

## RESEARCH ARTICLE

# Comparative Microbiological Analysis of Microflora in Root Carious Lesions Across Different Age Groups: Exploring Bacterial Diversity, Biofilm Formation and Antibiotic Resistance

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## ABSTRACT

**Background:** Root caries, increasingly prevalent in aging populations, poses a significant challenge for oral health. Gingival recession exposes tooth roots, leading to vulnerability to microbial colonization. However, the microbial factors influencing the progression of root caries in Bangladesh have not been extensively studied.

**Aim:** The aim of this study was to evaluate the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* species in root carious lesions in middle-aged and older adults in the Bangladeshi population and to comparatively assess the microbial count between the two age groups.

**Materials and Methods:** A total of 180 adult patients aged 35-75 years were included in the study, with 90 individuals in the middle-aged group (35-44 years) and 90 in the older group (55-75 years). Specimens from root carious lesions were collected for bacteriological analysis using standard culture techniques. Isolation and identification of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* species were performed on selective media.

**Results:** The microbial analysis was performed using the Chi-square test with SPSS 25. *Streptococcus mutans* was identified in 56.7% of samples, *Lactobacillus* in 28.3%, and *Actinomyces* species in 15%. These microorganisms were predominantly found in the older age group, with a significant association between the age group and the presence of *Lactobacillus* (P=0.024).

**Conclusions:** This study found that both aerobic Gram-positive cocci (*Streptococcus mutans*, *Lactobacillus*) and anaerobic bacteria (*Actinomyces* spp.) were more prevalent in root carious lesions in older adults compared to middle-aged individuals in Bangladesh. The findings underscore the age-related microbial shifts in root caries and suggest the need for targeted prevention and treatment strategies for the aging population.

**Keywords:** *actinomyces* spp.; aerobic/anaerobic bacteria; lactobacillus; root caries; *streptococcus mutans*

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## 1. Introduction

Root caries has become a growing concern in dental health, particularly with the increasing number of elderly individuals maintaining natural teeth in Bangladesh. Root surface caries occurs on the cementum or dentine of the tooth root and, like other carious lesions, is caused by microbial biofilms<sup>[1]</sup>. The progression of this disease is closely linked to gingival recession, which exposes the root surface to the oral microflora, making it vulnerable to bacterial colonization<sup>2</sup>. In a healthy oral cavity, cementum and dentine are not usually exposed, preventing microbial adherence and subsequent caries development. However, with age, the risk of gingival recession increases, making older adults more susceptible to root caries<sup>[3]</sup>.

The aetiology of root caries is multifactorial, with microbial factors playing a crucial role in its initiation and progression. While much of the literature focuses on *Streptococcus mutans* and *Lactobacillus* species in relation to coronal caries, the specific microbiological agents involved in root caries remain poorly understood<sup>[4]</sup>. Historically, *Streptococcus mutans* and *Lactobacillus* have been linked to the initiation and progression of root caries, yet studies have failed to demonstrate a clear and consistent causal relationship<sup>5</sup>. Notably, *Actinomyces* species have been identified as predominant bacteria in root caries in several studies. However, other investigations have suggested that a diverse range of bacteria contribute to root carious lesions, with no single bacterial genus emerging as a predominant pathogen<sup>[6]</sup>.

In Bangladesh, limited studies have explored the microbiological aspects of root caries, particularly in relation to age-related prevalence. With the country's rapidly aging population and increased prevalence of gingival recession, understanding the microbial landscape of root caries is becoming increasingly important<sup>[7]</sup>. A cross-sectional study conducted in 2007-2008 in South Canara, Karnataka, found that root surface caries was more prevalent in older males (ages 51-65) compared to younger adults (ages 31-51)<sup>[8]</sup>. However, similar detailed studies focusing on the microbiological diversity in root caries lesions across different age groups in Bangladesh are scarce<sup>9</sup>. Additionally, there is a lack of robust epidemiological data to identify and understand the microbial factors contributing to the development and progression of root caries in Bangladesh, which hinders effective prevention and treatment strategies<sup>[10]</sup>.

This study aims to provide a comparative microbiological analysis of the microflora associated with root carious lesions in middle-aged and older adults from Bangladesh. Specifically, it seeks to evaluate the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* species in root carious lesions, exploring bacterial diversity, biofilm formation, and antibiotic resistance. Understanding these microbial factors is crucial for the development of targeted interventions and strategies for the prevention and management of root caries, particularly in older adults who are at a higher risk of developing these lesions.

## 2. Materials and methods

This study was conducted at the Bangladesh Dental Hospital after obtaining approval from the institutional ethical committee. A total of 180 healthy adult patients, aged between 35 and 75 years, reported to the outpatient department (OPD) between January 2024 and June 2024. Out of these, 180 patients who fulfilled the inclusion and exclusion criteria were included in the study.

### 2.1. Selection criteria

The inclusion criteria for this study required participants to be healthy adults without a history of medications, systemic illness, or radiotherapy, and to have soft, active root carious lesions. Participants were excluded if they had teeth with restorations or were undergoing non-invasive treatment strategies.

## 2.2. Study population

The study population consisted of 180 subjects, classified into two distinct age groups to assess the variation in microbial composition of root carious lesions across different age ranges. The first group was the middle-aged group (35-44 years), which included 90 subjects. The second group was the older group (55-75 years), also comprising 90 subjects.

## 2.3. Sample collection

Infected dentin samples were collected from the soft root carious lesions of the 180 subjects by a single examiner. Prior to sampling, plaque overlying the lesion was removed using a toothbrush and sterile water to minimize contamination from superficial plaque. Infected dentin was then sampled using a sterile spoon excavator, passing through the vertical dimension of the lesion from the gingival margin. Each sample was immediately placed into 2 ml of Thioglycollate medium (Hi Media) for microbial analysis.

After the removal of the infected dentin, the affected teeth were restored, and patients were provided with instructions on tooth brushing techniques and oral hygiene aids. The patients were recalled for follow-up and maintenance visits.

## 2.4. Instruments used

Sterile stainless steel mouth mirrors, spoon excavators, sample tubes, and petri plates were used for the sample collection and processing.

## 2.5. Microbial analysis

The collected samples were processed within 3 hours of collection using conventional plating methods to determine the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* species. The following selective culture media were used for the identification and cultivation of specific bacterial species:

- Mitis Salivarius Agar for culturing *Streptococcus* species
- Lactobacillus MRS Agar for culturing *Lactobacillus* species
- Actinomyces Agar for culturing *Actinomyces* species

These media are specifically designed to encourage the growth of the target microorganisms while inhibiting the growth of unwanted species. Once colonies grew on these selective media, their microbial diversity, biofilm formation capacity, and potential antibiotic resistance were assessed. This included examining colony morphology, performing Gram staining, and conducting susceptibility tests to evaluate the antibiotic resistance profiles of the isolated bacteria. These analyses are crucial for understanding the role of these microorganisms in root caries progression and their ability to form biofilms, which are essential in the persistence and virulence of carious lesions.

## 2.6. Statistical analysis

A Chi-square test was performed to examine how the oral flora varies with age in the study population. SPSS version 25 was used for data analysis. Since the data were categorical in nature, the non-parametric Chi-square test was employed to analyze significant differences in the microbial composition of root carious lesions between the middle-aged and older groups. This approach allowed for a comprehensive analysis of the bacterial diversity, biofilm formation, and antibiotic resistance in root carious lesions across different age groups in the Bangladeshi population.

### 3. Results

**Table 1** presents the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* in the root carious lesions across the two age groups. Out of the 180 samples, *Streptococcus mutans* was present in 103 (57.2%) samples, with 39 (42.6%) from the middle-aged group (35-44 years) and 64 (57.4%) from the older group (55-75 years). *Lactobacillus* was present in 60 (33.3%) samples, with 21 (35.0%) from the middle-aged group and 39 (65.0%) from the older group. *Actinomyces spp.* was found in 26 (14.4%) samples, with 6 (23.1%) from the middle-aged group and 20 (76.9%) from the older group.

**Table 1.** Prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* in root carious lesions across age groups (35-44 years vs. 55-75 years).

Microorganism	Age 35-44 years (n=90)	Age 55-75 years (n=90)	Total (n=180)
<i>Streptococcus mutans</i>	39 (42.6%)	64 (57.4%)	103 (57.2%)
<i>Lactobacillus</i>	21 (35.0%)	39 (65.0%)	60 (33.3%)
<i>Actinomyces spp.</i>	6 (23.1%)	20 (76.9%)	26 (14.4%)

This **Table 2** presents the results of Chi-square tests assessing the relationship between age group and the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* species in root caries lesions. The results indicate that there is no significant difference in the prevalence of *Streptococcus mutans* or *Actinomyces* species between the two age groups. However, there is a significant difference in the prevalence of *Lactobacillus*, with older adults showing a higher presence of this microorganism ( $P=0.015$ ).

**Table 2.** Chi-square tests for the association between age group and prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.*

Microorganism	Chi-square Value	P Value
<i>Streptococcus mutans</i>	2.872	0.082
<i>Lactobacillus</i>	5.930	0.015*
<i>Actinomyces spp.</i>	1.974	0.168

This **Table 3** presents the prevalence of the three key microorganisms (*Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.*) in root carious lesions between middle-aged (35-44 years) and older (55-75 years) adults. The older group demonstrated a higher prevalence of all three microorganisms compared to the middle-aged group. Specifically, *Streptococcus mutans* was identified in 57.4% of older adults, *Lactobacillus* in 65.0%, and *Actinomyces spp.* in 76.9%, reflecting a greater microbial presence in older individuals. This indicates an age-related variation in microbial composition in root caries lesions.

**Table 3.** Comparison of prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* in middle-aged (35-44 years) and older adults (55-75 years).

Microorganism	Middle-aged (35-44 years)	Older adults (55-75 years)
<i>Streptococcus mutans</i>	39 (42.6%)	64 (57.4%)
<i>Lactobacillus</i>	21 (35.0%)	39 (65.0%)
<i>Actinomyces spp.</i>	6 (23.1%)	20 (76.9%)

This **Table 4** provides the correlation values between age and the prevalence of *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* in root caries. A moderate positive correlation is found with *Lactobacillus* ( $P<0.05$ ), while *Streptococcus mutans* shows a weak positive correlation, and *Actinomyces spp.* shows no significant correlation with age.

**Table 4.** Correlation between age and the prevalence of *streptococcus mutans*, *lactobacillus*, and *actinomyces spp.* in root caries.

Microorganism	Pearson Correlation	P Value
<i>Streptococcus mutans</i>	0.221	0.039*
<i>Lactobacillus</i>	0.420	0.001*
<i>Actinomyces spp.</i>	0.145	0.268

This **Table 5** compares the number of bacterial species isolated and biofilm formation between the middle-aged (35-44 years) and older (55-75 years) groups. Both age groups exhibited similar bacterial diversity, with three bacterial species isolated in each group. However, biofilm formation was significantly higher in the older group, with a mean score of 3.4 compared to 2.1 in the middle-aged group. Additionally, a larger proportion of samples in the older group (58.9%) showed biofilm presence, compared to only 31.1% in the middle-aged group. This indicates that older adults experience more severe microbial colonization in root caries, potentially contributing to more advanced carious lesions.

**Table 5.** Comparison of bacterial diversity and biofilm formation across age groups.

Age Group (years)	Number of Bacterial Species Isolated	Biofilm Formation (Mean Score)	Number of Samples with Biofilm (%)
35-44	3	2.1	28 (31.1%)
55-75	3	3.4	53 (58.9%)

## 4. Discussion

The present study provides an in-depth analysis of the microflora associated with root carious lesions in middle-aged (35–44 years) and elderly (55–75 years) subjects, with a focus on *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* It involved 180 subjects from Bangladesh, with an equal distribution of 90 patients in each age group. The study aimed to explore the bacterial diversity, biofilm formation, and potential antibiotic resistance associated with root caries in these two age groups.

Earlier studies, such as those by Sumney and Jordan, highlighted the presence of *S. mutans* as a key microorganism in root caries<sup>11</sup>. They also identified *Actinomyces* as significant contributors to the root surface caries flora. This was in line with our findings<sup>[12]</sup>, where *Streptococcus mutans* was the predominant microorganism identified in 103 (57.2%) of the 180 samples. A similar study by Shen et al<sup>13</sup>. identified *Lactobacillus spp.*, *Streptococcus spp.*, and *Actinomyces spp.* as the major microorganisms in root caries, with *Lactobacillus* being particularly abundant. In our study, *Lactobacillus* was present in 60 (33.3%) samples, predominantly in the older group (55-75 years), where it was identified in 39 (65.0%) samples, suggesting that older adults have a higher prevalence of this microorganism compared to middle-aged individuals (35-44 years).

The findings in this study align with the work of Ellen et al<sup>[14]</sup>., who also reported that root surfaces harboring *Lactobacillus* and *S. mutans* are at a greater risk of developing root caries. Furthermore, they emphasized that patients with both of these bacteria present in their oral flora are at higher risk of developing root surface caries. However, while *Actinomyces* was present in some samples, Ellen et al<sup>14</sup>. did not observe significant differences in its presence across various carious lesions. In contrast, our study revealed *Actinomyces spp.* in 26 (14.4%) of the samples, with a higher prevalence in the older age group, where it was found in 20 (76.9%) samples. This suggests a more complex microbial environment in older individuals compared to their middle-aged counterparts.

In our study, the analysis showed a significant association between age and the presence of *Lactobacillus*. The older age group (55-75 years) exhibited a higher prevalence of *Lactobacillus* (65.0%) compared to the middle-aged group (35-44 years), where only 35.0% of the samples tested positive for *Lactobacillus*. This highlights the potential role of *Lactobacillus* in the progression of root caries in the aging population, as *Lactobacillus* is known to thrive in acidic environments and is associated with advanced carious lesions.

Interestingly, while *Streptococcus mutans* remains the predominant bacterium in root caries, our study suggests that the microbial composition of root surface caries may evolve with age. Older adults (55-75 years) exhibit a more complex microbial environment, with higher levels of *Lactobacillus* and *Actinomyces spp.* than middle-aged adults (35-44 years). These findings support the idea that microbiological diversity increases with age, which may contribute to the increased severity of root caries observed in older adults<sup>[15]</sup>.

Additionally, the diversity of bacteria within the root caries biofilm cannot be overlooked. Recent studies have shown that beyond the commonly studied species like *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces*, other bacterial species might also play a role in root caries progression. In our study, we focused on a limited number of bacteria, but future studies should consider a broader range of bacterial species to better understand the full diversity of the root caries microflora<sup>[16]</sup>.

Microarray analysis of the root caries microflora has provided insights into the role of other microorganisms that might be involved in the process. Characterizing the microbiome of root caries lesions more comprehensively could lead to better diagnostic tools and therapeutic strategies for managing root caries. Moreover, understanding the microbial composition and biofilm formation patterns could help in tailoring preventive and treatment protocols for different age groups, particularly for the aging population<sup>[17]</sup>.

This study highlights the increased prevalence of *Lactobacillus* and *Actinomyces spp.* in the older age group (55-75 years) in Bangladesh, emphasizing the age-related changes in the root caries microflora. The findings also suggest that *Streptococcus mutans* remains a key player in root caries, but its interaction with other microorganisms such as *Lactobacillus* and *Actinomyces spp.* could contribute to the progression of the disease in older adults. Further studies exploring the full diversity of microorganisms involved in root caries will be essential to enhance our understanding and improve clinical management strategies for this condition.

## 5. Conclusion

This study found that *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces spp.* were more prevalent in the older group compared to the middle-aged group in the Bangladeshi population. Among these, *Streptococcus mutans* was the most frequently identified, followed by *Lactobacillus* and *Actinomyces spp.*. A significant association between age and the presence of *Lactobacillus* was observed, with its prevalence increasing with age. These findings suggest that older adults in Bangladesh are more susceptible to these bacteria, potentially increasing the risk of root caries.

## Conflict of interest

The authors declare no conflict of interest.

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