalised treatment strategies, improving oral health outcomes and potentially preventing oral cancer.

### Limitation(s)

The primary limitation of this study is the small sample size, which precludes robust statistical analysis. Future research with larger cohorts is warranted to validate these findings. Furthermore, the molecular characterization of *spaP* from *S. mutans* in OPMD patients offers valuable insights into its potential as a therapeutic target. This paves the way for the development of novel drugs, targeted therapies, and personalized medicine approaches. Ongoing investigations should explore these avenues to enhance the management and treatment of OPMD and related oral diseases.

## CONCLUSION(S)

The prevalence of *S. mutans* was highest among patients with OPMD and healthy individuals with caries. The findings of the study suggest a correlation of the *spaP* gene with *S. mutans*, showing an increased frequency in infected individuals and in the pathological conditions of the oromucosal and dentinal layers. Thus, dental research may progress further by targeting *spaP* genetic determinants to combat the complications caused by *S. mutans* in association with OPMD and caries. Additionally, *spaP* may be considered a vital target for both rapid diagnosis and therapeutic approaches.

## REFERENCES


[1] Yeladandi M, Thirupambaram Nataraja Sundaram U, Muthukumaran D. A cross-sectional study on oral potentially malignant disorders: Diagnostic challenges in early detection of dysplasia and the role of velscope. Cureus. 2024;16:e69542.
[2] Ashwin SP, Sinduja P, Jayaraman S, Priyadharshini R. Assessment of 8-OHDG, SOD, and GPx levels in OPMD and OSCC patients. Clin Cancer Investig J. 2023;12:01-08.
[3] Mello FW, Melo G, Guerra ENS, Warnakulasuriya S, Garnis C, Rivero ERC. Oral potentially malignant disorders: A scoping review of prognostic biomarkers. Crit Rev Oncol Hematol. 2020;153:102-986.
[4] Napimoga MH, Höfling JF, Klein MI, Kamiya RU, Gonçalves RB. Transmission, diversity and virulence factors of Streptococcus mutans genotypes. J Oral Sci. 2005;47:59-64.
[5] Girija ASS, Ganesh PS. Functional biomes beyond the bacteriome in the oral ecosystem. Jpn Dent Sci Rev. 2022;58:217-26.
[6] Banas JA. Virulence properties of Streptococcus mutans. IMR Press. 2004;9:1267-77.
[7] Love RM, McMillan MD, Jenkinson HF. Invasion of dentinal tubules by oral streptococci is associated with collagen recognition mediated by the antigen I/II family of polypeptides. Infect Immun. 1997;65:5157-64.
[8] You YO. Virulence genes of Streptococcus mutans and dental caries. IJOB. 2019;44:31-36.
[9] Lee SF, Progulske-Fox A, Erdos GW, Piacentini DA, Ayakawa GY, Crowley PJ, et al. Construction and characterization of isogenic mutants of Streptococcus mutans deficient in major surface protein antigen P1 (I/II). Infect Immun. 1989;57:3306-13.
[10] Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The biology of streptococcus mutans. Microbiology Spectrum. 2019;18:51-108.
[11] QIAamp DNA Mini Kit: For DNA Purification from Bacteria. June 2012.
[12] Kizhakkoottu S, Ramani P. Knowledge about the importance of early diagnosis and treatment of oral potentially malignant disorders among the South Indian population: An institutional retrospective study. Cureus. 2024;16:57-740.
[13] Burcher KM, Burcher JT, Inscore L, Bloomer CH, Furdui CM, Porosnicu M. A review of the role of oral microbiome in the development, detection, and management of head and neck squamous cell cancers. Cancers (Basel). 2022;14(17):4116.
[14] Nicolas GG, Lavoie MC. Streptococcus mutans and oral streptococci in dental plaque. Can J Microbiol. 2011;57:1-20.
[15] Avilés-Reyes A, Miller JH, Lemos JA, Abranches J. Collagen-binding proteins of Streptococcus mutans and related streptococci. Mol Oral Microbiol. 2017;32:89-106.
[16] Oda Y, Hayashi F, Okada M. Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients with intellectual disabilities. BMC Oral Health. 2015;15:102.
[17] Johansson I, Witkowska E, Kaveh B, Lif Holgerson P, Tanner AC. The microbiome in populations with a low and high prevalence of caries. J Dent Res. 2016;95:80-86.
[18] Durán-Contreras GL, Torre-Martínez HH, de la Rosa El, Hernández RM, de la Garza Ramos M. spaP gene of Streptococcus mutans in dental plaque and its relationship with early childhood caries. Eur J Paediatr Dent. 2011;12:220-24.
[19] Malini M, Thomas TK, Bhargava D, Girija S. Microbiology of the white coat in a dental operatory. Indian J Dent Res. 2012;23:841.
[20] Smiline GAS, Pandi SK, Hariprasad P, Raguraman R. A preliminary study on the screening of emerging drug resistance among the caries pathogens isolated from carious dentine. Indian J Dent Res. 2012;23:26-30.

[21] Smiline Girija AS. Delineating the immuno-dominant antigen vaccine peptides against gacSSensor kinase in: An investigational approach. Front Microbiol. 2020;11:2078.

[22] Sankar S. design of a multi-epitope Chimera from salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022;59:327-36.

**PARTICULARS OF CONTRIBUTORS:**
1. Undergraduate Student, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.
2. Professor and Head, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.
3. Associate Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.
4. PhD Scholar, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
AS Smiline Girija,
Professor and Head, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, P.H. Road, Chennai-600077, Chennai, Tamil Nadu, India.
E-mail: smilinejames25@gmail.com

**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
- Plagiarism X-checker: Sep 18, 2024
- Manual Googling: Mar 13, 2025
- iThenticate Software: Mar 15, 2025 (17%)

**ETYMOLOGY:** Author Origin

**EMENDATIONS:** 6

**AUTHOR DECLARATION:**
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 17, 2024**
Date of Peer Review: **Dec 18, 2024**
Date of Acceptance: **Mar 18, 2025**
Date of Publishing: **Sep 01, 2025**