ORIGINAL RESEARCH

Streptococci Biotypes in Primary and Permanent Caries: A Case–Control Study

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Abstract

Background and aim: Streptococci, mainly mutans streptococci, are known as the causative microbes of dental caries, but there is limited clarity about their impact on the tooth level and the distribution of streptococci species in different dentition stages. This study evaluates the distribution of streptococci species in primary and permanent teeth in children and adolescents with caries.

Materials and methods: The study population consisted of two groups: subjects with caries in primary teeth aged 2–5 years and adolescents with caries in permanent teeth aged 12–15 years. Age-matched controls were included in both groups. The decayed, missing, and filled teeth for primary teeth/decayed, missing, and filled teeth for permanent teeth (dmft/DMFT) index score was recorded according to World Health Organization (WHO) protocol. Dental biofilm samples were obtained from tooth sites under sterile conditions and placed in sterile transport media. Inoculation was performed on specific media, colonies were counted, and streptococci species were identified.

Results: In subjects with primary dentition caries, the streptococci species identified were *Streptococcus mutans* (*S. mutans*), *S. sobrinus*, *S. mitis*, *S. rattus*, and *S. sanguis*. In subjects with caries in permanent teeth, *S. salivarius*, *Enterococcus faecalis* (*E. faecalis*), *S. mitis*, and *S. agnosus* were seen in addition to *S. mutans* and *S. sobrinus*. The levels of mutans streptococci colony-forming units (CFU) were significantly higher in the primary caries group in children ($p < 0.01$). However, the CFU of streptococci in the healthy control group in children was not statistically different from the CFU/mL of streptococci in the healthy control group in adolescents.

Conclusion: A significant difference ($p < 0.01$) in the streptococci species profile was observed between primary and permanent teeth with caries.

Keywords: Caries, Oral streptococci, Permanent teeth, Primary teeth.

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Introduction

The genus *Streptococci* is of considerable importance in oral microbiology. The species commonly isolated from the healthy oral cavity are *Streptococcus salivarius* (*S. salivarius*), *S. sanguis*, *S. mitis*, and mutans streptococci.[1] The human mouth is sterile at birth, with *S. salivarius* being reported as the first species to establish on the tongue and oral mucosa within 18 hours after birth.[2] Dental decay or caries is known as a common chronic noncommunicable disease worldwide.[3] Data shows that 58% of adolescents aged 12–19 years have caries in permanent teeth, and 37% of children aged 2–8 years have caries in primary teeth.[4] Dental caries is primarily caused by mutans streptococci (*S. mutans* and *S. sobrinus*).[5] The less commonly isolated species of mutans streptococci from the human mouth and other mammals are *S. criceti*, *S. rattus*, *S. sobrinus*, *S. downei*, *S. macacae*, and *S. ferus*.[6]

A correlation between mutans streptococci levels and all types of caries has been observed in a number of studies.[7–9] These studies have confirmed the close relationship between mutans streptococci and dental caries. The keystone plaque hypothesis suggests a shift in bacterial profiles as dental caries advance.[10] In a preliminary study, species of the genera *Lactobacillus*, *Prevotella*, *Selomonas*, *Dialister*, *Fusobacterium*, *Eubacterium*, *Bifidobacterium*, *Propionibacterium*, and *Pseudoramibacter* were found to be present in rampant caries.[11] In another study involving cleft and noncleft children with differing caries in primary and permanent dentition, a higher total salivary streptococci count was observed in cleft subjects with caries. There was a positive correlation between decayed, missing, and filled teeth for primary teeth (dmft)/decayed, missing, and filled teeth for permanent teeth (DMFT) scores and salivary streptococci levels in dental caries.[12] Scant literature exists regarding the prevailing streptococci biotypes in primary and permanent dentition in dental caries. Moreover, the few existing studies have identified cariogenic streptococci using samples other than dental plaque, most commonly saliva.[13] Furthermore, the use of the 16S rRNA gene-based approach is not suitable for identifying oral streptococci, as oral streptococci are poorly differentiated by the 16S rRNA gene.[14,15]

Therefore, the present study aimed: (1) To isolate and identify biochemically the streptococci biotypes in caries of primary and permanent teeth in children and adolescents taking plaque

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samples; (2) To count the levels of streptococci [colony-forming units (CFU)] in the plaque of primary and permanent caries groups.

Materials and Methods

This current cross-sectional study was carried out between January 2023 and October 2023. The subject population was divided into two different groups: (1) Children with caries in primary teeth ($n = 15$) aged 2–5 years; (2) Adolescents with caries in permanent teeth ($n = 15$) aged 12–15 years. Thirty age-matched caries-free controls were included in both groups. The World Health Organization (WHO) criteria 2013 were followed for primary and permanent dentition age range and oral health behavior.[16]

The oral examination was performed by a well-trained dentist using a plane mouth mirror and explorer, with the subject sitting on the dental chair. The same examiner assessed all the subjects. The caries experience of the child/adolescent was assessed and recorded using the WHO criteria. The exclusion criteria included children who had been on antibiotics three months before dental biofilm collection and those using dentures or orthodontic appliances.

The study was conducted in the microbiology and pedodontic departments of Dr Harvansh Singh Judge Institute of Dental Science and Hospital, Panjab University, Chandigarh. Approval to commence the study was obtained from the university ethical board (PUIEC/2018/119/A-1/29/10). Written consent and assent were obtained from participants prior to the study. Participants were assured of data confidentiality.

Plaque Sampling

All the volunteers were asked to avoid brushing and eating in the morning before sample collection. Dental biofilms were collected from tooth sites in quadrants, pooled, and placed in sterile Eppendorf tubes containing sterile thioglycollate broth (HiMedia, Mumbai). After collection, the samples were sent to the microbiology laboratory.

Microbiological Processing

The sample was inoculated (0.1 mL) on MSB Agar (mitis salivarius–bacitracin) by spread plating and incubated at 37°C for 48 hours. After incubation, the colonies on the mitis salivarius agar plates were counted using a digital colony counter, and results were expressed as CFU/mL. The growth was visualized by Gram's stain, and a catalase test was performed. An inoculum was prepared from the growth and inoculated on the KB005A Hi Strep™ identification kit (HiMedia, Mumbai) for the differentiation of streptococci. The biochemical tests included in the kit and performed were fermentation of raffinose, mannitol, glucose, lactose, arabinose, sucrose, sorbitol, arginine hydrolysis, Voges-Proskauer, PYR, and ONPG utilization.[17]

Data Analysis

The data analysis was performed using Statistical Package for the Social Sciences (SPSS) package (version 22, IBM Corp, Armonk, New York, United States of America). The streptococci species distribution was analyzed by one-way analysis of variance (ANOVA) and the Kruskal–Wallis test. To compare dmft/DMFT scores with levels of mutans streptococci, the Wilcoxon signed-rank test was used.

Results

The growth on mitis salivarius agar was visualized by Gram staining. Gram-positive cocci in a chain-like structure and showing a catalase-negative test indicated that the colonies were of the genus *Streptococcus*. The biochemical tests in the Hi Strep kit identified each streptococci biotype. Oral streptococci were present in all the plaque samples from the 60 subjects. In children with caries in primary teeth, the streptococci biotypes isolated were *S. mutans* (53.3%), *S. sobrinus* (26.7%), *S. mitis* (6.7%), *S. rattus* (6.7%), and *S. sanguis* (6.7%). The mean count of the plaque samples in this group was 1.35 ± 1.99 CFU/mL with a mean dmft index of 8.60 ± 3.58. In the primary control group, the streptococci biotypes isolated were *S. mutans* (33.3%) and *S. mitis* (33.3%), with no *S. sobrinus* and *S. rattus* detected. The mean colony count of the plaque samples was 0.06 ± 0.03 CFU/mL, significant at $p < 0.001$. In children with caries in permanent teeth, the streptococci biotypes isolated were *S. mutans* (33.3%), *S. sobrinus* (20%), *S. salivarius* (26.7%), *Enterococcus faecalis* (*E. faecalis*) (6.7%), *S. mitis* (6.7%), and *S. agnisosus* (6.7%). The mean count of streptococci in these subjects was 0.08 ± 0.14 CFU/mL with a mean DMFT score of 3.67 ± 3.15. In the control group of adolescents, the streptococci biotypes isolated were *S. agalactiae* (16.7%), *S. mitis* (6.7%), *S. salivarius* (33.3%), and *S. sanguis* (33.3%). The mean colony count was 0.02 ± 0.01 CFU/mL, which was significant at $p < 0.001$ compared to the test group. The mean streptococci count of the control subjects in the primary caries group was 0.06 ± 0.03 CFU/mL, and the mean streptococci count of the control subjects in the permanent group was 0.02 ± 0.01 CFU/mL, which was not statistically significant (Fig. 1 and Table 1). The association between

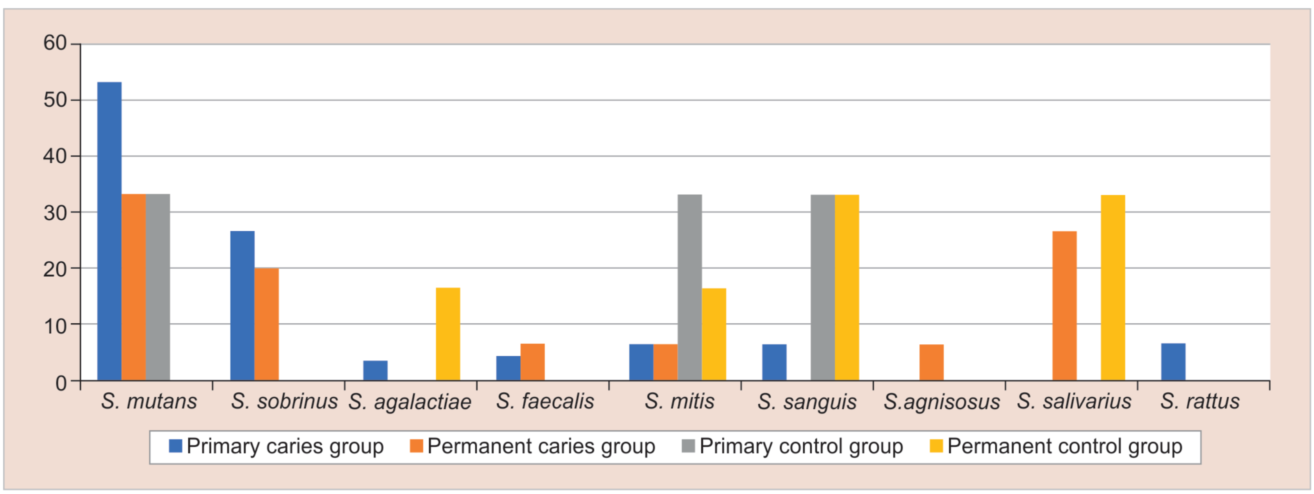

Fig. 1: Biotypes in different groups

Table 1: Mean salivary streptococci counts in different groups

Bacterial count (CFU/mL)	*Primary caries group [mean ± standard deviation (SD)]*	*Permanent caries group (mean ± SD)*	*Primary control group (mean ± SD)*	*Permanent control group (mean ± SD)*	*ANOVA*
Streptococci	1.35 ± 1.99	0.08 ± 0.14	0.06 ± 0.03	0.02 ± 0.01	0.004

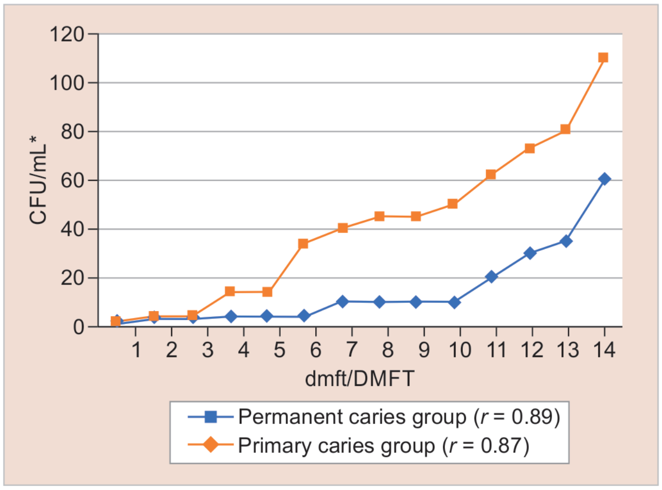

Fig. 2: Correlation of dmft/DMFT with streptococci counts in caries groups

the dmft/DMFT scores of subjects and the total streptococci counts was determined using Pearson correlation. The streptococci levels and the dmft score in children with caries in primary teeth were significantly positively correlated ($r = 0.87$). The streptococci counts and dmft/DMFT scores in adolescents with caries in permanent teeth were also positively correlated ($r = 0.89$) (Fig. 2).

Discussion

The isolation and biochemical identification of oral streptococci in plaque samples showed that species colonizing the mouth in children with primary teeth caries, aged 2–5 years, were predominantly *S. mutans* and *S. sobrinus*, with very low occurrences of *S. rattus*, *S. mitis*, and *S. sanguis*. The mean CFU/mL of oral streptococci in this group was 1.35 ± 1.99. The increased levels of mutans streptococci in children aged 2–5 years with caries correlate with findings from other studies.[18–22] However, these studies have reported salivary streptococci and our study reports streptococci from plaque. This shows streptococcus species isolation from saliva to plaque is similar. *S. rattus* was also isolated in a single sample from a child <5 years having caries. *S. rattus* has also been reported in Tanzanian children having caries.[23] It was also observed that there is a significantly high association of mutans streptococci in subjects with caries. In children with caries in primary dentition, *S. mutans* accounted for 53.3% of the isolates and *S. sobrinus* for 26.7%. Both *S. mutans* and *S. sobrinus* are considered initiators of caries, and their presence together can lead to the rapid development of early or severe caries.[24–26] The dmft scores of children with mutans streptococci were also higher than those with *S. mutans* alone. This indicates that children harboring both *S. mutans* and *S. sobrinus* exhibit significantly greater caries activity compared to those with *S. mutans* alone. *S. sanguis*, while isolated in both healthy mouths and in caries, showed a strong positive correlation with dmft/DMFT scores in caries of primary and permanent teeth. Other studies have similarly demonstrated a positive relationship between caries experience and levels of *S. mutans*.[11] A recent study indicates that the maximum caries is recorded in the first teeth to emerge, particularly the primary molars (D's), which are later replaced by permanent molars (6's). These teeth, emerging first and located toward the front of the mouth, provide a favorable environment for *S. mutans* to adhere and form dental biofilm, thereby initiating caries and contributing to high caries susceptibility.[27]

Strategies need to be developed to prevent mutans streptococci transmission and acquisition. A study has reported that preventing or delaying the colonization of *S. mutans* in young children by treating their mothers and diagnosing caries at age 19 resulted in 7 out of 19 children being caries-free compared to controls, where all had caries by this age.[28] In adolescents having caries in permanent teeth, the streptococci biotypes isolated were mainly *S. mutans*, *S. sobrinus*, *S. salivarius*, *S. faecalis*, *S. agnisosus*, and *S. mitis*. This indicates a change in the streptococci species profile with disease progression. Studies have shown that subjects with advanced decay in teeth may not have significant counts of *S. mutans*.[11] However, the total streptococci colony counts were not significantly different between candidates in the primary and permanent teeth caries groups. The DMFT index verifies the prevalence of decayed, missing, and filled teeth in the past but fails to differentiate between active and inactive caries.[29] This could explain the lower mutans streptococci count in children with caries in permanent teeth. In the control group of caries-free subjects, the rate of mutans streptococci isolation was 23.8%. Not all subjects who are carriers of *S. mutans* develop caries. Studies suggest that host susceptibility plays a crucial role in the initiation of tooth decay. The initiation of dental decay is influenced not only by the cariogenicity of *S. mutans* but also by factors such as genetic constitution, tooth anatomy and quality, nature of saliva, and the oral microbiome of the host. Although the sample size in our study is small, it indicates that mutans streptococci are more prevalent in caries among children compared to adolescents, where nonmutans streptococci are more common. Therefore, strategies need to be developed to prevent the transmission and acquisition of mutans streptococci at an early age, such as the development of an effective caries vaccine to correct the imbalance of mutans streptococci.

Conclusion

Mutans streptococci are more prevalent in the initial stages of caries development. As the disease progresses, the streptococci profile shifts to include non-*S. mutans* streptococci. Therefore, more studies on both cultivable and yet-to-be-cultivated oral streptococci are needed to better understand their roles in caries disease.

Author Contributions

Study conception and design: SBB.
Data collection and analysis: SBB, US.
Original manuscript draft: SBB, US, MM, JS.
All authors approve the final version of the manuscript.

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References

1. Thurnheer T, Gmur R, Giertsen E, et al. Automated fluorescent in situ hybridization for the specific detection and quantification of oral streptococci in dental plaque. J Microbiol Methods 2001;44:39–47. DOI: 10.1016/s0167-7012(00)00226-8
2. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett 2014;162(2):22–38. DOI: 10.1016/j.imlet.2014.08.017
3. Dye BA, Thornken-Evans G, Li X, et al. Dental caries and sealant prevalence in children and adolescents in the United States, 2011-2012. NCHS Data Brief 2015;(191):1–8.
4. Saman HA, Senthil Kumar R, Imran K, et al. Isolation and typing of *Streptococcus mutans* and Streptococcus sobrinus from caries-active subjects. Contemp Clin Dent 2017;8:587–593. DOI: 10.4103/ccd.ccd_610_17
5. Gábris K, Nagy G, Madléna M, et al. Associations between microbiological and salivary caries activity tests and caries experience in Hungarian adolescents. Caries Res 1999;33:191–195. DOI: 10.1159/000016516
6. Peterson SN, Snesrud E, Liu J, et al. The dental plaque microbiome in health and disease. PLoS One 2013;8(3):e58487. DOI: 10.1371/journal.pone.0058487
7. Hong X, Hu DY. Salivary *Streptococcus mutans* level: value in caries prediction for 11-12-year-old children. Community Dent Health 2010;27:248–252.
8. Hebbal MI, Ankola AV, Metgud SC. Association between socio-economic status, salivary *S. mutans*, Lactobacilli, and dental caries among 12-year-old school children in Belgaum city. World J Dent 2011;2:316–320.
9. Pannu P, Gambhir R, Sujllana A. Correlation between the salivary *Streptococcus mutans* levels and dental caries experience in adult population of Chandigarh, India. Eur J Dent 2013;7:191–195. DOI: 10.4103/1305-7456.110169
10. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994;8:263–271. DOI: 10.1177/08959374940080022001
11. Aas JA, Griffin AL, Dardis SR, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008;4:1407–1417.
12. Ravindran S, Chaudhary M, Gawande M. Enumeration of salivary streptococci and lactobacilli in children with differing caries experience in adult population of Chandigarh, India. Eur J Dent 2013;7:191–195. DOI: 10.5402/2013/476783
13. Simon-Soro A, Tomás I, Cabrera-Rubio R, et al. Microbial geography of the oral cavity. J Dent Res 2013;92:616–621.
14. Bishop CJ, Aanensen DM, Jordan GE, et al. Assigning strains to bacterial species via the internet. BMC Biol 2009. DOI: 10.1186/1741700773
15. Hiergeist A, Reischl U, Priority Program 1656 Intestinal Microbiota Consortium/ quality assessment participants, et al. Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability. Int J Med Microbiol 2016;306:334–342. DOI: 10.1016/j.ijmm.2016.03.005
16. World Health Organization. Oral Health Surveys: Basic Methods, 5th edition. Geneva, Switzerland; World Health Organization; 2013.
17. https:/www.cdc.gov/streplab/strep-doc/general–methods-section2.html.2017.
18. Rashkovo MP, Tonchea AA. Gingival disease and secretory immunoglobulin A in non-stimulated saliva in children. Folia Med 2010;52:48–55. DOI: 10.2478/v10153-010-0017-y
19. Sidi AD, Ashley FP. Influence of frequent sugar intakes on experimental gingivitis. J Periodontol 1984;55:419–423. DOI: 10.1902/jop.1984.55.7.419
20. Roters FJM, Van De Hoeven JS, Burgersdiij K RCW, et al. Lactobacilli, mutants streptococci and dental caries: a longitudinal study in 2-year-old children up to the age of 5 years. Caries Res 1995;29:272–279. DOI: 10.1159/000262081
21. Pradopo S. The colony number of *S. mutans* and Lactobacillus in saliva of dental caries and free children. Dent J 2008;41:535.
22. Sakeenabi B, Hiremath SS. Dental caries experience and salivary *Streptococcus mutans*, lactobacilli scores, salivary flow rate, and salivary buffering capacity among 6-year-old Indian school children. J Int Soc Prev Community Dent 2011;1(2):45–51. DOI: 10.4103/2231-0762.97697
23. Matee MIN, Mik XFHM, Frencken JEFN, et al. Selection of a micromethod and its use in the estimation of salivary *Streptococcus mutans* and lactobacillus counts in relation to dental caries in Tanzanian children. Caries Res 1985;19:497–506. DOI: 10.1159/000260888
24. Okada M. Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and Streptococcus sobrinus in pre-school children. J Med Microbiol 2005;54;661–665. DOI: 10.1099/jmm.0.46069-0
25. Jiang Q, Yu M, Min Z, et al. AP-PCR detection of *Streptococcus mutans* and *Streptococcus sobrinus* in caries free and caries active subjects. Mol Cell Biochem 2012;365;159–164. DOI: 10.1007/s11010-012-1255-5
26. Soni H, Vasavada M. Distribution of *S. mutans* and *S. sobrinus* in caries-active and caries-free children by PCR approach. Int J Oral Craniofac Sci 2015;1:27–30.
27. Dinis M, Traynor W, Argenello M, et al. Tooth specific *Streptococcus mutans* distribution and associated microbiome. Microorganisms 2022;10:1129. DOI: 10.3390/microorganisms10061129
28. Kohler B, Andreen I. Mutans streptococci and caries prevalence in children after early maternal caries prevention: a follow-up at 19 years of age. Caries Res 2012;46:474–480. DOI: 10.1159/000339665
29. Chokshi A, Mahesh P, Sharada P, et al. A correlative study of the levels of salivary *Streptococcus mutans*, lactobacilli and Actinomyces with dental caries experience in subjects with mixed and permanent dentition. J Oral Maxillofac Pathol 2016;20:25–28. DOI: 10.4103/0973-029X.180916