



## Original Article

# Detection of *Streptococcus Mutans* and *Streptococcus sobrinus* by Polymerase Chain Reaction (PCR) in Saliva Samples

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### Abstract

**Background:** Dental caries has been a major problem in Bangladesh for decades. Oral diseases are the most common non-communicable diseases related to severe local and systemic disorders. Oral microorganisms can grow and spread in the oral mucosae and most commonly in biomaterials under polymicrobial biofilms, leading to several diseases such as dental caries and periodontal disorders.

**Aims:** This study aimed to detect *S. mutants* and *S. sobrinus* by polymerase chain reaction (PCR) amplification and to relate the link between their presence and dental caries.

**Materials and Methods:** *Streptococcusmutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) strains were isolated from individuals with no caries. However, they were isolated with high counts of those organisms in saliva samples. The saliva samples were collected from the patients who attended the outdoor Dhaka Dental College Hospital from March 2018 to December 2018. PCR was performed to detect the presence of different organisms.

**Results:** Prevalence of *S. mutans* and *S. sobrinus* was, respectively, 19% and 04%. About 68% of the saliva sample was not either positive for both bacterial species, whereas 09% was positive for both bacterial species.

**Conclusion:** This study demonstrated that PCR is an easy, quick, and reliable method for detecting *S. mutans* and *S. sobrinus* in epidemiological studies. It was also observed that *S. mutans* was the most common organism than *S. sobrinus* to develop caries.

**Keywords:** *Streptococcus Mutans*, *Streptococcusobrinus*, Polymerase Chain Reaction, and Saliva Samples.

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### Introduction

According to the report of the World Health Organization (WHO), oral diseases are the most common non-communicable diseases which cause

discomfort, pain, and disfigurement. Dental caries, the most prevalent condition, derives from microbial biofilms (plaque) formed on the surface of the tooth.<sup>1</sup> At present, it has been calculated that about 2.4 billion people have caries in their

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permanent teeth, whereas about 486 million children have caries in primary teeth.<sup>2</sup> At the same time, periodontal disease and dental caries are also related to bacterial and fungal infections, which are significant causes of the decay of deciduous teeth in the case of about 560 million people, involving hundreds of billions of dollars of expenses per year, all over the globe.<sup>1,3</sup>

In low-income groups, the majority of dental caries are left untreated, for which affected teeth are most often extracted because of pain and discomfort.<sup>4,5</sup>

The pain, along with inflammation of dental caries, can impair eating and sleeping, and overall life quality. The aims of this study were to detect *S. mutans* and *S. sobrinus* by polymerase chain reaction (PCR) amplification and to relate the link between their presence with dental caries.

## Materials and Methods

### Collection of Saliva Samples:

The saliva was collected with a sterile paper point (no.50), held with forceps with sterile beaks, and placed on the tip of the tongue for 1 minute until it was soaked with saliva. After that, each point was transferred to a sterile Eppendorf tube with forceps. The tubes were stored at -80°C. Ethical clearance was taken from the proper authority.

### Calculation of the copy number:

## Results

Among the 100 patients, 68 (68%) saliva samples were negative for any organisms. By far, the highest was positive for *S. Mutans*, which was about 19 (19%). Whereas 04 (4%) was positive for *S. sobrinus*. In the case of the 09 (09%) population, both *S. Mutans* and *S. Sobrinus* were present (Table 1).

A total of 100 patients were included in this study. Out of those majority, 79 (79%) were females. The study population's age ranged from 05 years to 15 years was the highest group with 66 (66%), whereas the percentage decreased with age reversely. Out of the total respondents, 59(59%) were from the rural population. In contrast, only 41(41%) were from urban areas. Regarding their income level, low socio-economic condition patient was by far the highest number with 74(74%), followed by middle socio-economic and higher socio-economic groups with 22(22%) and 04(04%), respectively. At the same time, the highest number of the population was illiterate, 54(54%), in contrast, 32(32%) had completed their primary/secondary education, and only 12(12%) had completed their higher degrees (Table 2).

Standard curves were obtained by using the bacterial strains of *S. mutans* by using (CCUG 11877T, serotype c; Clarke1924-AL) and the *S. sobrinus* (CCUG 27507, serotyped; Coykendall 1983-VP).

The bacterial strains were cultured on the blood agar media and then subjected to the 0.5McFarland suspension (which is equivalent to 1.5 x 10<sup>8</sup> CFU/ml). Serial dilutions were done to obtain the bacterial suspensions from 1 x 10<sup>10</sup> to 1 x 10<sup>7</sup> CFU. Quantitative detection of *S. mutans* and *S. sobrinus* was performed according to the manufacturer's instructions using the TaqMan assay. The gene-specific primer pairs (200 nm each) and the probes (250 nM) for *mutans* and *S. sobrinus* were used with the 1X TaqManUniversal PCR Master Mix (Applied Biosystems) and 5µl of isolated bacterial DNA in a 20 µl reaction volume. The PCR was run at 95°C for 10 min for enzyme activation, followed by 45 two-step cycles (15 sec 95°C and 1 min at 58°C). We used the ABI PRISM 7900 Sequence Detection System for detection. Each sample was analyzed, and the Ct value of each sample was converted to the quantity of *S. mutans* and *S. sobrinus* using the standard curves, which were measured in the same experiment. The specificity and the sensitivity of the assay were also determined from these standard curves by qPCR.

**Table 1. Prevalence of *S. Mutans* and *S. Sobrinus* (n = 100).**

Streptococcus carrier status group	Number of cases	Percentage (%)
S. Mutans and S. Sobrinus free group	68	68%
Presence of only the S. Mutans group	19	19%
Presence of only the S. Sobrinus group	04	04%
Both S. Mutans and S. sobrinus presence group	09	09%

**Table 2: Socio-demographic data of the study population (n= 100)**

	Variables	Total Number	Percentage(%)
Sex	Male	21	21
	Female	79	79
Age	05-15	66	66
	16-30	24	24
	31-50	10	10
Residence	Rural	59	59
	Urban	41	41
Socio-economic condition	Low	74	74
	Middle	22	22
	High	04	04
Educational level	Illiterate	54	54
	Primary to Secondary	32	32
	Up to graduation	12	12

## Discussion

Caries is the most common polymicrobial infection of the oral cavity.<sup>6</sup> Each oral microorganism plays a major role in determining the cariogenicity of the biofilm or dental caries. In this study, we analyzed the unstimulated saliva samples to determine the presence of *S. mutans* and *S. sobrinus* and to relate it to whether there are caries or not.

Determination of *S. mutans* and *S. sobrinus* CFU/ml levels by real-time quantitative PCR is an easy, reliable, fast method that is applicable in epidemiological studies.<sup>7-9</sup> *S. mutans* and *S. sobrinus* are the species that have been isolated most frequently from the human oral cavity, and

causes have been implicated as the main germs causing dental caries in humans.<sup>10,11</sup>

In this study, among 100 cases, 32(32%) were positive for *mutans* and *S. sobrinus* infection, and among them, females were predominant, 79(79%). The majority of the cases were detected between the age group 05 and 15 years which was 66(66%).

## Conclusion

This study demonstrated that PCR is the reliable method for the detection of *S. mutans* and *S. sobrinus* in any of the epidemiological studies in any country. It was also established that *S. mutans* was by far the most common organism than *S. sobrinus* to develop caries in humans.

**Conflict of interest:** None declared

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