



Original Research Article

To evaluate the variations in oral bacterial species associated among the adolescent population of Uttarakhand

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Abstract

Background: Adolescence is a developmental period of vital importance with hormonal shift, changing lifestyle, and behavior, which impinges importantly upon oral health and microbiota. It is crucial to comprehend these microbial differences to design potent, age-specific preventive oral health measures.

Objective: To examine diversity and oral bacterial species prevalence among adolescents in Uttarakhand and discuss their relation with demographic variables and oral hygiene practices.

Materials and Methods: A cross-sectional design was conducted using 70 adolescents between 10 and 19 years old. Unstimulated saliva samples were obtained and cultured on selective medium.

Bacterial isolates were characterized by Gram staining and biochemical assays such as catalase, methyl red-Voges Proskauer, indole, and citrate utilization tests. Statistical correlations between the presence of bacteria and factors like age, gender, and oral hygiene were evaluated with Chi-square tests through SPSS v23.

Results: Streptococcus spp. dominated (24%), followed by Lactobacillus spp. (20%) and Actinomyces spp. (17%). In 7% of the adolescents, mixed colonization by several species was observed, and in 32% of the samples, no bacterial growth was found, predominantly in females and regular brushers. Actinomyces spp. were significantly more common in older adolescents ($\chi^2 = 4.63$, $p = 0.031$). Streptococcus spp. prevalence was positively related to poor oral hygiene ($\chi^2 = 6.12$, $p = 0.013$), with no statistically significant variation by gender ($\chi^2 = 0.47$, $p = 0.49$).

Conclusions: Oral microbiota composition among adolescents of Uttarakhand is largely determined by oral hygiene and age, not gender. These findings point to the need for adolescent-specific oral health education and preventive care measures to reduce risks related to dental caries and periodontal disease.

Keywords: Adolescents, Oral microbiota, Streptococcus, Lactobacillus, Actinomyces, Preventive dentistry, Uttarakhand.

Received: 26-08-2025; **Accepted:** 28-09-2025; **Available Online:** 28-10-2025

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1. Introduction

Oral health is an integral part of systemic health and quality of life. The oral cavity contains one of the most complex microbial ecosystems in the human body, supporting oral homeostasis but potentially serving as a reservoir for upcoming pathogens when ecological equilibrium is upset.^{1,3} Dysbiosis of this community has been associated with the etiologies of dental caries, periodontal infections, and inflammatory conditions affecting the whole body, thus underscoring its clinical relevance.

Adolescence is a pivotal transitional stage marked by significant physiological, hormonal, and psychosocial alterations. Oral hygiene behaviors, food choices, and microbial colonization patterns are all affected by these

changes.⁴⁻⁶ During adolescence, oral microbiologic changes have been correlated with increased risks for dental caries and periodontal disorders, which, if left untreated, can lead to systemic complications like cardiovascular disease and diabetes via inflammation pathways^{7,8}

In the face of growing worldwide interest in charting oral microbiota, research on Indian teens is particularly lacking. The north Indian mountain state of Uttarakhand, with its specific eating habits, lifestyle patterns, and local environmental exposures, is a special population in this regard. These characteristics might contribute to the microbial composition in a distinct way, but regional information is inadequately represented in the literature⁹⁻¹¹

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This study aims to fill the above gap by characterizing the oral bacterial species prevalent among adolescents in Uttarakhand and studying the influence of demographic factors, such as age and gender, and oral hygiene behaviors on microbial distribution. Baseline epidemiological evidence from this study is expected to inform adolescent-oriented preventive oral health strategies in the region.

2. Materials and Methods

2.1. Ethics

Review and approval of the study protocol was given by the Institutional Ethics Committee before the start. Written informed consent from the guardians of participants and assent from adolescents were taken before sample collection, in line with the Declaration of Helsinki and standard ethical guidelines.¹²

2.2. Study design and participants

This investigation employed a cross-sectional observational design and was conducted in 2025. A total of 70 adolescents (40 males, 57.1%; 30 females, 42.9%), aged 10–19 years, were recruited through a stratified sampling strategy from schools and community health centers across Uttarakhand.

Recruitment aimed to ensure representation across different socioeconomic strata to enhance the generalizability of findings.

2.3. Inclusion criteria

Teenagers living in Uttarakhand, no recent exposure to antibiotics in the last 4 weeks, and willing participation with informed consent to give saliva samples.

2.4. Exclusion criteria

Systemic disease, active orthodontic treatment, or non-participation.

3. Sample Collection and Processing

Unstimulated whole saliva was collected under standardized conditions into sterile containers. Instructions were given to the participants to avoid food or drink (except water) for a period of 30 minutes prior to collection in order to reduce variability.² All the samples were transported in cold chain conditions (on ice) and processed within 2 hours within the microbiology laboratory.

Cultivation was done in nutrient agar, blood agar, and selective media and incubated aerobically at 37 °C for 48 hours. Isolated colonies were subjected to morphological description, Gram staining, and confirmatory biochemical tests such as catalase, methyl red–Voges Proskauer (MR–VP), indole, and citrate utilization tests, as per routine diagnostic procedures. Identification of genera and species was directed towards priority oral bacteria of concern for adolescents.

3.1. Statistical analysis

Descriptive statistics were utilised to calculate prevalence rates of bacterial species. The associations of microbial distribution with demographic/ behavioral variables (age, gender, oral hygiene habits) were examined using Chi-square analysis (χ^2) within SPSS v23. The significance level of $p < 0.05$ was set for all analyses. Where appropriate, effect sizes were calculated to signify the strength of associations.

4. Results

4.1. Demographic profiles (Table 1)

70 adolescents participated in the study, including 40 males (57.1%) and 30 females (42.9%). The participants' mean age was 15.2 years (SD = 2.1), ranging from 10 to 19 years. The participants were stratified to reflect the range of socioeconomic backgrounds across schools and community health centers of Uttarakhand.

4.2. Bacterial species pattern and frequency (Table 2)

Culture-based detection of unstimulated saliva samples detected several clinical-relevant bacterial genera. *Streptococcus* spp. were the most common isolates (24%), followed by *Lactobacillus* spp. (20%) and *Actinomyces* spp. (17%). Mixed colonization, represented as the detection of two or more bacterial species, was found in 7% of the participants, while no detectable bacterial growth under the conditions used was found in 32% of the adolescents.

The following distinct patterns of distribution were observed. *Streptococcus* spp. were isolated more often in adolescents who had poor oral hygiene habits. *Lactobacillus* spp. were detected in all groups regardless of demographics or behavior. *Actinomyces* spp. were found with increased prevalence in older adolescents (≥ 16 years old). Irregular brushing habits had mixed species colonization, whereas a lack of bacterial growth was more often seen among females and adolescents who brushed their teeth regularly.

4.3. Statistical associations (Table 3)

Chi-square analysis revealed strong associations between bacterial prevalence and some independent variables. A strong association was found between age and *Actinomyces* spp. colonization ($\chi^2 = 4.63$, $p = 0.031$). The presence or absence of oral hygiene was strongly associated with *Streptococcus* spp., with higher frequency in adolescent patients with bad oral hygiene ($\chi^2 = 6.12$, $p = 0.013$). A lack of influence of gender on microbial distribution was found ($\chi^2 = 0.47$, $p = 0.49$).

Taken together, these results suggest age and oral health habits, not gender, are primary predictors of oral bacterial variation in this teenage group.

Table 1: Demographic data

Parameter	Number	Percentage (%)
Total Participants	70	100
Male	40	57.1
Female	30	42.9
Mean Age (years)	15.2	(SD = 2.1)

Table 2: Bacterial species distribution and prevalence

Bacterial Species	Prevalence (%)	Notable Observations
Streptococcus spp.	24	Higher prevalence with poor hygiene
Lactobacillus spp.	20	Present uniformly across demographics
Actinomyces spp.	17	Significantly higher in older age group
Mixed species (≥2)	7	Linked to inconsistent brushing
No bacterial growth	32	More frequent in females and regular brushers

Table 3: Statistical associations with age, oral hygiene and gender

-	Bacterial Species	χ^2 Value	p-value	Interpretation
Age	Actinomyces spp.	4.63	0.031	Higher prevalence in older adolescents
Oral Hygiene	Streptococcus spp.	6.12	0.013	More common with poor hygiene
Gender	All species	0.47	0.49	No significant gender-based difference

5. Discussion

The findings of this research strongly support the current literature that characterizes *Streptococcus*, *Lactobacillus*, and *Actinomyces* as the most prevalent bacterial genera of the oral microbiota in adolescents.⁴⁻⁶ The dominance of *Streptococcus* species, specifically, is consistent with such risk factors as higher exposure to dietary sugars and poor oral hygiene habits—both established causes for dental caries.⁷ The increased prevalence among older adolescents of *Actinomyces* species probably mirrors puberty- related physiological and hormonal changes that affect the development of gingival.

Tissues and the associated microbial ecology.⁸ Most importantly, our results show that bacterial prevalence is not influenced by gender, reaffirming the current belief that

behavioral determinants, particularly oral hygiene practices, place more influence on microbial colonization than biological sex.⁹ This really left a notable mark in our study: a really huge proportion of adolescents really had no culturable bacterial growth. This comprises the majority of those who continually practiced oral hygiene. This really underlined how keeping good oral hygiene resulted in microbial homeostasis and impeded pathogenic bacterial colonization.

Although culture-based microbiological approaches employed in this work allow for the detection of culturable oral pathogens, one major limitation still exists: such methods do not detect fastidious, slow-growing, or uncultivable microorganisms that are relevant to the oral microbiome. Sophisticated molecular techniques, such as next-generation sequencing (NGS), allow for detailed profiling of the oral microbial community, including the detection of uncharacterized taxa.¹⁰⁻¹² These methods give a better understanding of the intricacies of microbial interactions, ecological dynamics, and their connection to oral disease and health. Therefore, future studies that involve metagenomics and other high-throughput molecular techniques are needed to document the entire oral microbiota diversity in adolescents. These studies will enhance our knowledge of microbial ecology in key windows of development and allow for the development of evidence-based targeted interventions aimed at maintaining oral health and disease prevention.

These advanced methods also have promise in identifying microbial biomarkers for early risk assessment, treatment outcome monitoring, and even tailoring oral health care - especially crucial for populations like adolescents in Uttarakhand where unique microbial signatures may be shaped by cultural and environmental factors. By probing the oral ecosystem with research beyond traditional cultivation-based techniques, we can better understand the oral ecosystem, inform public health policies, and ultimately improve population-level oral health outcomes.

6. Conclusion

Teenagers in Uttarakhand show oral microbial patterns largely determined by the oral hygiene behavior and age, with minimal gender influences. This highlights the urgent requirement for specific oral health education programs aiming to promote good and regular hygiene habits tailored for teenagers. Incorporation of such programs within school curricula has been proved effective in enhancing oral health knowledge, attitudes, and behaviors and has been found to produce dramatic declines in caries and periodontal disease. Regular professional dental examinations supplement educational approaches by providing for early recognition and treatment of oral diseases. Public health programs adopting these age-based and behavior-focused approaches can thus realize significant oral health gains and create lifelong prevention habits among the vulnerable group of adolescents.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Cite this article: Ahuja D, Kumar V, Seth J Arya G. To evaluate the variations in oral bacterial species associated among the adolescent population of Uttarakhand. *J Dent Panacea*. 2025;7(3):138-141.