

# Evaluation of microflora of root carious lesions in different age groups: A microbiological study

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## ABSTRACT

**Aim:** To evaluate the prevalence of *mutans streptococci*, *lactobacilli* and *Actinomyces* species in root carious lesions in middle-aged and older adults of South Indian population and to comparatively evaluate the microbial count in two different age groups. **Materials and Methods:** Specimens of root carious lesions were collected from 60 adults for bacteriological studies. Amongst which 28 subjects belonged to middle age group (35-44 years) and 32 subjects to older age group (55-75 years). Standard procedures of culture, isolation and identification of aerobic and anaerobic bacteria were adopted in the study. **Results:** The analysis of results was performed using Chi-square test. SPSS 20 has been used to analyze the data. Among the isolated microorganisms, *streptococci* (56.7%) and *lactobacilli* (28.3%) were more prevalent in root caries lesions compared to *actinomyces* species (15.0%) and most frequently occurred in older adults. There is also a statistically significant association between the age group and the presence of *lactobacillus* ( $P=0.024$ ). **Conclusions:** Aerobic Gram-positive cocci (*Streptococcus mutans*, *lactobacillus*) as well as anaerobic bacteria (*Actinomyces* spp.), occurred most frequently in root carious lesions in older adults as compared to middle-aged population.

## Key words

*Actinomyces* spp, aerobic/anaerobic bacteria, *lactobacilli*, root caries, *streptococcus mutans*

## INTRODUCTION

Root caries is an important dental disease, especially since the number of elderly dentate people in institutions is increasing.<sup>[1,2]</sup> Root surface caries, as the name implies, occurs on root cementum or dentine and is caused by a microbial biofilm. The disease is secondary to gingival recession, since in a healthy mouth, cementum and dentine are not exposed to the microflora and therefore, are not prone to colonization.<sup>[3-5]</sup>

The aetiology of root caries is multifactorial of which microbiological factor plays a critical role.<sup>[3-5]</sup> While there is ample evidence to imply a strong association between *mutans* group streptococci and coronal caries,<sup>[3-7]</sup> similar

data on microbiological agents in root caries is poorly understood.

Historically, *mutans streptococci* and *lactobacilli* have been associated with the initiation and progression of root caries. Despite the significantly raised levels of *mutans streptococci*, there is no reliable evidence that they initiate or cause the progression of root lesions, either on their own or even in concert with other bacteria.<sup>[8]</sup> Some epidemiological studies have shown that *Actinomyces* spp. are predominant bacteria in root caries,<sup>[3-5,9-11]</sup> while others have demonstrated a variety of bacteria and failed to implicate any single bacterial genus as a predominant isolate.<sup>[5,7,11]</sup>

A cross sectional study conducted in the South Canara District of Karnataka in the year 2007-2008 has revealed that root surface caries was more prevalent in males, in the age group of 51-65 as compared to the age group of 31-51 and has been positively associated with age.<sup>[12]</sup> However, the information that is available on the nature of the microflora associated with root surface lesions is in some instances superficial.<sup>[13]</sup> There are no statistical data of any microbiological study on root carious lesions

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on the South Indian population. Hence, there is a need to identify the micro-organisms involved in the cause and progression of root caries for its prevention and management.

This study evaluated whether the prevalence of various micro-organisms were dependant on the age of the individuals. Its aims were to evaluate the prevalence of *mutans streptococci*, lactobacilli and Actinomyces species in root carious lesions in middle-aged and older adults of South Indian population and to compare the microbial prevalence in adults in 2 different age groups.

## MATERIALS AND METHODS

This study was conducted in the University, after the approval from institutional ethical committee.

Twelve thousand and five hundred healthy adult patients under the age group of 35-75 years had reported at the OPD between December 2011-June 2012. Out of these, only 60 patients fulfilling the inclusion and exclusion criteria were reported.

### Inclusion criteria

- Healthy adults with no history of medications, systemic illness and radiotherapy
- Soft, active root carious lesions.

### Exclusion criteria

- Teeth with restorations
- Teeth undergoing non-invasive treatment strategies.

Samples of infected dentin were collected from these 60 subjects by a single examiner. Among them, 60 adults were classified into middle-aged group (35-44 years) with 28 subjects and older group (55-75 years) with 32 subjects. After the removal of infected dentin, the teeth were restored and patients were given instructions about tooth brushing techniques and oral hygiene aids and were later recalled for follow up of maintenance.

Instruments used in the study were sterile stainless steel mouth mirror, spoon excavator, sample tubes, petri plates.

### Sample collection

Carious dentin was sampled from individual soft root caries lesions. To minimize contamination of the underlying infected dentin with superficial plaque, which has a different microbial composition, the plaque overlying the lesion was removed using a toothbrush and sterile water. The infected dentin was sampled by means of a sterile excavator passing through the vertical dimension of the lesion, from the gingival margin. Each sample was placed into 2 ml of Thioglycollate medium (Hi Media, Mumbai, India).

## Microbial analysis of samples

Using conventional plating methods, each sample was processed within 3 hours to determine the prevalence of Streptococci, Lactobacilli and Actinomyces species. Mitis Salivarius Agar, *Lactobacillus* MRS Agar and Actinomyces Agar (Hi Media, Mumbai, India) were used to culture Streptococci, Lactobacilli and Actinomyces species respectively.

## Statistical analysis

A Chi-square test was conducted to understand how the oral flora varies with aging population. SPSS 20 has been used to analyze the data. Due to categorical nature of observed data, non-parametric test, Chi-square test has been used to analyze the significant differences in the microflora of root caries patients as a function of age.

## RESULTS

Table 1 shows that *Streptococcus mutans* were present in 34 (56.7% of the total) samples. Specifically, 13 samples (38.2%) in the age group of 35-44 years identified the presence of *Streptococcus mutans* and 21 samples (61.8%) found its existence in the age group of 55-75 years. The Chi-square examines whether there is an association between the two categorical variables, age group and presence of the bacteria *Streptococcus mutans* in root caries patients. In this case, the *P* value is 0.134 [Table 2]. Hence, stating there is no significant difference in the presence of *Streptococcus mutans* among the age groups in root caries patients. Table 1 also suggests that *lactobacillus* was present in 17 (28.3% of the total) cases. Among these, *lactobacillus* was present in four samples (23.5%) in the age group of 35-44 years and in 13 samples (76.5%) in the age group of 55-75 years. In this case, the *P* value is 0.024 [Table 2], thus stating there is a significant difference in the presence of *lactobacillus* among the age groups in root caries patients.

Actinomyces spp. was present in 9 (15.0% of the total) samples [Table 1]. Specifically, in the age group of 35-44,

**Table 1: Comparison of the prevalence of *Streptococcus mutans*, *Lactobacillus* and *Actinomyces* spp. in middle aged (35-44 years) and older adults (55-75 years)**

	<i>Streptococcus mutans</i>	<i>Lactobacillus</i>	<i>Actinomyces</i> spp.
Age 35-44 years count	13	4	2
% within microorganism	38.2	23.5	22.2
% of total	21.7	6.7	3.3
55-75 years count	21	13	7
% within microorganism	61.8	76.5	77.8
% of total	35.0	21.7	11.7
Total count	34	17	9
% within microorganism	100.0	100.0	100.0
% of total	56.7	28.3	15.0

**Table 2: Chi-square tests**

	<i>Streptococcus mutans</i>		<i>Lactobacillus</i>		<i>Actinomyces spp.</i>	
	Value	Asymp. Sig. (2-sided)	Value	Asymp. Sig. (2-sided)	Value	Asymp. Sig. (2-sided)
Pearson Chi-square	2.241	0.134	5.102	*0.024	2.542	0.111
N of valid cases	60		60		60	

\* $P < 0.05$ 

*Actinomyces spp.* was present in 2 samples (22.2%) and in the age group of 55-75 years identified 7 samples (77.8%). In this case, the  $P$  value is 0.111 [Table 2]. Hence, stating there is no significant difference in the presence of *Actinomyces spp.* among the age groups in root caries patients.

The predominant microorganism isolated from 60 root carious lesions was *Streptococcus mutans* followed by *Lactobacillus* and *Actinomyces spp.* and all the three microorganisms were predominant in older group (55-75 years) as compared to middle-aged group (35-44 years). Also, there is a significant association between the age group and the presence of *Lactobacillus* [Table 1 and Figure 1].

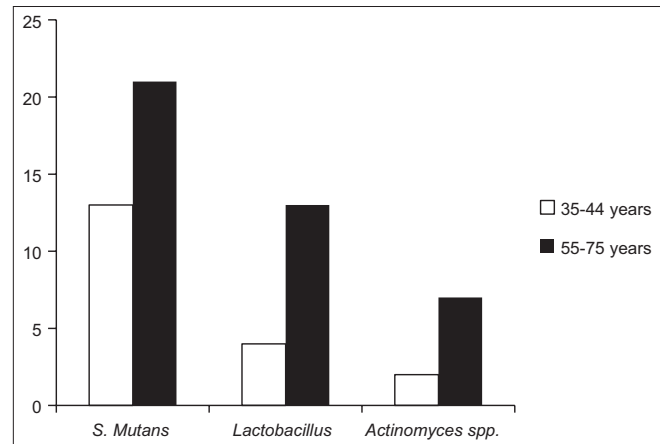
## DISCUSSION

The present study offers the evaluation of the microflora associated with root surfaces in middle-aged and elderly subjects based on conventional plating methods. Among the 60 samples collected, 28 samples belonged to 35-44 years (middle age) and 32 samples to 55-75 years (older age).

The first studies of lesions of root surface caries in humans made by Sumney and Jordan identified *S. mutans* as a significant component of the flora. However, *Actinomyces* were also identified. This study stated that a unique bacterial flora exists for caries that originates on different surfaces of the dentition, emphasizing a unique flora from root surface caries lesions.<sup>[14]</sup>

The predominant microorganisms isolated by Shen *et al.*<sup>[15]</sup> from 30 root caries lesions were *Lactobacillus spp.* (90%), *Streptococcus spp.* (100%) and *Actinomyces spp.* (63%). This result of the present Chinese subject was in agreement with studies, most of which conducted on Caucasians subjects, at other parts of the world.<sup>[16,17]</sup>

Study by Ellen *et al.*, showed that root surfaces harboring *Lactobacillus* and *S. mutans* were at greater risk of becoming carious and that persons with both bacteria in their plaque were at higher risk of developing root surface caries. Although *A. viscosus* was commonly isolated, Ellen *et al.* could not detect differences between the isolation or numbers of this organism.<sup>[14]</sup>



**Figure 1:** Comparison of prevalence of *Streptococcus mutans*, *Lactobacillus* and *Actinomyces spp.* in middle aged (35-44 years) and older adults (55-75 years)

However, it was known from the earlier studies of Jordan and Hammond that the flora was more complex and included 'intermediate' strains of *Actinomyces*.

In the present study, the predominant microorganism isolated was *Streptococcus mutans* followed by *Lactobacillus* and *Actinomyces spp.* which were also identified in few subjects. Among the 60 samples collected, *Streptococcus mutans* were identified in 34 (56.7% of the total) samples. Out of these, 13 (38.2%) samples belonged to 35-44 years (middle age) and 21 (61.8%) samples- to 55-75 years (older age). *Lactobacillus* was present in 17 (28.3% of the total) samples whereas *Actinomyces spp.* was identified in 9 (15.0% of the total) samples. Specifically, in the age group of 55-75 years, *Lactobacillus* was identified in 13 samples (76.5%) and *Actinomyces spp.* in 7 samples (77.8%). This suggests that *Streptococcus mutans*, *Lactobacillus* and *Actinomyces spp.* were predominant in older group (55-75 years) as compared to middle-aged group (35-44 years). Also, there is a significant association between the age group and the presence of *Lactobacillus*.

Apparently, a stage has been reached in the analysis of the microbiology of plaque associated with root caries where workers are locked into the enumeration of a limited number of bacteria dictated by selective media. Yet there is considerable information on diversity among even those species that we accept as 'target' organisms, the '*mutans streptococci*', oral *Streptococcus*, *Actinomyces*, and *Lactobacillus*. Consideration of this diversity in future studies may reveal other associations between bacteria

and root surface caries. Recently, microarray analysis of the microflora of root caries also demonstrated that other species in addition to *Streptococcus mutans*, *Lactobacilli* and *Actinomyces* may be also involved in the root caries process.

Given careful characterization of the flora and the state of the lesions, it is likely that microbiology will be useful in diagnosing the state of a root surface lesion and in determining the mode of treatment in control of the progression of root surface lesions.<sup>[13]</sup>

## CONCLUSION

*Streptococcus mutans*, *Lactobacillus* and *Actinomyces* spp. were higher in older group (55-75 years) as compared to middle-aged group (35-44 years) of South Indian population with *Streptococcus mutans* being the highest in number followed by *Lactobacillus* and *Actinomyces* spp. respectively. Also, there is a significant association between the age group and the presence of *Lactobacillus*.

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