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PREVALENCE AND REDUCTION OF *Streptococcus mutans, Streptococcus sobrinus,* AND *Streptococcus sanguinis* IN VIETNAMESE STUDENTS FOLLOWING FLUORIDE VARNISH APPLICATION

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ABSTRACT

Dental caries is a multifactorial disease influenced by microbial composition, host-related factors, and environmental conditions. *Streptococcus mutans* and *Streptococcus sobrinus* are key contributors to caries progression, while *Streptococcus sanguinis* has a more complex role in caries development. This study aimed to determine the prevalence of *S. mutans*, *S. sobrinus*, and *S. sanguinis* in dental plaque among Vietnamese students and evaluate the antibacterial effects of fluoride varnish over a six-month period.

A cross-sectional analytical study was conducted at Vo Truong Toan Primary School, Can Tho City, Vietnam. Dental plaque samples were collected from 77 children with active caries and analyzed using PCR. Fluoride varnish (Clinpro™ White Varnish) was applied at baseline and three months post-treatment. Bacterial presence was re-evaluated at three and six months. The initial prevalence of *S. mutans*, *S. sobrinus*, and *S. sanguinis* was 50.6%, 14.3%, and 1.3%, respectively. After three months, *S. mutans* prevalence significantly declined to 19.5%, while *S. sobrinus* remained stable at 13.0%, and *S. sanguinis* increased slightly to 3.9%. At six months, *S. mutans* further decreased to 11.7%, and *S. sobrinus* to 6.5%, while *S. sanguinis* was detected in 2.6% of cases. The proportion of bacteria-free samples increased from 23.4% before treatment to 77.9% after six months. The findings confirm the dominant role of *S. mutans* in dental caries and highlight the sustained antibacterial effects of fluoride varnish in reducing its prevalence. *S. sobrinus* was detected less frequently but remained a significant contributor to caries, while *S. sanguinis* showed variable presence. These results support the use of fluoride varnish as a preventive measure for caries management. Future studies should explore bacterial load quantification, metagenomic profiling, and the long-term impact of fluoride varnish on microbial communities.

Keywords: Bacterial prevalence, caries prevention, dental plaque, fluoride varnish, oral microbiome, pediatric dentistry.

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INTRODUCTION

Dental caries is a microbiologically driven disease resulting from complex interactions between the host, oral microbiota, and environmental factors. The primary bacterial species responsible for caries progression include *Streptococcus* spp., *Lactobacillus* spp., and *Actinomyces* spp. These microorganisms interact within biofilms, utilizing fermentable carbohydrates to produce acidic metabolic byproducts, which initiate enamel demineralisation and facilitate lesion progression (Pharmacy, 2021; Zhou, 2016; Zubaidah et al., 2022). The progression of caries is characterized by alternating cycles of demineralization and remineralization, where bacterial acid production leads to mineral loss, while salivary components facilitate enamel recovery. However, an imbalance favouring prolonged acid exposure results in irreversible enamel destruction (Nguyen et al., 2021; Organization, 2013; Pharmacy, 2021). The morphological and biochemical properties of enamel play a critical role in caries susceptibility. Newly erupted enamel contains a high proportion of carbonate-rich hydroxyapatite, which is highly soluble in acidic environments. Over time, post-eruptive maturation occurs, with carbonate ions being replaced by hydroxyl or fluoride ions, increasing enamel resistance to acid dissolution (Wang & Liu, 2013; Zhou, 2016). Additionally, developmental anomalies such as enamel hypoplasia, defects in mineralization, and inadequate fluoride incorporation further predispose teeth to caries formation (Pharmacy, 2021).

The *Streptococcus* genus, particularly *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis*, plays a dominant role in biofilm formation and caries pathogenesis. *S. mutans* was first identified by Louis Pasteur in 1887, and its role in acidogenesis and biofilm stability has been extensively studied (Zhang et al., 2022). These bacteria produce extracellular polysaccharides (EPS) such as dextran, which enhance their adhesion to the tooth surface, promoting biofilm maturation and increasing their resistance to environmental changes (Zhang et al., 2022). While *S. mutans* is frequently detected in both active and inactive carious lesions, *S. sobrinus* is predominantly isolated from active decay sites and has been reported to play a crucial role in the initiation of smooth-surface caries (Zhang et al., 2022). Studies suggest that the presence of both *S. mutans* and *S. sobrinus* and *S. sanguinis* correlates with increased caries severity and a higher number of affected teeth. However, research on *S. sobrinus* remains limited compared to *S. mutans*, highlighting the need for further microbiological and genomic investigations (Zhang et al., 2022).

Lactobacillus spp. and *Actinomyces* spp., though not primary colonisers in initial caries formation, play important roles in later stages of caries progression. Lactobacillus spp. are highly acidogenic and acid-tolerant, contributing significantly to the acidic environment in deep dentinal lesions. *Actinomyces* spp., particularly *A. naeslundii*, are frequently associated with root caries due to their saccharolytic metabolism and preference for exposed root surfaces in older individuals or those with gingival recession (Yuan et al., 2020). While these species were not the focus of this study, their roles support the multifactorial nature of caries and the complexity of microbial interactions in dental biofilms.

The ability of cariogenic bacteria to persist and thrive in the oral cavity is largely attributed to their ability to form biofilms and adapt to acidic conditions. *S. mutans* and *S. sobrinus*, and *S. sanguinis* possess several key virulence factors that contribute to their pathogenic potential. Acid production via carbohydrate fermentation is one of the primary mechanisms, where *S. mutans* and *S. sobrinus* and *S. sanguinis* metabolize sucrose, glucose, maltose, and other fermentable sugars into organic acids, primarily lactic acid, which reduces plaque pH and promotes enamel demineralization (Lemos et al., 2019). *S. sobrinus* is particularly efficient at acid production, contributing to a lower pH and higher cariogenic potential than *S. mutans*

(Lemos et al., 2019). Unlike commensal oral bacteria, *S. mutans* has evolved acid tolerance mechanisms, including proton pumps, acid-neutralizing enzymes, and stress response pathways, which enable it to survive in acidic conditions that are lethal to non-aciduric bacteria. While *S. sobrinus* lacks well-characterized acid tolerance mechanisms, it has been shown to modulate host immune responses, produce hydrogen peroxide, and enhance glucan synthesis, further supporting its role in caries progression (Lemos et al., 2019).

Dental caries does not result from a single bacterial species but rather from a highly adapted microbial consortium that creates a low-pH, EPS-enriched environment. The coexistence of *S. mutans* and *S. sobrinus*, and *S. sanguinis* is associated with higher caries prevalence and increased dmft (decayed, missing, and filled teeth) scores, particularly in early childhood caries (Lemos et al., 2019). Compared to *S. mutans*, *S. sobrinus* exhibits greater acid production and biofilm-forming capacity, accelerating caries progression. *S. mutans* has also been linked to systemic infections such as infective endocarditis, while specific strains have been associated with neurological diseases, IgA nephropathy, and atherosclerosis, highlighting its potential role in systemic health (Lemos et al., 2019).

The application of molecular diagnostics and sequencing technologies in cariology research has significantly expanded our understanding of the oral microbiome. These advancements provide a foundation for biotechnological interventions aimed at preventing and managing dental caries. Traditional culture-based methods have long been used to isolate and identify bacterial species within dental biofilms (Nieminen et al., 1996), but these techniques are often time-consuming, labor-intensive, and limited by the viability of bacteria within the sample. Advances in molecular diagnostics, including polymerase chain reaction (PCR) and next-generation sequencing (NGS), have revolutionized microbial identification, enabling the detection and quantification of cariogenic species with high specificity and sensitivity (Nieminen et al., 1996; Pandey et al., 2022). These biotechnological tools provide critical insights into the composition, genetic diversity, and functional capabilities of oral microbiota, paving the way for novel microbiome-based therapeutic interventions (Nieminen et al., 1996; Pandey et al., 2022).

Future research should focus on developing point-of-care diagnostic tools. These may include compact PCR-based kits for rapid chairside detection of bacteria, as well as biosensors and microfluidic devices for real-time monitoring of the oral microbiome. Additionally, synthetic biology approaches could be leveraged to engineer beneficial oral bacteria for biofilm modulation, and probiotic strains capable of outcompeting *S. mutans* and *S. sobrinus* and *S. sanguinis* could be identified for therapeutic use. Advances in microbiome science, molecular biology, and synthetic biology will be crucial in designing next-generation caries prevention technologies, holding the potential to modulate microbial ecosystems and provide long-term solutions for oral health maintenance beyond traditional chemical-based interventions (Conrads et al., 1999; Nguyen et al., 2023).

MATERIALS AND METHODS

Study design and setting

This study employed a cross-sectional analytical design to investigate the prevalence of dental caries, the presence of cariogenic bacteria, and the efficacy of fluoride varnish in bacterial reduction and caries management. The study was conducted at Vo Truong Toan Primary School, An Hoa Ward, Ninh Kieu District, Can Tho City, Vietnam, while all microbiological analyses were performed at the Molecular Biology Laboratory of Can Tho University of Medicine and Pharmacy (CTUMP). The research was carried out between December 2022 and January 2024.

Study population and participant selection

The study included students from Vo Truong Toan Primary School, selected based on the following inclusion and exclusion

criteria. Only children whose legal guardians provided written informed consent were eligible. Children with at least one active carious lesion were further analyzed for bacterial presence and fluoride varnish treatment response.

Exclusion criteria included students with systemic diseases, acute infections, ongoing fluoride treatment, use of antibacterial mouthwash, or allergies to fluoride-based products. Children with physical or mental disabilities that prevented full participation in the study were also excluded.

Caries examination and clinical assessment

Dental caries was diagnosed using the WHO caries detection criteria, which involved visual-tactile assessment under natural light (Organization, 2013). Each tooth was classified as healthy, carious, restored, or missing due to caries. The Decayed, Missing, and Filled Teeth (DMFT) index was determined for permanent teeth, while the DMFT index was used for primary teeth.

Caries prevalence was analyzed by gender, age group, type of teeth, jaw location, and the number of affected teeth. Caries activity was classified into active and inactive lesions based on lesion characteristics such as color, texture, plaque accumulation, and enamel integrity.

Sample collection and processing

Dental plaque sampling

Plaque samples were collected from the cervical region of mandibular molars using sterile curettes. Each sample was transferred into 1 mL phosphate-buffered saline (PBS, pH 7.4) and stored at 4 ^{o}C before being transported to the Molecular Biology Laboratory at CTUMP for further analysis.

DNA extraction and preparation

Total bacterial DNA was extracted using the heat-shock method. Samples were vortexed and centrifuged at 13,000 rpm for 5 minutes, followed by two PBS washes. The bacterial pellet was resuspended in 50 µL TE buffer and incubated at 95°C for 10 minutes to lyse bacterial cells (Le et al., 2021). DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher, USA).

PCR detection of Streptococcus mutans, Streptococcus sobrinus, and Streptococcus sanguinis

Multiplex PCR was performed to detect *S. mutans*, *S. sobrinus*, and *S. sanguinis* using species-specific primers. The reaction mixture (25 µL) contained 12.5 µL 2X Master Mix (Thermo Fisher, USA), 0.5 µL of each primer pair (10 µM), 3 µL of DNA template, and DEPC-treated water.

PCR Thermal Cycling Conditions: Initial denaturation at 95 ^{o}C for 5 min, 40 cycles of denaturation at 95 ^{o}C for 30 sec, annealing at 58 ^{o}C for 30 sec, extension at 72 ^{o}C for 45 sec. Final extension at 72 ^{o}C for 5 min. PCR products were analyzed by 2% agarose gel electrophoresis at 90V for 45 min, visualized under UV transillumination (Gel Doc XR+, Bio-Rad, USA), and compared with a 100 bp DNA ladder (Thermo Fisher, USA) (Le et al., 2021).

Table 1. Primer sequences and expected product sizes

Target bacteria	Primer sequences (5'→3')	Product size (bp)
Streptococcus mutans	F- AGCCATGCGCAATCAACAGGTT	415
	R- CGCAACGCGAACATCTTGATCAG	
Streptococcus sobrinus	F- CGGACTTGCTCCAGTGTTACTAA	546
	R- CCAATGCCTTTAACTTCAGACTTAC	
Streptococcus sanguinis	F- GGATAGTGGCTCAGGGCAGCCAGTT	313
	R- GAACAGTTGCTGGACTTGCTTGTC	

Fluoride varnish application and treatment evaluation

Students with active carious lesions received 5% fluoride varnish (Cinpro™ White Varnish, USA) applications twice (at baseline and after three months). The varnish was applied using a microbrush, with cotton roll isolation and air-drying of teeth before application.

Caries activity was re-evaluated at 3 months and 6 months post-treatment using the WHO diagnostic criteria. PCR analysis was repeated to determine changes in bacterial presence following fluoride application.

Statistical analysis

Data were analyzed using SPSS 22.0 (IBM, USA). Descriptive statistics were used to determine frequencies, percentages, and mean values. The chi-square test was applied to compare categorical variables, while t-tests were used for continuous data.

Multivariate logistic regression was conducted to assess the association between bacterial colonization and caries severity, with results expressed as Odds Ratios (OR) and 95% Confidence Intervals (CI). A p-value < 0.05 was considered statistically significant.

Ethical considerations

This study was approved by the Ethical Committee of Can Tho University of Medicine and Pharmacy (Approval No. 22.339.HV/PCT-HĐĐĐ, August 11, 2022). Participation was voluntary, and informed consent was obtained from all legal guardians. As part of the study, students received oral health counselling and preventive care recommendations.

RESULTS

PCR amplification and gel electrophoresis analysis of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis*

The agarose gel electrophoresis results provide a clear visualization of PCR amplification for the target bacterial species *S. mutans*, *S. sobrinus*, and *S. sanguinis*, as specified in Table 1. The gel image (Fig. 1) reveals distinct bands corresponding to the expected amplicon sizes: 415 bp for *S. mutans*, 546 bp for *S. sobrinus*, and 313 bp for *S. sanguinis*. A molecular weight marker (DNA ladder) is present in one lane, allowing for accurate band size estimation.

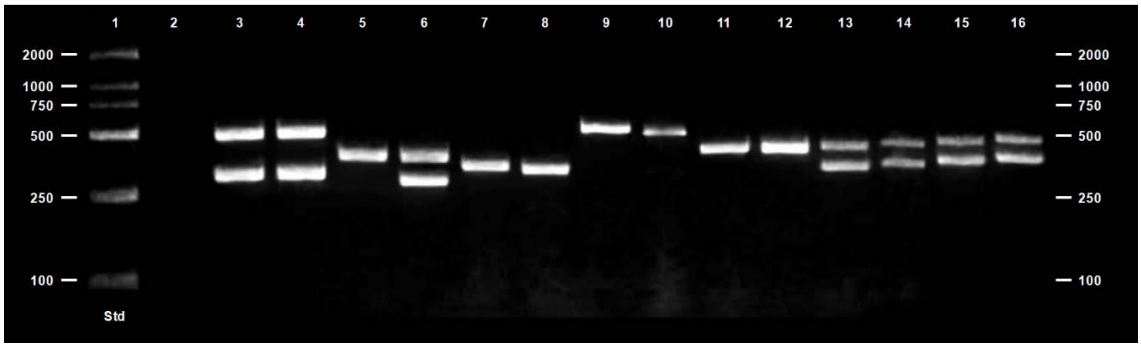

Figure 1. Agarose Gel Electrophoresis of PCR Products for *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis*. Lane 1: Marker 100–2,000 bp; Lane 2: Negative control sample; Lane 3: Positive control sample of *Streptococcus sanguinis* (~313 bp) and *S. sobrinus* (~546 bp); Lane 6: Positive control sample of *Streptococcus sanguinis* and *Streptococcus mutans* (~415 bp); other lane: sample collected

For *S. mutans*, strong and well-defined bands appeared at 415 bp in the lanes corresponding to positive samples, confirming the presence of this bacterium in the tested plaque samples. The intensity of the bands varies among different samples, which may

indicate differences in bacterial load or slight variations in PCR amplification efficiency. The absence of additional or smeared bands suggests high specificity of the primer set for *S. mutans*, minimizing the possibility of non-specific amplification.

Similarly, for *S. sobrinus*, bands were visible at the expected 546 bp region. The presence of these bands in multiple lanes confirmed the identification of *S. sobrinus* in certain samples. However, compared to *S. mutans*, the bands for *S. sobrinus* appear in fewer lanes, indicating a lower prevalence of this species in the tested samples. The clarity and sharpness of the bands suggest that the PCR reaction was well-optimized for *S. sobrinus*.

For *S. sanguinis*, bands were observed at 313 bp in some lanes, corresponding to the expected amplicon size. The lower intensity of some *S. sanguinis* bands compared to *S. mutans* and *S. sobrinus* might reflect a lower bacterial concentration in the plaque samples or a slightly lower amplification efficiency of this primer set.

Negative control lanes do not show any bands, confirming that there was no contamination or primer-dimer formation in the PCR reaction. The absence of unintended bands further supports the specificity of the primer sets used. The results align well with the molecular diagnostic approach, reinforcing the reliability of PCR-based identification of cariogenic bacterial species in plaque samples. The observed variation in bacterial prevalence among samples highlights the differential colonization patterns of *S. mutans*, *S. sobrinus*, and *S. sanguinis*, which may be influenced by factors such as oral hygiene, diet, and individual susceptibility to bacterial adhesion.

Prevalence of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* in dental plaque by age group and gender

The distribution of *S. mutans*, *S. sobrinus*, and *S. sanguinis* in dental plaque varied among different age groups and genders (Table 2). Among male participants, *S. mutans* was detected in 48.8% of cases, *S. sobrinus* in 14.6%, and *S. sanguinis* in 2.4%. A combination of *S. mutans* and *S. sobrinus* was present in 4.9% of cases, while *S. mutans* and *S. sanguinis* coexisted in 2.4%. Additionally, *S. sobrinus* and *S. sanguinis* were found together in 4.9% of cases. The percentage of samples without any of these bacteria was 22.0%. Among female participants, *S. mutans* was detected in 52.8%, *S. sobrinus* in 13.9%, while *S. sanguinis* was not identified in any sample. Dual infections of *S. mutans* and *S. sobrinus* were present in 5.6%, and *S. mutans* and *S. sanguinis* in 2.8%. No cases of *S. sobrinus* and *S. sanguinis* co-infection were observed. The proportion of negative samples in females was 25.0%. No statistically significant difference was observed between males and females across bacterial groups ($p > 0.05$).

Regarding age groups, *S. mutans* was detected in 60.6% of children aged 6−7 years, compared to 43.2% in those aged 8−10 years ($p = 0.130$). The prevalence of *S. sobrinus* was 15.2% in the younger group and 13.6% in the older group ($p = 1.000$). *S. sanguinis* was only detected in 3.0% of children aged 6−7 years and absent in those aged 8−10 years ($p = 0.429$). Co-infection of *S. mutans* and *S. sobrinus* was more common in the younger age group (9.1%) compared to 2.3% in the older group ($p = 0.308$). The prevalence of *S. mutans* and *S. sanguinis* co-infection was similar between groups ($p = 0.836$), while *S. sobrinus* and *S. sanguinis* were only detected in 4.5% of older children ($p = 0.504$). The percentage of children without any of the three bacteria was significantly higher in the older age group (34.1%) than in the younger group (9.1%) ($p = 0.010$).

The findings indicate that *S. mutans* was the most prevalent species in both genders and age groups, while *S. sanguinis* was detected at lower frequencies. The proportion of bacterial co-infections remained relatively low. Notably, the absence of all three bacterial species was significantly higher in older children compared to younger children, suggesting a possible age-related shift in microbial colonization.

Prevalence of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* by number of carious teeth and caries location

The distribution of *S. mutans*, *S. sobrinus*, and *S. sanguinis* varied according to the number of decayed teeth and the location of carious lesions (Table 3). Among students with fewer than six carious teeth, *S. mutans* was the most frequently detected species, present in 75.0% of cases, followed by *S. sobrinus* in 12.5%. No cases of *S. sanguinis* or bacterial co-infections were observed in this group, and 12.5% of students were negative for all three bacterial species. In students with 6–10 decayed teeth, *S. mutans* was identified in 41.4%, while *S. sobrinus* was present in 13.8%. No cases of *S. sanguinis* were found, while dual infections of *S. mutans* and *S. sobrinus* were detected in 3.4%, *S. mutans* and *S. sanguinis* in 6.9%, and *S. sobrinus* and *S. sanguinis* in 6.9%. The percentage of students without detectable bacterial colonization in this group was 27.8%.

The analysis of bacterial prevalence by caries location showed that among students with caries limited to molars, *S. mutans* was the most prevalent at 47.2%, followed by *S. sobrinus* at 13.2% and *S. sanguinis* at 1.9%. The prevalence of *S. mutans* and *S. sobrinus* co-infection was 3.8%, while *S. mutans* and *S. sanguinis* co-infection was found in 3.8%, and *S. sobrinus* and *S. sanguinis* in 3.8% (Table 3). The proportion of students without detectable bacterial presence was 26.4%. In students with both anterior and molar caries, *S. mutans* was present in 58.3% of cases, *S. sobrinus* in 16.7%, while *S. sanguinis* was absent in this group. Co-infection with *S. mutans* and *S. sobrinus* was observed in 8.3% of cases (Table 3). No samples showed co-infection with *S. mutans* and *S. sanguinis*, or with *S. sobrinus* and *S. sanguinis*. The proportion of students without any of the three bacterial species was 16.7%. No statistically significant differences in bacterial prevalence were observed between the two groups categorized by caries location ($p > 0.05$).

Among students with 11 or more decayed teeth, *S. mutans* was found in 52.5%, *S. sobrinus* in 15.0%, and *S. sanguinis* in 2.5%. The prevalence of *S. mutans* and *S. sobrinus* co-infection was 7.5%, while *S. mutans* and *S. sanguinis* co-infection was not observed in this group. No cases of *S. sobrinus* and *S. sanguinis* co-infection were recorded, and 22.5% of students in this category tested negative for all three bacteria. The differences between bacterial prevalence among the three groups based on the number of carious teeth were not statistically significant ($p > 0.05$).

The results indicate that *S. mutans* remains the most prevalent species across all categories, with variations in its detection rate depending on the number of carious teeth and lesion location. While bacterial co-infections were present, they remained relatively low. The percentage of students without bacterial colonization increased with higher numbers of decayed teeth, but did not reach statistical significance. The absence of *S. sanguinis* in students with both anterior and molar caries suggests possible variations in bacterial colonization patterns depending on lesion location.

Prevalence of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* based on oral hygiene and snacking habits

The distribution of *S. mutans*, *S. sobrinus*, and *S. sanguinis* varied according to oral hygiene and snacking habits (Table 4). Among students who brushed their teeth fewer than two times per day, *S. mutans* was the most frequently detected species, present in 57.1% of cases, while *S. sobrinus* was identified in 7.1%. No cases of *S. sanguinis* were found in this group. Co-infection of *S. mutans* and *S. sobrinus* was observed in 7.1%, whereas *S. mutans* and *S. sanguinis*, as well as *S. sobrinus* and *S. sanguinis*, were not detected. The proportion of students without any of these bacteria was 28.6%. Among those who brushed at least twice daily, *S. mutans* was found in 49.2%, *S. sobrinus* in 15.9%, and *S. sanguinis* in 1.6%. Co-infections of *S. mutans* and *S. sobrinus* were present in 4.8%, *S. mutans* and *S. sanguinis* in 3.2%, and *S. sobrinus* and *S. sanguinis* in 3.2%. The proportion of students testing negative for all three bacteria was 22.2%. No statistically

significant differences were observed between these groups ($p > 0.05$).

Regarding snacking habits, *S. mutans* was found in 51.4% of students who frequently consumed snacks and 50.0% of those who rarely consumed snacks. The prevalence of *S. sobrinus* was slightly higher in the frequent snacking group (16.2%) than in the low-frequency group (12.5%). *S. sanguinis* was absent among frequent snackers but was detected in 2.5% of those who rarely consumed snacks. Co-infections of *S. mutans* and *S. sobrinus* were more frequent among those who rarely consumed snacks (7.5%) compared to frequent snackers (2.7%). The co-occurrence of *S. mutans* and *S. sanguinis* was detected in 5.0% of the low-snacking group but was absent in frequent snackers. *S. sobrinus* and *S. sanguinis* were found in 5.4% of frequent snackers but were absent in those who snacked less frequently. The proportion of students without any of the three bacteria was 24.3% among frequent snackers and 22.5% in those who rarely snacked. No significant differences were observed in bacterial prevalence based on snacking habits ($p > 0.05$).

The findings indicate that *S. mutans* remained the dominant species regardless of oral hygiene or snacking habits. Although *S. sobrinus* was more prevalent among those who brushed more frequently, and *S. sanguinis* was only detected in those with better oral hygiene and less frequent snacking, these differences were not statistically significant. The absence of *S. sanguinis* in students who frequently snacked may suggest an altered microbial profile associated with dietary habits.

Changes in *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* presence in dental plaque over time

The bacterial profile in dental plaque changed significantly over the study period following fluoride varnish application (Table 5). At baseline (pre-intervention), *S. mutans* was detected in 50.6% of cases, *S. sobrinus* in 14.3%, and *S. sanguinis* in 1.3%. Co-infection of *S. mutans* and *S. sobrinus* was found in 5.2%, while *S. mutans* and *S. sanguinis* co-occurred in 2.6%. The presence of *S. sobrinus* and *S. sanguinis* together was recorded in 2.6%, and 23.4% of participants had no detectable bacterial colonization.

After three months, *S. mutans* prevalence dropped to 19.5%, while *S. sobrinus* remained stable at 13.0%. The detection of *S. sanguinis* increased slightly to 3.9%. Co-infections of *S. mutans* and *S. sobrinus* were observed in 3.9%, while *S. mutans* and *S. sanguinis* co-infection was no longer present. Similarly, *S. sobrinus* and *S. sanguinis* were undetected after three months. The proportion of bacteria-negative samples significantly increased to 59.7% ($p < 0.001$).

At six months post-treatment, *S. mutans* prevalence further declined to 11.7%, while *S. sobrinus* dropped to 6.5% and *S. sanguinis* to 2.6%. Only 1.3% of cases exhibited *S. mutans* and *S. sobrinus* co-infection, while no cases of *S. mutans* and *S. sanguinis* or *S. sobrinus* and *S. sanguinis* co-infection were detected. The proportion of bacteria-negative samples increased further to 77.9% ($p < 0.001$).

Statistical analysis indicated a significant reduction in *S. mutans* prevalence over time ($p < 0.001$), while *S. sobrinus* and *S. sanguinis* did not exhibit statistically significant variations ($p > 0.05$). The percentage of bacteria-free samples showed a significant increase from baseline to six months ($p < 0.001$), indicating a strong effect of fluoride varnish in reducing cariogenic bacterial presence.

These findings demonstrate a significant reduction in *S. mutans* prevalence over time, with a corresponding increase in bacteria-free samples, particularly after six months of fluoride varnish application. The lack of significant changes in *S. sobrinus* and *S. sanguinis* suggests that fluoride treatment primarily impacts *S. mutans*, which is considered the most cariogenic species. The marked increase in bacteria-free samples highlights the potential effectiveness of fluoride varnish in modulating the oral microbiome and reducing the risk of dental caries.

Table 2. Distribution of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* in Dental Plaque by Age Group and Gender

Group	*Streptococcus mutans* (%)	*Streptococcus sobrinus* (%)	*Streptococcus sanguinis* (%)	*Streptococcus mutans* + *Streptococcus sobrinus* (%)	*Streptococcus mutans* + *Streptococcus sanguinis* (%)	*Streptococcus sobrinus* + *Streptococcus sanguinis* (%)	No Infection (%)	*p*-value
Gender								
Male (n = 41)	48.8	14.6	2.4	4.9	2.4	4.9	22.0	0.466
Female (n = 36)	52.8	13.9	0.0	5.6	2.8	0.0	25.0	
p-value	0.726	0.926	1.000	1.000	1.000	0.496	0.752	
Age Group								
6–7 years (n = 33)	60.6	15.2	3.0	9.1	3.0	0.0	9.1	0.050
8–10 years (n = 44)	43.2	13.6	0.0	2.3	2.3	4.5	34.1	
p-value	0.130	1.000	0.429	0.308	0.836	0.504	0.010	

Table 3. Prevalence of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* by Number of Carious Teeth and Caries Location

Group	*Streptococcus mutans* (%)	*Streptococcus sobrinus* (%)	*Streptococcus sanguinis* (%)	*Streptococcus mutans* + *Streptococcus sobrinus* (%)	*Streptococcus mutans* + *Streptococcus sanguinis* (%)	*Streptococcus sobrinus* + *Streptococcus sanguinis* (%)	No Infection (%)	*p*-value
Number of Carious Teeth								
< 6 (n = 8)	75.0	12.5	0.0	0.0	0.0	0.0	12.5	0.397
6–10 (n = 29)	41.4	13.8	0.0	3.4	6.9	6.9	27.8	
≥ 11 (n = 40)	52.5	15.0	2.5	7.5	0.0	0.0	22.5	
p-value	0.231	1.000	1.000	0.766	0.337	0.337	0.793	
Caries Location								
Molars only (n = 53)	47.2	13.2	1.9	3.8	3.8	3.8	26.4	0.470
Anterior and molars (n = 24)	58.3	16.7	0.0	8.3	0.0	0.0	16.7	
p-value	0.364	0.732	1.000	0.585	1.000	1.000	0.349	

Table 4. Prevalence of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* Based on Oral Hygiene and Snacking Habits

Group	*Streptococcus mutans* (%)	*Streptococcus sobrinus* (%)	*Streptococcus sanguinis* (%)	*Streptococcus mutans* + *Streptococcus sobrinus* (%)	*Streptococcus mutans* + *Streptococcus sanguinis* (%)	*Streptococcus sobrinus* + *Streptococcus sanguinis* (%)	No Infection (%)	*p*-value
Brushing Frequency								
< 2 times/day (n = 14)	57.1	7.1	0.0	7.1	0.0	0.0	28.6	0.788
≥ 2 times/day (n = 63)	49.2	15.9	1.6	4.8	3.2	3.2	22.2	
p-value	0.591	0.678	1.000	0.560	1.000	1.000	0.728	
Snacking Frequency								
Frequent (n = 37)	51.4	16.2	0.0	2.7	0.0	5.4	24.3	0.240
Rare (n = 40)	50.0	12.5	2.5	7.5	5.0	0.0	22.5	
p-value	1.000	0.642	1.000	0.616	0.494	0.228	1.000	

Table 5. Changes in *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* Presence in Dental Plaque Over Time

Group	*Streptococcus mutans* (%)	*Streptococcus sobrinus* (%)	*Streptococcus sanguinis* (%)	*Streptococcus mutans* + *Streptococcus sobrinus* (%)	*Streptococcus mutans* + *Streptococcus sanguinis* (%)	*Streptococcus sobrinus* + *Streptococcus sanguinis* (%)	No Infection (%)	*p*-value
Timepoint								
Before Treatment (n = 77)	50.6	14.3	1.3	5.2	2.6	2.6	23.4	< 0.001
After 3 Months (n = 77)	19.5	13.0	3.9	3.9	0.0	0.0	59.7	
After 6 Months (n = 77)	11.7	6.5	2.6	1.3	0.0	0.0	77.9	
p-value	< 0.001	0.260	0.585	0.359	0.109	0.109	< 0.001	

DISCUSSION

Prevalence and distribution of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* in dental plaque

Dental caries is a multifactorial disease influenced by host factors, diet, and microbial composition, with *S. mutans* and *S. sobrinus* playing major roles in its development. The detection of these bacteria in dental plaque samples has been increasingly favored over saliva-based methods due to their higher accuracy in identifying bacterial colonization at carious lesion sites. The results of this study indicate significant variations in bacterial prevalence compared to previous research, potentially influenced by regional differences in diet, oral hygiene habits, and host genetics.

The prevalence of *S. mutans* was found to be 50.6%, a rate higher than the 10% reported by Pandey et al. (2022) in children aged 6–9 years and the 23.5% reported by Singla et al. (2016) in children aged 3–5 years, but lower than the 82.6% observed in Brazilian children aged 5–6 years by Franco et al. (2007). *S. sobrinus* was present in 14.3% of cases, exceeding the 13% reported by Okada et al. (2005) in Japanese children and the 8.8% observed by Singla et al. (2016), but slightly lower than Franco et al. (2007) (17.4%). Co-infection with *S. mutans* and *S. sobrinus* was found in 5.2% of cases, significantly lower than the 58% reported by Piszko et al. (2023), the 58.8% by Singla et al. (2016), and the 18% observed in infants by Soyolmaa et al. (2011).

The lower co-infection rates compared to international studies suggest potential regional differences in microbial colonization patterns, host immune responses, or oral hygiene behaviors. Factors such as fluoride exposure, diet, and oral health education may also contribute to variations in bacterial distribution. Notably, *S. mutans* was the most prevalent species in this study, reaffirming its dominant role in caries pathogenesis.

Research on cariogenic bacteria in Vietnamese populations remains limited, particularly in the Mekong Delta region. The findings of this study contribute to the growing body of literature on bacterial prevalence in Vietnamese children. A previous study by Luu Hong Lat (2018) reported *S. mutans* prevalence at 47.48% in deep carious lesions from patients at the National Hospital of Odonto-Stomatology in Ho Chi Minh City, which aligns with the current findings (Luu et al., 2018). Additional research by Nguyen Huu Tuyen et al. demonstrated that nano-berberine exhibited strong inhibitory effects against *S. mutans*, indicating potential applications for antimicrobial interventions (Nguyen et al., 2023).

A study conducted by Vu Dinh Tuyen (2024) on 6–7-year-old students in Hai Duong province reported *S. mutans* in 82% and *S. sobrinus* in 24% of cases, both higher than the prevalence rates observed in this study (Vu, 2024). Studies in Indonesia by Zubaidah et al. (2022) (94% *S. mutans*, 30% *S. sobrinus*) reported higher prevalence rates, as did studies conducted in India (68% *S. mutans*, 67% *S. sobrinus*) (Pandey et al., 2022). These differences may be attributed to variations in dietary patterns, oral hygiene practices, and genetic predisposition to microbial colonization.

Gender and age differences in bacterial prevalence

No significant gender-based differences were found in the prevalence of *S. mutans* or *S. sobrinus*. The combined prevalence of *S. sobrinus* was 31.7% in boys and 19.5% in girls, lower than the 48.3% in boys and 50.2% in girls reported by Pandey et al. (2022), and slightly higher than the 20% in boys and 9% in girls reported by Franco et al. (2007). The prevalence of *S. mutans* in boys (48.2%) was slightly lower than that observed by Pandey et al. (2022) (51.7%) but higher in girls (61.2%) compared to their findings (47.9%). These variations suggest that gender-related differences may be influenced more by dietary and oral hygiene behaviors rather than intrinsic biological factors.

Age-related variations were also observed, with *S. sobrinus* detected in 27.3% of children

aged 6–7 years and 25% of those aged 8–10 years. The prevalence of *S. mutans* was higher in younger children (66.7%) compared to older children (45.5%), a trend consistent with findings from Okada et al. (2005) in Japanese children, where *S. mutans* was detected in 74.2% of those aged ≥ 5 years (Okada et al., 2005). The higher bacterial prevalence in younger children may be attributed to less effective oral hygiene practices, greater consumption of cariogenic foods, and the transition from primary to permanent dentition.

Effectiveness of fluoride varnish in reducing *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis*

Fluoride varnishes have long been recognized for their ability to promote remineralization and prevent dental caries. Clinpro™ White Varnish, used in this study, contains tricalcium phosphate, which enhances remineralization and exhibits antibacterial properties against cariogenic bacteria (Coelho, 2020). The findings confirmed the effectiveness of fluoride varnish in reducing *S. mutans* and *S. sobrinus* in dental plaque over time. The antibacterial effects observed align with previous studies, such as those by Erkmen Almaz and Akbay Oba (2020), which demonstrated that fluoride varnishes containing Tricalcium Phosphate significantly reduced *S. mutans* and *Lactobacillus* levels after three months (Erkmen & Akbay, 2020).

The data reveal a notable decrease in *S. mutans* and *S. sobrinus* prevalence following fluoride varnish application. Prior to treatment, *S. mutans* was present in 55.8% of plaque samples, and *S. sobrinus* in 26%. After three months, these percentages decreased to 23.4% and 16.9%, respectively, and further declined to 13% and 7.8% after six months. The progressive reduction over time underscores the sustained antibacterial effect of fluoride varnish, which is consistent with studies by Nano Silver Fluoride Varnish reduced *S. mutans* prevalence from 93.3% to 20% over three months (Wang & Liu, 2013). Similarly, research by Ben Khadra et al. (2019) and Paul et al. (2014) demonstrated significant declines in *S. mutans* levels in response to fluoride varnish application, emphasizing its potential for long-term microbial control (Ben Khadra et al., 2019; Paul et al., 2014).

Implications of *Streptococcus mutans*, *Streptococcus sobrinus*, and *Streptococcus sanguinis* in caries prevention

The marked reduction in *S. mutans* prevalence supports its role as a primary etiological agent in dental caries. The study by Acevedo et al. (2009) found that *S. mutans* was detected in 62.5% of children with caries and 37.5% of caries-free individuals, reinforcing its strong association with disease progression (Acevedo et al., 2009). The current study aligns with these findings, demonstrating a significant decline in *S. mutans* prevalence after fluoride varnish application. The sustained antibacterial effects observed over six months suggest that fluoride varnish application can serve as an effective strategy for managing *S. mutans* populations in pediatric patients.

The reduction in *S. mutans* also aligns with previous research in Southeast Asia. Zubaidah et al. (2022) reported *S. mutans* prevalence rates of 94% in children and 52% in adults, suggesting that childhood represents a critical period for microbial intervention (Zubaidah et al., 2022). Pandey et al. (2022) similarly observed *S. mutans* positivity in 68% of cases, indicating that targeted interventions during early childhood could have significant long-term benefits (Pandey et al., 2022).

While *S. mutans* is widely recognized as a key cariogenic bacterium, *S. sobrinus* has been linked to more aggressive and rapidly progressing caries lesions (Zhou, 2016). The present study demonstrated a notable reduction in *S. sobrinus* levels following fluoride varnish application, which is crucial given its association with severe caries development.

Previous studies have highlighted *S. sobrinus* as a significant risk factor for

extensive caries. Okada et al. (2005) reported that *S. sobrinus* was present in 61.3% of children aged ≥ 5 years, a much higher prevalence than that observed in this study (Okada et al., 2005). Additionally, research by Wang & Liu (2013) indicated that *S. sobrinus* was more prevalent in cases of rapidly progressing caries, suggesting that its presence may indicate a more aggressive disease phenotype (Zhou, 2016). The decline in *S. sobrinus* levels after fluoride varnish treatment suggests that this intervention may be particularly effective in high-risk populations where *S. sobrinus* contributes to caries progression.

Unlike *S. mutans* and *S. sobrinus*, *S. sanguinis* is often considered a commensal species with potential protective effects against caries. However, its role remains controversial, as some studies have suggested that its presence may contribute to early enamel demineralization. The findings of this study show that *S. sanguinis* was present in only 1.3% of plaque samples before intervention, with a slight increase to 3.9% after three months, before decreasing again to 2.6% at six months. This fluctuation suggests that while fluoride varnish may reduce *S. mutans* and *S. sobrinus*, its effect on *S. sanguinis* is less pronounced.

Studies have indicated that *S. sanguinis* competes with *S. mutans* for colonization sites on enamel surfaces, potentially inhibiting *S. mutans*-mediated caries progression (Zhou, 2016). However, other research has suggested that *S. sanguinis* may still play a role in caries initiation by promoting an acidic microenvironment (Acevedo et al., 2009). The present study's findings suggest that *S. sanguinis* may be less responsive to fluoride varnish treatment than *S. mutans* or *S. sobrinus*, warranting further investigation into its role in caries prevention strategies.

Implications for future research and study limitations

This study provides valuable insights into the microbial composition of dental plaque in Vietnamese students, but several limitations should be considered for future research. While *S. mutans* and *S. sobrinus* are well-established cariogenic bacteria, they act within a broader microbial community, including *S. sanguinis*, *Lactobacillus* spp., and *Actinomyces* spp. Future studies should employ metagenomic sequencing to comprehensively analyze the oral microbiome and its role in caries progression.

The results confirm *S. mutans* as the predominant species in dental plaque, while *S. sobrinus* has a lower prevalence, differing from international studies suggesting a stronger role for *S. sobrinus* in rapidly progressing caries (Zhou, 2016). No significant correlations were observed between *S. mutans* and *S. sobrinus* presence with the number of decayed teeth, tooth type, or jaw location, indicating that additional host and environmental factors influence bacterial distribution (Okada et al., 2005; Setyarini et al., 2020; Yuan et al., 2020).

The study utilized PCR-based bacterial identification for its accuracy and efficiency; however, bacterial load was not quantified. Future research should incorporate quantitative PCR (qPCR) or next-generation sequencing (NGS) to assess bacterial abundance and its correlation with caries severity. A notable proportion of children with active caries lacked detectable *S. mutans* or *S. sobrinus*, suggesting involvement of other cariogenic bacteria. Expanding microbiological investigations will enhance understanding of the diversity of caries-associated species in Vietnamese children.

The findings also support the clinical use of fluoride varnish as an effective intervention for reducing *S. mutans* and *S. sobrinus* in students. The sustained antibacterial effects observed over six months highlight its potential for inclusion in preventive care programs, particularly for children at high risk of dental caries. However, the study did not evaluate long-term caries incidence following treatment. Future research should examine whether bacterial reductions translate into lower caries progression rates. Additionally, while fluoride varnish significantly reduced

bacterial levels, the optimal frequency of application remains uncertain. A study by Nguyen et al. (2021) reported a reduction in caries prevalence from 66.4% to 40% following fluoride varnish application, but the necessary reapplication interval requires further investigation (Nguyen et al., 2021).

Further studies should explore broader microbial shifts induced by fluoride varnish. The presence of caries-active children without detectable *S. mutans* or *S. sobrinus* suggests other bacterial species play key roles in caries development. Metagenomic sequencing could provide a more detailed understanding of the caries-associated microbiome and identify additional bacterial targets for intervention.

Variations in fluoride varnish formulations should also be examined. While Clinpro™ White Varnish demonstrated strong antibacterial effects, comparisons with other formulations, such as those containing chlorhexidine-thymol or nano-silver fluoride, could optimize microbial control. Previous research by Paul et al. (2014) has shown differences in antibacterial efficacy between fluoride varnishes, emphasizing the need for further comparative studies (Paul et al., 2014; Wang & Liu, 2013).

CONCLUSION

This study highlights the prevalence of *S. mutans*, *S. sobrinus*, and *S. sanguinis* in Vietnamese students, with *S. mutans* playing a dominant role in caries progression. Fluoride varnish containing tricalcium phosphate significantly reduced *S. mutans* and *S. sobrinus* over six months, supporting its use as a preventive measure for high-risk children.

Fluoride varnish should be implemented in school-based caries prevention programs, with further research on bacterial load, long-term effectiveness, and the potential integration of complementary strategies such as probiotics for enhanced oral health outcomes.

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