



Comparative Analysis of Colony Counts of Different Species of Oral Streptococci in Saliva of Dentulous, Edentulous and in those Wearing Partial and Complete Dentures

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ABSTRACT

Objectives: To study and compare the number of colony forming units of *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus milleri* in dentulous, edentulous and in those wearing partial and complete dentures by using semi-quantitative culture method of saliva samples with calibrated standard loop.

Materials: Sterile specimen collection bottles, Mitis salivarius agar plates, Standard loop, Candle jar, Incubator, Colony counter.

Methodology: Study population consisted of 100 subjects with 25 in each group, with an age range of 40 to 80 years, who were attending the Department of Community Dentistry and Prosthodontics at MNR Dental College, Sangareddy, Hyderabad. Unstimulated saliva samples were collected from patients and inoculated on to Mitis salivarius agar plates using calibrated standard loop. The plates were then incubated anaerobically at 37°C for 24 hours and left at room temperature for further 24 hours. Using a colony counter, the number of colonies of each species was counted.

Results: *Streptococcus mutans* and *Streptococcus mitis* predominates in the dentulous group, *Streptococcus sanguis* in complete denture group, *Streptococcus salivarius* in edentulous group and *Streptococcus milleri* in removable partial denture group.

Conclusion: The results of our study are in accordance with the previous studies, which have sought to differentiate different groups of mutans streptococci using a simple calibrated standard loop.

Keywords: Streptococci, Saliva, Culture, Complete and partial dentures.

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INTRODUCTION

Various studies have shown that mutans streptococci in saliva can be used as an index of the degree of colonization on teeth. The study of microorganisms of the genus streptococci is of great clinical interest due to their pathogenic potential.¹ They cause a wide variety of diseases which include dental caries and also serious systemic diseases like bacterial endocarditis, rheumatic fever, purpuric fever and various pyogenic infections.

The warm and moist condition in the oral cavity, combined with its variety of sites suited for prospective bacterial colonization offers oral streptococci, an optimal environment for their growth.² The composition of oral microflora at different surfaces within the mouth is based on physical and biological properties like presence of receptors for microbial adhesion, the redox potential of the site and provision of essential nutrients.³ Saliva bathes both hard and soft tissues of the oral cavity and maintains the ecologic balance in the mouth.

Microbes that were formerly associated only with oral diseases have been shown to be increasingly pathogenic in general. Almost 50% of the oral microflora is constituted by oral streptococci. Bacteremia may occur after dental treatment, but also after vigorous tooth brushing especially in patients with periodontitis. Thus, for many microorganisms, oral cavity acts as an important pathway into the human body.⁴

Taking into account, the important role of mutans streptococci in the etiopathogenesis of dental caries,⁵⁻⁸ their quantification and identification are relevant for epidemiological and early intervention studies.⁹ Detection and identification of mutans streptococci have been performed by different methods, namely microbial culture techniques,^{10,11} biochemical identification,^{12,13} bacteriocin

typing^{14,15} and molecular techniques.¹⁶ Considering the simplicity of the microbial culture technique, the present study was aimed to analyse and compare the number of colony forming units of *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus milleri* in dentulous, edentulous and in those wearing partial and complete dentures by using semi-quantitative culture method of saliva samples with a calibrated standard loop.

METHODOLOGY

Study population consisted of 100 subjects with 25 in each group of age range 40 to 80 years, who attended the Departments of Community Dentistry and Prosthodontics at MNR Dental College, Sangareddy, Hyderabad, Andhra Pradesh. The study was conducted during the period of September to November, 2012. Informed consent was obtained prior to the study. After obtaining approval from the institutional ethical committee board, the subjects were enrolled for the study.

Criteria for inclusion in the study:

1. Edentulousness without dentures for past 3 months for edentulous group.
2. Minimum 20 teeth for dentulous group.
3. Partial dentures in either maxillary or mandibular arches for partial denture group.
4. Wearing complete dentures for the past 3 months for complete denture group.
5. Absence of active dental caries.
6. No history of antibiotics or steroidal therapy for the past 3 months.
7. No history of diabetes or any systemic metabolic disease.

Among 1-2 ml of unstimulated saliva was collected in sterile bottles at least 2 hours after ingestion of food or beverage. Samples were inoculated on to Mitis salivarius agar plates by impregnating 0.001 ml of saliva using calibrated standard loop. The agar plates were then incubated at 37°C under anaerobic conditions for 24 hours and left at room temperature for further 24 hours for better appreciation of colony characteristics of oral streptococci. Using a colony counter, the number of colonies of different species of streptococci produced by 1 µl of saliva was counted based on colony morphology (Figs 1 to 3).

RESULTS

The highest counts of *Streptococcus mutans* (Fig. 1) was obtained from saliva samples of dentulous group followed by slightly lower counts in the complete denture group. The levels of *S. mutans* in the edentulous group was very low.

The distribution of *Streptococcus sanguis* (Fig. 1) was highest in the complete denture group, followed in

decreasing order by dentulous, removable partial denture and edentulous groups. Levels of *S. sanguis* were similar to that of *S. mutans* except for slight predominance in the complete denture group.

The pattern of distribution of *Streptococcus salivarius* (Fig. 2) was contradictory to those of *S. mutans* and *S. sanguis*. Significantly higher levels of *S. salivarius* were seen in the edentulous group, in comparison to the other groups. Least counts were observed in the dentulous group. The complete and partial denture groups showed values in between the extremes.

Highest salivary counts of *Streptococcus mitis* (Fig. 3) were shown by the dentulous group followed in decreasing order by complete denture, edentulous and removable partial denture groups. On contrary to all the above *Streptococcal* species, *Streptococcus milleri* (Fig. 3) gave the highest counts in removable partial denture group followed by the dentulous and complete denture groups which showed similar counts. However, *S. milleri* levels in the edentulous group were significantly low.

DISCUSSION

As sampling of saliva is easy and noninvasive, this technique has been used to evaluate the caries susceptibility and caries activity of different individuals.^{17,18} Numerous semi-quantitative tests for mutans streptococci in saliva are now commercially available.^{19,20} However, it is impossible to differentiate mutans group of streptococci using these methods.

The pattern of distribution of *S. mutans* is consistent with the studies by Fitzgerald et al 1983, which proved that the prevalence patterns of *S. mutans* in the saliva of naturally dentate individuals is similar to that of full denture wearers. The edentulous individuals without dentures had no detectable mutans streptococci in their saliva. *S. mutans* is

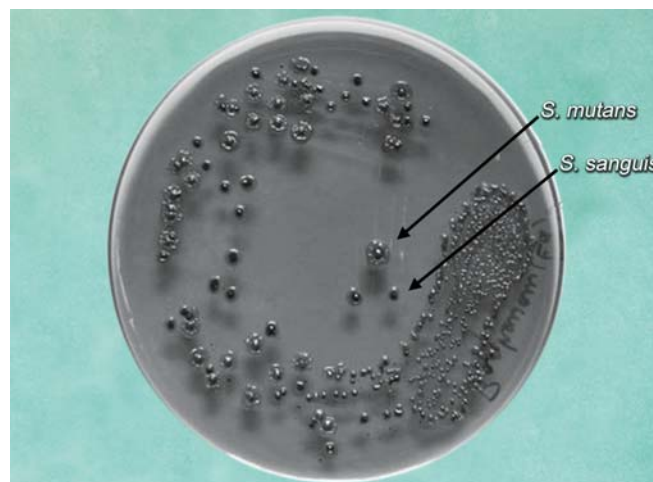


Fig. 1: Colony counts of *Streptococcus mutans* and *sanguis*

considered by most experts to be the prime etiologic agent involved in human dental caries. Thus the elderly and especially those wearing dentures can harbor high levels of potentially cariogenic organisms and could therefore continue to remain at risk of caries and could act as vectors for the transmission of these bacteria to young children in close family situations.²¹

Distribution patterns of *Streptococcus sanguis* closely agrees with the results of Loesche et al. *Streptococcus sanguis* levels increased both in the presence of dentures and with increased number of teeth.²² Predominance in complete denture group is in accordance with the fact that *S. sanguis* prefers hard surfaces, but is capable of colonizing mucosal surfaces also. Previous studies have shown that *S. sanguis* represents one half of the oral streptococci involved in bacterial endocarditis.²

Very high levels of *Streptococcus salivarius* in edentulous mouths prove that the organism has a clear cut predilection for mucosal surfaces. This also supports the fact that the sterile mouth of newborn infant is first colonized by *S. salivarius*. They have been isolated from the mouth

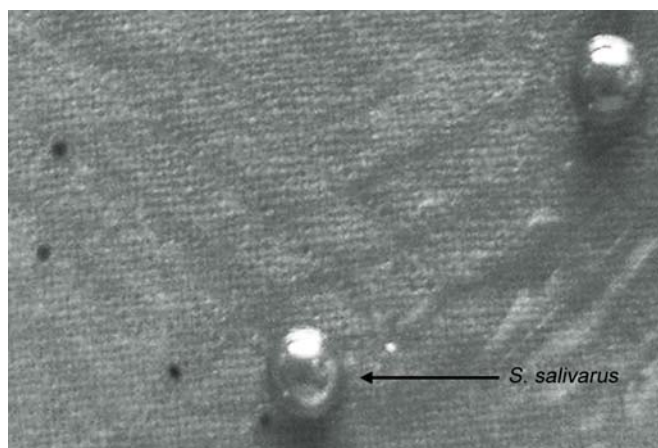


Fig. 2: Colony counts of *Streptococcus salivarius*

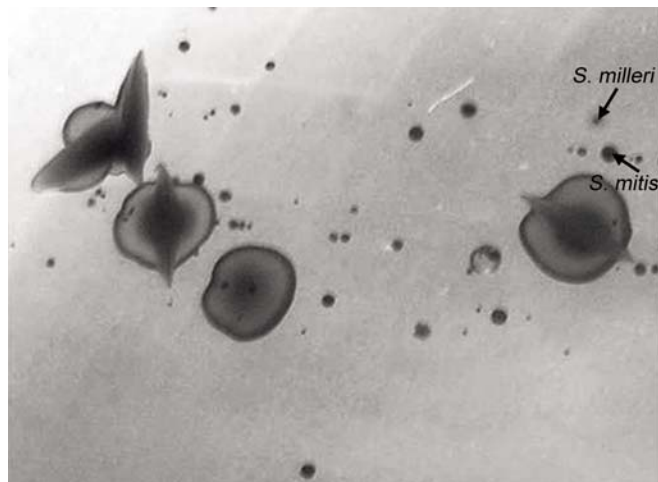


Fig. 3: Colony counts of *Streptococcus mitis* and *milleri*

of infants 18 hours after birth, and continues to predominate on tongue and mucosa with age.¹

Streptococcus mitis comprises a major percentage of microorganisms in plaque.² It is well known that plaque easily accumulates in mouths with dentures and teeth. This explains the increased counts of *S. mitis* in the dentulous and complete denture groups. However, slightly elevated counts in the edentulous group could not be explained.

The study results show significantly high counts of *S. milleri* in the removable partial denture group. Previous studies have shown that *S. milleri* is associated with oral and systemic pyogenic infections.² This could probably explain the result as most of the removal partial denture patients in the study had periodontal conditions.

CONCLUSION

Each species of oral streptococci is unique in its preference of surfaces or sites within the oral cavity. From the study, it can be concluded that *S. mutans* and *S. mitis* predominates in dentulous group, *S. sanguis* in complete denture group, *S. salivarius* in edentulous group and *S. milleri* in removable partial denture group. The presence of *S. mutans* and *S. sanguis* has an antagonistic effect on *S. salivarius*. Presence of dentures and increase in the number of teeth gives increased counts of *S. mutans* and *S. sanguis* and decreased counts of *S. salivarius*.

In conclusion, speciation of different groups of oral streptococci would enable us to obtain information on the quantity and quality of infecting species of mutans streptococci, which would be very useful for planning dental caries prevention programs.

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