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# Molecular identification and genotyping of *Streptococcus mutans* from saliva samples of children in Medellin, Colombia*

## Identificación molecular y genotipificación de *Streptococcus mutans* de muestras de saliva de niños de Medellín, Colombia

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## Abstract

### Introduction and objective:
Tooth decay is one of the most prevalent infectious diseases of the oral cavity. The aim of this study was to determine the prevalence of *Streptoccocus mutans* and its genotypes in saliva samples from 6 and 7 year old children with and without dental caries.

### Materials and methods:
Forty seven saliva samples were obtained from 6-7 year-old children, randomly selected from a Public School in Medellin, Colombia. Oral examinations in order to determine the DMF-T Index (Decay, Missing and Filling Teeth) were carried out. Isolates were identified by the *Streptoccocus sobrinus* and *Streptococcus mutans* PCR, API 20STREP and 16S rRNA sequence analysis. Genotypes *c, e, f* and *k* were detected by PCR.

### Results:
Prevalence of *S. mutans* was 14.9%. Of the 47 saliva samples, 57.4% (27) corresponded to children with dental caries, and 8.5% (4) were positive for *S. mutans* genotype *c*, 2.1% (1 each) genotype *f*, genotype *k*, and genotype *c* and *k*, respectively.

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**Conclusion:**
Prevalence of *S. mutans* was lower than in previous studies of Colombian children with similar demographic characteristics. *Streptoccocus mutans* genotype *c*, *f* and *k* were found in children with caries but not in the group without caries. This is the first report of *S. mutans* genotype *k* in Colombia; this genotype requires further study to clarify its relation with dental caries and cardiovascular disease in Colombia.

**Key words:**
*Streptococcus mutans*, Tooth decay, Polymerase Chain Reaction, genotyping, 16S rRNA.

## Resumen

**Introducción y objetivo:**
La caries dental es una de las enfermedades infecciosas de la cavidad oral más comunes en el mundo. El objetivo de este estudio fue determinar la prevalencia de *Streptococcus mutans* y sus genotipos en muestras de saliva de niños de 6 y 7 años, con y sin caries dental.

**Materiales y métodos:**
Cuarenta y siete (47) muestras de saliva fueron obtenidas de niños de 6-7 años, seleccionados aleatoriamente de una escuela pública en Medellín, Colombia. Se realizaron evaluaciones orales para determinar el índice COP-D (número de dientes permanentes cariados, obturados y perdidos). Los aislamientos fueron identificados mediante PCR para diferenciación de *S. sobrinus* y *S. mutans*, API-20STREP y análisis de ARNr 16S. Se determinaron mediante PCR los genotipos *c, e, f* y *k* en las cepas identificadas como *S. mutans*.

**Resultados:**
La prevalencia de *S. mutans* fue 14,9%. De las 47 muestras de saliva, 57,4% (27) correspondieron a niños con caries dental, y 8,5 % (4), fueron positivas para *S. mutans* genotipo *c*, 2,1 % (1 cada una) genotipo *f*, genotipo *k* y genotipo *c* y *k*, respectivamente.

**Conclusión:**
La prevalencia de *S. mutans* fue menor que la reportada en estudios previos de niños colombianos con características demográficas similares. Se encontró presencia de genotipos *c, f* y *k* de *S. mutans* en niños con caries dental, pero no en el grupo de niños sin caries dental. Este es el primer estudio en reportar la presencia del genotipo *k* en Colombia. Es necesario realizar más investigaciones en nuestro país para aclarar la relación de *S. mutans* genotipo *k* con caries dental y enfermedad cardiovascular.

**Palabras clave:**
*Streptococcus mutans*, Caries dental, Reacción en Cadena de la Polimerasa, Genotipificación, ARNr 16S.

## Introduction

Tooth decay is one of the most common diseases of the oral cavity, affecting especially children between 5 and 12 years, and it is considered the most recurrent infectious disease worldwide (1, 2); it is an infectious pathological process, multifactorial, localized, post-eruptive and transmissible, that finally destroys hard dental tissues (3). The initiation and development of this disorder is linked to the presence of abundant microorganisms which vary depending on the stage of the lesion (4). Oral bacteria colonizing teeth are organized in biofilms which exists in a dynamic balance with the organism immune defenses (5). In addition to the presence of bacterial communities associated with fermentative metabolism, an increase in dietary carbohydrates, particularly sucrose (4,5), result in further acid production that may exceed both the capacity of the saliva to remove acid end-products and the neutralizing power of the salivary/plaque buffer system, producing additional acidification of the biofilm (6).

At each stage of progression of the carious lesion, succession of different bacterial species occurs. At the onset and progression of the lesion, species belonging to the Mutans streptococci group, particularly *Streptococcus mutans* and *Streptococcus sobrinus* are the main microorganisms found, while *Lactobacillus* and *Bifidobacterium* dominate in the advanced stages of the disease (4,5). Usually, the emergence of *S. mutans* in the tooth cavities is followed by the presence of caries after the infective window of 6 to 24 months (6).

The important role of *S. mutans* in caries initiation is due to its acidogenic and aciduric potential (7, 8). In addition, *S. mutans* generate an extracellular matrix polysaccharide in dental biofilms, the exopolysaccharides. These are glucans synthesized by the products of the glucosyltransferases genes *gtf*B and *gtf*C , and provide binding sites that promote the establishment of pathogenic biofilms (9,10).

Mutans streptococci are classified serologically on the basis of the chemical composition of its exopolysaccharides, also known as cell surface serotype-specific rhamnose–glucose polymers (RGPs). Six genetic groups and seven serotypes are recognized: *S mutans* serotypes *c, e, f, k*; *S. sobrinus* serotype *g*; *S. downei* serotype *g*; *S. rattus* serotype *b*; *S cricetus* serotype *a* and *S. macacae* serotype *c* (isolated from monkeys) (11,12). Data regarding the distribution and prevalence of *S. mutans* serotypes are limited. In Japan, the prevalence of *S. mutans* serotypes in oral isolates, is primarily serotype *c* (76 %), followed by serotype e (26 %), serotype *f* (8 %), and serotype *k* (2 - 5%) (12,13). Studies on acidogenicity of *S. mutans* serotypes had shown it is higher in subjects with dental caries and that the *c* serotypes strains are the most acidogenic (14,15).

Serotype *k* has been found in patients with infective endocarditis, a complication that can lead to heart valves replacement (16). Importantly, the frequency of serotype *k* was significantly higher (75%) in samples of dental plaque from patients with infectious endocarditis compared with oral samples from healthy patients (20%), suggesting a possible association with the development of cardiovascular disease (17-20).

In Colombia, some studies have analyzed the relationship between dental caries and counts of *S. mutans* in children with and without dental caries; however no statistically significant differences in *S. mutans* counts among these groups were found (21). Thirty years ago, presence of *S. mutans* serotype *c* and *f*, and other serotypes *d* and *g* classified as *S. sobrinus* according to Coykendall were reported in school-age population in the

country (22,23). Since then, there are no reports on studies evaluating prevalence of *S. mutans* and its serotypes in school-age population in Colombia. The aim of the present study was to determine the prevalence of *Streptococcus mutans* and its genotypes in saliva of children aged 6 and 7 years with and without dental caries in a public school in Medellin.

## Materials and methods

**Saliva sampling:** One saliva sample was obtained from 47 children of ages 6 and 7, randomly selected from a public primary school of Medellin city. Each child received oral examination to determine the Decay, Missing and Filling Teeth (DMF-T index). Saliva was collected in 50 mL sterilized conical tubes, after minimum an hour of food intake for 3 minutes. Evaluated children chewed 1 gr of wax (Ivoclar Vivadent AG FL-9494, Schaan, Liechtenstein). The saliva samples were stored at 4°C and processed within the following two hours.

**Bacterial cultures:** Serial dilutions of the saliva samples in sterilized PBS (10-1 to 10-6) were seeded in Mitis Salivarius Agar medium (Difco Laboratorios, Detroit MI, USA), added with 0.2 U/mL bacitracin (Sigma, St Louis, MO, USA), and subsequently incubated at 37°C, 5% CO2 for 48-72 hours. One hundred and sixteen isolates of gram-positive cocci with colony morphology, positive mannitol and sorbitol fermentation compatible with *S. mutans* were stored at -80°C in 20% glycerol brain–heart infusion broth (Difco, Detroit, MI, USA).

**PCR for differentiation of *Streptococcus sobrinus - Streptococcus mutans:*** Bacterial DNA for PCR amplification was extracted using DNeasy® Blood & Tissue kit following the manufacturer's instructions (DNeasy® Blood & Tissue Kit, Qiagen, Germantown, USA). Differentiation between *S. sobrinus* and *S. mutans* was performed using a multiplex PCR (24). Two primer pairs amplified *gtf* I and *gtf* B genes for *S. sobrinus* and *S. mutans*, respectively (Table 1). Each PCR mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 1.5 mM MgCl2, 0.5 µM of each oligonucleotide primer, 0.2 mM of each dNTP (Bioline, London, UK), 0.65U Taq DNA polymerase (Bioline, London, UK) and 50-100 ng DNA. Samples were amplified in an iCycler thermocycler (BioRad Laboratories, Hercules, CA, USA) under the following PCR conditions, initial denaturation at 96°C for 2 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 59°C for 30 sec and extension at 72°C for 1 min.

**In silico primer analysis:** Specificity of *S. mutans* specific primers (24) were evaluated using *in silico* analyses because PCR amplification of a fragment similar to the size expected for *S. mutans* was found in other species of oral streptococci. Analyses were done using Primer-BLAST (25) and sequences from the *Streptococcus* genus in GenBank, including complete genomes of *S. mitis* strain B6 (GI: 288906474), *S. salivarius* strain 57.I (GI: 339291081) and *S. parasanguinis* FW213 strain (GI: 387878539) and ATCC 15912 (GI:335369081).

**Biochemical identification:** To corroborate the PCR results particularly on isolates not identified by the PCR, 39 out of the 116 isolates were identified using biochemical tests. These strains corresponded to isolates identified as *S. mutans* and strains not identified by the *S. sobrinus - S. mutans* PCR, randomly selected using the program EPIDAT (SERGAS, Xunta of Galicia, PAHO, WHO). The isolates stored at -80°C were recovered on 5% blood agar and incubated at 37°C, 5% CO2 for 48-72 hours. Negative catalase test and gram-positive cocci in the Gram staining helped to define the *Streptococcus* genus. Biochemical identification was performed using API-20STREP (bioMérieux SA. Inc., Durham, USA) and readings were performed at 4 and 24 hours using the software api-WebTM (bioMérieux SA. Inc., Durham, USA).

**Amplification and sequencing of the 16S rRNA:** Because the biochemical identification of several isolates was not accurate, amplification and sequencing of the 16S rRNA for 29 and a control strain was performed to confirm bacterial identification. Universal primers for 16S rRNA Eubac 27F and 1492R were used (Table 1) (26-28). Each reaction mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 1.5 mM MgCl2, 0.2 mM each dNTPs (Bioline), 0.5 µM of each oligonucleotide primer, 0.65U of Taq Polymerase (Bioline) and 50-100ng DNA. Samples were amplified in a thermocycler iCycler (BioRad) by PCR conditions described previously (28).

These products were purified using the QIAquick Spin Kit (QIAGEN, Hilden, Germany) and subsequently sequenced in the forward and reverse directions. Sequences were edited using the software Geneious (Geneious Pro 5.5.6 created by Biomatters. (Available from http://www.geneious.com). Sequences with a Q value ≥ 20 were assembled and compared to the sequences reported in GeneBank (National Center for Biotechnology Information NCBI) (29).

**Genotype analysis:** Genotypes *c, e* and *f* of isolates identified as *S. mutans* were detected using the multiplex PCR (30). Primers used in the PCR assay were SC-F, SC-R, SE-F, SE-R, SF-F, SF-R (Table 1). Each 50 µL reaction mixture contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 2 mM de MgCl2, 0.2 mM of each dNTP, 0.5 µM of each oligonucleotide primer, 1U Taq DNA polymerase and 50-100ng DNA. Samples were amplified in a thermocycler iCycler (Bio-Rad). PCR conditions were described previously (30). *S. mutans* genotype *k* was identified by conventional PCR using a set of specific primers CEFK-F and KR based on the sequence of genes *rgp*F and r*gp*E (Table 1) and the PCR conditions previously described.

**Table 1.** Primers and control strains used in the PCR reactions

| Primers | Sequence (5´ to 3´) | Target | Size (bp) | Control Strain | Reference |
|---|---|---|---|---|---|
| GTFB-F | ACTACACTTTCGGGTGGCTTGG | *gtf* B | 517 | *S. mutans* LM7<br>*S. mutans* UA159<br>*S. mutans* MT6219 | Oho et al., 2000 |
| GTFB-R | CAGTATAAGCGCCAGTTTCATC | | | | |
| GTFI-F | GATAACTACCTGACAGCTGACT | *gtf* I | 712 | - | |
| GTFI-R | AAGCTGCCTTAAGGTAATCACT | | | | |
| SC-F | CGGAGTGCTTTTTACAAGTGCTGG | *rgp*H | 727 | *S. mutans* UA159 (serotype c) | |
| SC-R | AACCACGGCCAGCAAACCCTTTAT | | | | |
| SE-F | CCTGCTTTTCAAGTACCTTTCGCC | ORFe3 | 517 | *S. mutans* LM7 (serotype e) | Shibata et al., 2003 |
| SE-R | CTGCTTGCCAAGCCCTACTAGAAA | | | | |
| SF-F | CCCACAATTGGCTTCAAGAGGAGA | ORF2f | 316 | *S. mutans* MT6219 (serotype f) | |
| SF-R | TGCGAAACCATAAGCATAGCGAGG | | | | |
| CEFK-F | ATTCCCGCCGTTGGACCATTCC | *rgp*E and *rpg*F | 294 | *S. mutans* YK1<br>*S. mutans* FT1 (serotype k) | Nakano et al., 2004 |
| K-R | CCAATGTGATTCATCCCATCAC | | | | |
| Eubac 27F | AGAGTTTGATCCTGGCTCAG | 16S rRNA | 1500 | | Dunbar et al., 2001 |
| 1492R | GGTTACCTTGTTACGACTT | 16S rRNA | | | |

## Statistical analysis

Data analyses were performed using the Statistical Software SPSS® (Statistical Package for Social Sciences) for Windows, Version 19 (SPSS Inc., Chicago, IL). Quantitative variables were analyzed using measures of central tendency (mean and standard deviation) and qualitative variables in percentages. The Kolmogorov-Smirnoff test was used as normality test. Because the variables assumed a non-normal distribution, non-parametric statistical tests were performed with a significance of $p<0.05$.

## Results

**Demographic and oral characteristics of patients:** From the 47 children evaluated, 38% (18) were six and 62% (29) were seven years old, 62% (29) were male. The overall pattern of dental caries experience and history was of 8% and 13%, respectively. The average DMF-T was of 2.7 (SD ± 3.2), suggesting very low levels of caries prevalence and severity; similar to the results obtained during the sixth oral health monitoring in Medellin (31).

***Streptococcus mutans* identification and genotyping:** *S. sobrinus - S. mutans* PCR differentiated 16 isolates as *S. mutans* and four isolates as *S. sobrinus* (Figure 1). Although, differences in species identification of the isolates by the *S. sobrinus – S. mutans* PCR and 16S RNA analysis were found, in silico analyses of *S. mutans* specific primers against *Streptococcus* sequences in GenBank revealed the putative amplification of a 517 bp DNA fragment of the glucosyltransferase gene exclusively in five *S. mutans* strains. These results showed the expected size of this gene in *S. mutans* and hence support the species specificity of the primers used. Likewise, *in silico* analyses of *S. sobrinus* specific primers revealed the putative amplification of 712 bp in *S. sobrinus*, *S. downei*, *S. dentirousetti*, *S. dentisuis*, *S. orisuis* and *S. cricetti*. These results indicate the need of laboratory confirmed strains to check the specificity of these primers.

By API-20STREP 16 isolates and by 16S rRNA sequencing 12 isolates were identified as *S. mutans* (Table 2). The Kappa concordance test value between API-20STREP and 16S rRNA species identification was of 0.73. Overall, agreement between biochemical and molecular identification (16S rRNA) was observed for *S. mutans* isolates; however, contradictory results of species identification were found in 9 strains by API- 20STREP and 16S rRNA sequencing. Seven out of nine strains showed a lower percentage of identification by API (58.7% – 95.8%) than 16S rRNA sequencing (99% - 100%), suggesting higher accuracy with the last method. 2 out of 7 strains that showed discrepancies were identified by 16S rRNA sequence analysis with 100% similarity as *Streptococcus gordonii* and *Streptococcus pseudoneumoniae* (species not included in the API-20STREP database), while the API system identified those isolates as *Streptococcus oralis* (95.8%) and *Streptococcus salivarius* (81%). Likewise, 2 other strains were identified by 16S rRNA sequence analysis with 99% similarity as *Streptococcus oralis* and *Streptococcus parasanguinis* (the latter not included in API-20STREP database), while those isolates were identified by the API system as *S. mutans* with 99.9% similarity.

Regarding the genotype analysis, the most frequent *S. mutans* genotype detected was genotype c (n= 4, 8.5%) (Figure 2, Table 3). Notably, genotype *k* (n=2, 4.2%) was detected for the first time in Colombia in this work (Figure 3).

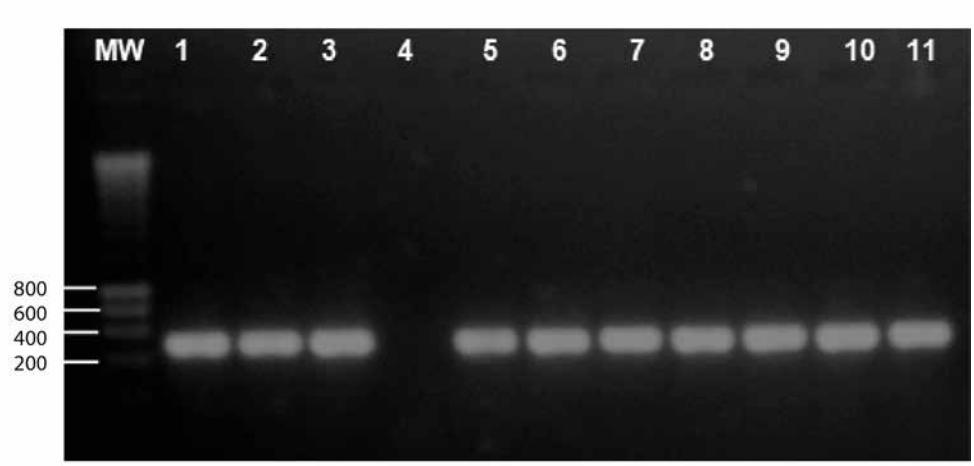


**Figure 1.** PCR differentiation for *S. sobrinus – S. mutans*. Lane 1 through 3: *S. mutans* control strains (LM7, MT 6219 and UA 159). Lane 4: negative control. Lane 5 through 11: isolates evaluated

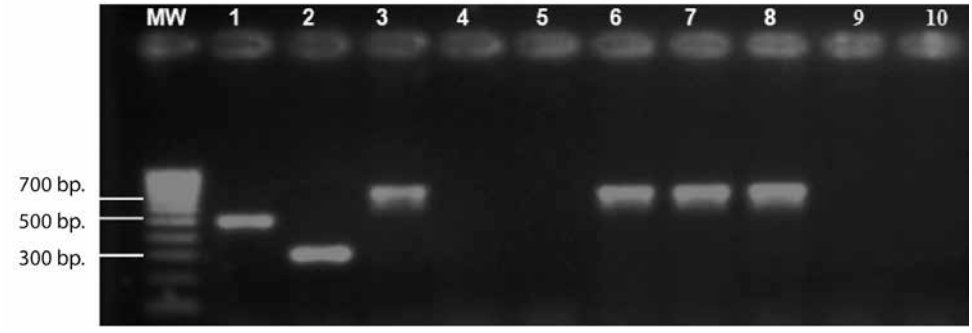


**Figure 2.** Detection of *S. mutans* genotypes *c, e* and *f* by PCR. MW: Molecular weight marker, Lane 1: control strain LM7 (serotype *e*), Lane 2: control strain MT 6219 (serotype *f*) , Lanes 3: control strain UA 159 (serotype *c*), Lane 4: negative control, Lane 5 through 10: *S. mutans* isolates evaluated


**Figure 3.** Detection of *S. mutans* genotype *k* by PCR. MW: Molecular weight marker.
Lane 1: negative control.Lanes 2 through 6: *S. mutans* isolates. Lane 18: *S. mutans* control strain YT serotype *k*.
Lane 19: *S. mutans* control strain FT serotype *k*.

**Prevalence of *Streptococcus mutans* and its genotypes in children with and without caries:** Overall, prevalence of *S. mutans* based on 16S rRNA species identification was 14.9 % (Table 3). In the group without dental caries *S. mutans* was not detected. There was no significant statistical relationship (p> 0.05) in the presence of *S. mutans* between the groups with and without dental caries. The subjects harboring *S. mutans* genotype *c*, had an average DMF-T of 6.7 (SD ± 1.6) and the subjects with genotypes *f, k* and co-infection of *c* and *k* had a DMF-T of 6. Oral examination of one patient with genotype *c* and *k* showed the presence of dental caries in permanent teeth.

## Discussion

Tooth decay is a multifactorial disease strongly associated with socio-cultural, economic, environmental and behavioral factors; its distribution and severity varies from region to region (32). Although several works have studied the relationship between dental caries and the presence of *S. mutans* (33-35); in Colombia, studies on *S. mutans* prevalence and circulating genotypes comparing children with and without dental caries are scarce.

An approximation to the complete history and experience of dental caries in the population is achieved by the measure of the DMF-T index. Oral examination in this study detected a low dental caries index (DMF-T 0.04 ± 0.3), indicating low dental caries prevalence. However, is also frequent to find a DMF-T low in younger children because change to permanent teeth occurs between six and seven years old (36), in older children this probability increases because of the higher number of permanent teeth exposed to disease.

In general, the DMF-T index and prevalence of dental caries in this study (57.4 %) was lower than previously reported in Medellin for children of 2.5 - 4 years with a prevalence of 74.9 % (37). Similarly, a Monitoring Oral Health Survey of 2006 found a lower prevalence of dental caries (24%) in children aged 6 and 7 (31). Contrary, higher prevalence of dental caries (77,8%) has been reported in children 1-5 years old (38). It is important to mention that in the former study, an inclusion criteria was of children with a low socioeconomic status, aspect associated with higher prevalence and severity of dental caries (39); this variable was not consider in the present study.

Considering the possible role of *S. mutans* in the initiation and progression of dental caries (4), it is important not only to described its prevalence, but also characterize the strains present in children with dental caries. Therefore, it is important to guaranty the proper identification of *S. mutans* (40). This study evidenced the problems occurring during biochemichal identification of the viridans group streptococci; for example, the identification of some *S. mutans* isolates showed incongruent results between biochemical and genetic tests (Table 2). Furthermore, the *in silico* analyses with the *S. mutans-S. sobrinus* PCR, several isolates were identified as *S. mutans*; however, by 16S rRNA comparisons they showed a high sequence identity with *S. mitis, S. oralis* and *S. parasanguinis* (Table 2), which indicated that this PCR is not as specific as anticipated.

**Table 2.** Comparison of species identification by PCR, API 20STREP and 16S rRNA

| Sample N° | 16S rRNA | | API 20STREP | | PCR |
|---|---|---|---|---|---|
| | Species | Max. Identity | Species | % Identity | *S. mutans – S. sobrinus* |
| 1 | *S. mutans* | 100 % | *S. mutans* | 98.0 % | *S. mutans* |
| 2 | *S. mutans* | 100 % | *S. mutans* | 87.1% | *S. mutans* |
| 3 | *S. mutans* | 100 % | *S. mutans* | 71.3 % | *S. mutans* |
| 4 | *S. mutans* | 95.0 % | *S. mutans* | 98.0 % | *S. mutans* |
| 5 | *S. mutans* | 100 % | *S. mutans* | 99.9% | *S. mutans* |
| 6 | *S. mutans* | 100 % | *S. mutans* | 98.0 % | *S. mutans* |
| 7 | *S. mutans* | 100 % | *S. mutans* | 99.9 % | *S. mutans* |
| 8 | *S. mutans* | 100 % | *S. mutans* | 99.9 % | *S. mutans* |
| 9 | *S. mutans* | 100 % | *S. mutans* | 99.9 % | *S. mutans* |
| 10 | *S. mutans* | 100 % | *S. mutans* | 87.1% | *S. mutans* |
| 11 | *S. mutans* | 100 % | *S. mutans* | 87.1% | *S. mutans* |
| 12 | *S. mutans* | 100 % | *S. mutans* | 99.9 % | *S. mutans* |
| 13 | *S. oralis* | 99.0 % | *S. mutans* | 99.9% | *S. mutans* |
| 14 | *S. parasanguinis* | 99.0 % | *S. mutans* | 99.9% | *S. mutans* |
| 15 | *b* | - | *S. mutans* | 98.0 % | *S. mutans* |
| 16 | *b* | - | *S. mutans* | 97.4 % | *S. mutans* |
| 17 | *S. salivarius* | 100 % | *S. mitis* | 89.2% | *a* |
| 18 | *S. mitis* | 100 % | *S. mitis* | 80.5% | *a* |
| 19 | *S. mitis* | 100 % | *S. oralis* | 95.8% | *S. mutans* |
| 20 | *S. mitis* | 100 % | *Gemella morbillorum* | 69.9% | *a* |
| 21 | *S. mitis* | 100 % | *S. oralis* | 79.9% | *a* |
| 22 | *S. mitis* | 100 % | *S. mitis* | 60.0% | *a* |
| 23 | *S. seudopneumoniae* | 100 % | *S. oralis* | 95.8% | *a* |
| 24 | *S. salivarius* | 100 % | *S. salivarius* | 36.7% | *a* |
| 25 | *S. gordonii* | 100 % | *S. salivarius* | 81.0% | *a* |
| 26 | *S. mitis* | 99.0 % | *S. sanguinis* | 58.7% | *a* |
| LM 7 | *S. mutans* | 100 % | *S. mutans* | 99.1% | *S. mutans* |

a. No amplication detected in the *S. mutans* and *S. sobrinus* PCR.

b. Sequences not analyzed due to low quality (Q value ≤ 20).

In general, in the present study, high concordance (0.61 - 0.80) in species identification was found between API-20STREP and 16S rRNA sequencing (k=0.73, p< 0.001); indicating that both methods appropriately identify these oral bacteria, especially *S. mutans*. However, caution should be taken when using API-20STREP, because in some occasions, isolates identified as *S. mutans* by this system were identified as other streptococci by 16S rRNA; this occurred more frequently with species not included in the API-20STREP database. Given that the 16S rRNA is highly conserved within bacterial species and had proved to be highly accurate for bacteria identification and especially useful for discriminating

among isolates difficult to distinguish (41,42), in the present study, species were assigned according to the 16S rRNA sequencing results. This is supported by the consideration that the 16S rRNA is the suggested barcode for identification of both culture and non-culture bacterial species (43).

**Table 3.** *Streptococcus mutans* and its genotypes in children with and without dental caries.

| Groups | n (%) |
|---|---|
| **Children with dental caries** | **27 (57.4%)** |
| *S. mutans* detected | 7 (14.9%) |
| Genotype *c* | 4 (8.5%) |
| Genotype *f* | 1 (2.1%) |
| Genotype *k* | 1 (2.1%) |
| Genotype *c* and *k* | 1 (2.1%) |
| *S. mutans* no detected | 20 (42.6%) |
| **Children without dental caries** | **20 (42.6%)** |
| *S. mutans* no detected | 20 (42.6%) |
| **Total children** | **47 (100%)** |

In our study, all children with *S. mutans* had dental caries; similarly, 66.7% of the children with *S. sobrinus* (identified by the species PCR), had caries. Prevalence of *S. mutans* was lower (14.9%) than in a previous study of Colombian children with similar demographic characteristics (51.4%) (22). Multiple could be the reasons for the difference in prevalence; among them, is the nature of the techniques used that could vary in sensitivity and specificity; specifically, Thompson et al. (22), used a fluorescent antibody based methodology, in which the antisera exhibited a strong cross reaction with other oral bacteria (22). In general, in this study the prevalence of *S. mutans* and *S. sobrinus* was lower than in previous surveys in school-age children with dental caries (35,44). These results suggest the importance of evaluating the role of *S. sobrinus* in dental caries.

Regarding the distribution of serotypes, it is known that serotype *c* strains predominate in the human oral cavity (45). Our results showed that the prevalence of *S. mutans* genotype *c* (8.5%) and genotype *f* (2.1%) was lower than reports in Japan, Finland and Thailand (12, 17, 33, 46). In Colombia, higher prevalence of *S. mutans* serotype *c* (65.1%), serotype *e* (14.1%) and serotype *f* (25.4%) was reported in plaque specimens of children with ages ranging from 8 to 14 years, of the Heliconia and Don Matias municipalites in Antioquia Department (22).

Remarkably, this study reports for the first time the presence of genotype *k* in Colombia. Prevalence of genotype *k* was 4.2%, similar to reports from Japan and Thailand (1-5%) (12, 13, 20, 33, 46), but lower than reported in México (16.9%) (47). *Streptococcus mutans* serotype *k* has been found in saliva and blood isolates (12, 19). Study on properties of *k* strains support their lower cariogenicity (19) and its ability to survive for longer periods in blood, due to resistance to phagocytosis (48). Also, this new serotype has been related with bacteremia, systemic inflammation and was described as one of the risk factors of infective endocarditis and hemorrhagic stroke (49). Thus, future studies in Colombia are required for understanding the role of *S. mutans* genotype *k* in the development of cardiovascular infective disease.

## Conflict of interest

The authors declare that they have no competing interests.

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