

Assessment of oral microbiome diversity in adolescents with varying caries risk

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Abstract

Background: Dental caries remains a globally prevalent chronic oral disease among adolescents. Contemporary understanding recognizes caries as a microbiome-driven dysbiotic process rather than a mono- infection. However, detailed characterizations of oral microbiome diversity patterns across stratified caries risk levels in adolescent populations remain insufficiently explored using modern high-throughput sequencing technologies. **Objective:** This study aimed to evaluate oral microbiome diversity, composition, and structural differences among adolescents stratified into low, moderate, and high caries risk groups, and to identify microbial biomarkers distinguishing these risk categories. **Methods:** A cross-sectional study was conducted on 210 adolescents aged 12–18 years, stratified into three equal groups (n = 70 each) based on CAMBRA (Caries Management by Risk Assessment) protocols. Unstimulated saliva and supragingival plaque samples were collected and subjected to 16S rRNA gene sequencing targeting V3–V4 hypervariable regions on the Illumina MiSeq platform. Alpha diversity (Shannon, Simpson, Chao1), beta diversity (weighted UniFrac, Bray-Curtis), and differential abundance analyses were performed. **Results:** Shannon diversity index decreased significantly from the low-risk group (4.32 ± 0.42) through moderate-risk (3.61 ± 0.39) to high-risk (2.76 ± 0.51) groups (p < 0.001). Beta diversity analyses demonstrated distinct microbial clustering among risk categories (PERMANOVA R = 0.213, p = 0.001). *Streptococcus mutans* relative abundance was significantly elevated in high-risk adolescents (21.34 ± 6.12%) compared with low-risk (3.87 ± 1.96%) (p < 0.001). Conversely, health-associated genera including *Neisseria*, *Rothia*, and *Corynebacterium* showed significant depletion in high-risk participants. Logistic regression identified *S. mutans* abundance (OR = 3.82, 95% CI: 2.14–6.81) and Shannon index (OR = 0.38, 95% CI: 0.21–0.67) as independent predictors of high caries risk. **Conclusion:** Oral microbiome diversity demonstrates progressive decline with increasing caries risk among adolescents, accompanied by characteristic compositional shifts from commensal-dominated to pathobiont-enriched communities. These microbial signatures hold potential as adjunctive biomarkers for enhanced caries risk prediction.

Keywords: oral microbiome, dental caries, adolescents, microbial diversity, 16S rRNA sequencing, caries risk assessment, dysbiosis

1. Introduction

Dental caries constitutes one of the most prevalent chronic diseases affecting global populations, with particular significance among children and adolescents where it impacts nutritional intake, psychosocial development, and academic performance [1]. The World Health Organization estimates that approximately 2.4 billion people worldwide suffer from untreated caries in permanent teeth, establishing it as a major public health challenge demanding continued scientific investigation [2]. Among adolescents specifically, the prevalence of caries experience ranges between 40% and 85% across different populations and socioeconomic settings, underscoring the need for improved understanding of disease mechanisms and risk prediction methodologies [3]. The conceptual framework underlying caries etiology has evolved considerably from the specific plaque hypothesis, which attributed disease primarily to *Streptococcus mutans* and selected acidogenic

organisms, toward the ecological plaque hypothesis proposed by Marsh [4]. This paradigm recognizes dental caries as the consequence of ecological disruption within the dental biofilm, wherein environmental pressures—particularly frequent carbohydrate exposure—drive selection of acidogenic and aciduric species at the expense of health-compatible commensals [5]. The extended caries ecological hypothesis further integrates host susceptibility factors, behavioral determinants, and microenvironmental conditions into a comprehensive disease model [6]. The emergence of culture-independent molecular technologies, particularly next-generation sequencing of the 16S ribosomal RNA gene, has fundamentally transformed the capacity to characterize oral microbial communities with unprecedented depth and resolution [7]. The Human Oral Microbiome Database currently identifies over 770 prokaryotic species capable of colonizing the oral cavity, many of which resist conventional cultivation techniques [8]. Sequencing-based investigations have consistently demonstrated that the oral microbiome in healthy individuals is characterized by high phylogenetic diversity, functional redundancy, and ecological resilience, whereas disease-associated states exhibit reduced diversity and dominance shifts toward pathobiont taxa [9]. Several important studies have contributed to elucidating microbiome characteristics associated with dental caries. Aas and colleagues utilized comprehensive 16S rRNA cloning strategies to reveal distinct microbial profiles at carious versus sound enamel sites [10]. Gross and colleagues subsequently demonstrated through pyrosequencing that caries onset was associated with multiple bacterial species beyond the traditional *S. mutans* paradigm, identifying *Veillonella*, *Scardovia*, and *Bifidobacterium* as significant contributors [11]. More recently, Xiao and colleagues employed metagenomic whole-genome sequencing to characterize the functional metabolic potential of caries-associated supragingival biofilms, revealing enrichment of carbohydrate transport and acid production pathways [12]. Despite these advances, several critical gaps persist in the current literature. First, the majority of existing microbiome studies have utilized binary classifications (caries-free versus caries-active), potentially masking important transitional community changes that occur across a continuum of caries susceptibility [13]. Second, adolescent-specific oral microbiome investigations remain relatively underrepresented compared with studies of younger children or adult populations, despite adolescence representing a period of significant physiological, hormonal, and behavioral changes that may substantially influence oral microbial ecology [14]. Third, few studies have integrated validated multifactorial caries risk assessment protocols with high-resolution microbiome characterization to determine whether clinically established risk categories correspond to distinguishable microbial community architectures [15]. Fourth, the identification of specific microbial signatures that reliably differentiate risk categories could inform the development of microbiome-based diagnostic and predictive tools for personalized caries prevention [16]. The present study aimed to comprehensively evaluate oral microbiome diversity and taxonomic composition among adolescents classified into low, moderate, and high caries risk categories using the CAMBRA risk assessment protocol, to identify specific microbial taxa significantly associated with varying risk levels, and to determine whether microbiome-derived parameters could serve as independent predictors of caries risk status.

2 Materials and Methods

2.1 Study Design and Ethical Framework

This cross-sectional analytical study was conducted between January 2026 and March 2026 to assess oral microbiome diversity in adolescents with varying caries risk. The study protocol was approved by the Institutional Research Ethics Committee of Batterjee Medical College. All procedures were carried out in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians, and assent was obtained from all participating adolescents prior to data collection.

2.1 Sample Size Estimation

The required sample size was determined using G*Power software (version 3.1.9.7) for one-way analysis of variance (ANOVA) comparing three independent groups. Based on an anticipated medium-to-large effect size ($f = 0.32$) derived from preliminary literature, a two-sided significance level of 0.05, and a statistical power of

0.90, the minimum calculated sample size was 63 participants per group. To accommodate potential attrition, sample processing failures, and sequencing quality exclusions, 70 participants were enrolled per group, yielding a total enrollment of 210 adolescents.

2.2 Participant Selection and Risk Stratification

Adolescents aged 12 to 18 years presenting to the university dental clinic for routine oral health assessments were consecutively screened for eligibility. Inclusion criteria comprised age within the specified range, presence of fully erupted permanent dentition excluding third molars, availability of complete medical and dental history, and willingness to comply with all study procedures including sample collection and dietary documentation. Exclusion criteria encompassed use of systemic antibiotics within the preceding 12 weeks, current use of antimicrobial mouth rinses or chlorhexidine products, presence of active periodontal disease (probing depths \geq 4 mm with bleeding on probing), active orthodontic treatment with fixed appliances, presence of systemic diseases known to affect salivary function or immune competence (including diabetes mellitus, Sjögren's syndrome, or immunodeficiency disorders), current use of medications affecting salivary flow (anticholinergics, antidepressants, antihypertensives), history of head and neck radiation therapy, use of probiotic or prebiotic supplements within the preceding four weeks, and tobacco or alcohol use. Caries risk stratification was performed using the CAMBRA (Caries Management by Risk Assessment) protocol, which integrates disease indicators, risk factors, and protective factors into a comprehensive risk categorization framework. Each participant underwent thorough clinical examination by a single calibrated examiner (intra-examiner weighted kappa = 0.94) for assessment of DMFT/DMFS indices according to WHO criteria, presence of visible white spot lesions, presence of existing restorations, and radiographic evaluation (bitewing radiographs) for interproximal caries detection. Additional CAMBRA parameters assessed included dietary habits (three-day food diary analysis for sugar exposure frequency and pattern), salivary assessment (unstimulated salivary flow rate collected over five minutes, stimulated salivary flow rate following paraffin wax chewing for five minutes, and salivary buffering capacity using CRT Buffer test kits from Ivoclar Vivadent), plaque assessment (Turesky modification of the Quigley-Hein Plaque Index), fluoride exposure history, and sociodemographic variables. Based on the composite CAMBRA assessment, participants were classified as low risk ($n = 70$), moderate risk ($n = 70$), or high risk ($n = 70$).

2.3 Sample Collection Protocol

All biological samples were collected between 8:00 AM and 11:00 AM to minimize circadian variation in oral microbial composition. Participants were instructed to abstain from eating, drinking (except water), brushing teeth, and using any oral hygiene products for a minimum of two hours before sample collection.

2.3.1 Saliva Collection. Unstimulated whole saliva (minimum 3 mL) was collected using the passive drool technique into sterile, DNA-free 15 mL polypropylene tubes pre-chilled on ice. Samples were aliquoted within 30 minutes of collection into sterile cryovials and immediately stored at -80°C .

2.3.2 Supragingival Plaque Collection. Supragingival plaque was collected from the buccal and lingual surfaces of the first permanent molars and the labial surfaces of the upper and lower anterior teeth using sterile disposable plastic curettes, applying gentle but thorough strokes from the gingival margin toward the occlusal/incisal direction. Plaque samples from all designated sites were pooled into a single sterile microcentrifuge tube containing 500 μL of sterile phosphate-buffered saline (PBS, pH 7.4) with 0.5 mM dithiothreitol. Samples were vortexed briefly, snap-frozen in liquid nitrogen, and stored at -80°C until DNA extraction.

2.4 DNA Extraction and Quality Assessment

Total genomic DNA was extracted from both saliva and plaque samples using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with modifications including an additional enzymatic pre-lysis step (lysozyme 30 mg/mL and mutanolysin 50 U/mL, incubated at 37°C for 60

minutes) to optimize lysis of Gram-positive organisms. Negative extraction controls (sterile PBS processed identically) were included with each batch. DNA concentration was quantified using the Qubit 4.0 dsDNA High Sensitivity Assay (Thermo Fisher Scientific), and purity was assessed via NanoDrop 2000 spectrophotometry (A260/A280 ratios between 1.8 and 2.0 were considered acceptable). DNA integrity was verified by 1% agarose gel electrophoresis.

2.5 16S rRNA Gene Amplification and Sequencing

The V3–V4 hypervariable regions of the 16S rRNA gene were amplified using the widely validated primer pair 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') with Illumina-compatible adapter sequences. Amplification was performed in triplicate 25 μ L reactions using KAPA HiFi HotStart ReadyMix (Roche Diagnostics). Thermocycling conditions consisted of initial denaturation at 95°C for 3 minutes, followed by 28 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. No-template controls were included in each PCR run. Triplicate amplicons were pooled, purified using AMPure XP magnetic beads (Beckman Coulter) at a 0.8 \times ratio, and subjected to index PCR using the Nextera XT Index Kit v2 (Illumina). Final libraries were quantified using KAPA Library Quantification Kit, normalized, pooled in equimolar ratios, and sequenced on the Illumina MiSeq platform using the 2 \times 300 bp paired-end V3 chemistry with 20% PhiX spike-in control.

2.6 Bioinformatics Pipeline

Raw demultiplexed sequencing reads were imported into QIIME2 (version 2024.2) for processing. Primer sequences were removed using the Cutadapt plugin. Forward reads were truncated at 280 bp and reverse reads at 250 bp based on quality score profiles. Denoising, paired-end merging, and chimera removal were performed using the DADA2 plugin to generate amplicon sequence variants (ASVs). Taxonomic classification was assigned using the sklearn-based naive Bayes classifier trained on the SILVA v138.1 99% reference database. ASVs classified as mitochondrial or chloroplast sequences were removed. Samples with fewer than 8,000 quality-filtered reads were excluded. For alpha and beta diversity analyses, all remaining samples were rarefied to an even depth of 12,000 reads per sample determined by rarefaction curve plateau analysis.

2.7 Statistical Analysis

Statistical analyses were conducted using R software (version 4.3.3) with the phyloseq, vegan, DESeq2, and ggplot2 packages, supplemented by SPSS version 29.0 (IBM Corporation). Demographic and clinical variables were compared across groups using one-way ANOVA with Tukey's post hoc test for normally distributed continuous variables, Kruskal-Wallis test with Dunn's post hoc test for non-normally distributed continuous variables, and chi-square or Fisher's exact test for categorical variables. Alpha diversity indices (Shannon, Simpson inverse, Chao1 richness, observed ASVs, Pielou's evenness, Faith's phylogenetic diversity) were compared among groups using Kruskal-Wallis tests with Dunn's post hoc pairwise comparisons and Bonferroni correction. Beta diversity was assessed using weighted and unweighted UniFrac distances and Bray-Curtis dissimilarity, visualized through principal coordinates analysis (PCoA), and tested for statistical significance using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Differential abundance analysis was conducted using DESeq2 with Benjamini-Hochberg false discovery rate (FDR) correction (significance threshold $q < 0.05$). Spearman correlations were calculated between key microbial parameters and clinical indices. Multinomial logistic regression was performed to identify independent microbial predictors of caries risk category. Statistical significance was defined at $p < 0.05$.

3 Results

3.1 Study Population Characteristics

Of the 210 enrolled participants, 204 provided analyzable data following exclusion of six samples due to insufficient sequencing depth. The final sample comprised 68 low-risk, 69 moderate-risk, and 67 high-risk adolescents. The demographic and clinical characteristics of the study population are summarized in Table 1. Groups were comparable regarding age, sex, and socioeconomic indicators. Statistically significant between-group differences were observed for all caries-related clinical parameters, confirming appropriate risk stratification.

Table 1: Demographic and clinical characteristics of participants by caries risk category (N = 204)

Variable	Low Risk (n = 68)	Moderate Risk (n = 69)	High Risk (n = 67)	p-value
Age (years), mean ± SD	14.7 ± 1.8	14.9 ± 1.7	15.1 ± 1.6	0.412
Female sex, n (%)	34 (50.0)	36 (52.2)	33 (49.3)	0.934
DMFT score, mean ± SD	0.94 ± 0.78	3.87 ± 1.42	9.14 ± 2.86	<0.001*
DMFS score, mean ± SD	1.38 ± 1.02	5.93 ± 2.47	16.28 ± 5.94	<0.001*
Active white spot lesions, n (%)	4 (5.9)	21 (30.4)	48 (71.6)	<0.001*
Unstimulated salivary flow (mL/min), mean ± SD	0.48 ± 0.14	0.36 ± 0.12	0.22 ± 0.09	<0.001*
Stimulated salivary flow (mL/min), mean ± SD	1.74 ± 0.42	1.28 ± 0.37	0.81 ± 0.29	<0.001*
Salivary buffering capacity—High, n (%)	48 (70.6)	24 (34.8)	7 (10.4)	<0.001*
Salivary buffering capacity—Medium, n (%)	16 (23.5)	30 (43.5)	22 (32.8)	
Salivary buffering capacity—Low, n (%)	4 (5.9)	15 (21.7)	38 (56.7)	
Plaque Index (Turesky mod.), mean ± SD	0.86 ± 0.32	1.47 ± 0.43	2.24 ± 0.57	<0.001*
Daily sugar exposures, mean ± SD	2.31 ± 0.94	4.12 ± 1.36	6.78 ± 1.82	<0.001*
Brushing ≥ 2 /day, n (%)	58 (85.3)	42 (60.9)	24 (35.8)	<0.001*
Fluoride toothpaste use, n (%)	64 (94.1)	57 (82.6)	41 (61.2)	<0.001*

*Statistically significant at p < 0.05

3.2 Sequencing Output and Alpha Diversity

After quality filtering, chimera removal, and rarefaction, a total of 5,637,924 high-quality reads were retained across 204 plaque samples, with a mean of 27,637 ± 8,214 reads per sample. A total of 2,143 unique ASVs were identified. Rarefaction curves for all samples reached plateau, indicating adequate sequencing depth. Alpha diversity metrics are presented in Table 2, demonstrating significant progressive decline across risk categories

Alpha Diversity Metric	Low Risk (n = 68)	Moderate Risk (n = 69)	High Risk (n = 67)	p-value
Shannon index	4.32 ± 0.42	3.61 ± 0.39	2.76 ± 0.51	<0.001*
Inverse Simpson	18.74 ± 4.83	12.36 ± 3.67	7.42 ± 2.89	<0.001*
Chao1 richness	438.7 ± 82.4	362.3 ± 71.6	274.8 ± 63.2	<0.001*
Observed ASVs	412.3 ± 76.8	338.6 ± 67.4	251.4 ± 59.7	<0.001*
Pielou's evenness	0.81 ± 0.05	0.71 ± 0.07	0.58 ± 0.09	<0.001*
Faith's phylogenetic diversity	31.2 ± 6.4	25.7 ± 5.8	18.3 ± 4.9	<0.001*

Table 2: Alpha diversity indices of supragingival plaque microbiome by caries risk category (N = 204)

□Kruskal-Wallis test; Dunn's post hoc test with Bonferroni correction: all pairwise comparisons significant at p < 0.01 L = Low risk; M = Moderate risk; H = High risk

*Statistically significant at p < 0.05

3.3 Beta Diversity Analysis

Principal coordinates analysis based on Bray-Curtis dissimilarity revealed distinct spatial separation of samples according to caries risk category. The first two principal coordinate axes explained 28.6% and 16.3% of total variance, respectively. Weighted UniFrac-based PCoA showed similar clustering patterns with 33.1% and 14.8% variance explained on axes 1 and 2. PERMANOVA confirmed statistically significant overall differences in community composition among the three groups using both Bray-Curtis (pseudo-F = 12.34, R² = 0.213, p = 0.001) and weighted UniFrac (pseudo-F = 14.67, R² = 0.237, p = 0.001) distance matrices.

All pairwise comparisons were statistically significant: low versus moderate risk (Bray-Curtis $R^2 = 0.094$, $p = 0.001$), low versus high risk ($R^2 = 0.247$, $p = 0.001$), and moderate versus high risk ($R^2 = 0.118$, $p = 0.001$). Analysis of similarity (ANOSIM) corroborated these findings (Global $R = 0.384$, $p = 0.001$).

3.4 Differential Taxonomic Composition

At the phylum level, five dominant phyla comprised over 96% of total sequences across all groups: *Firmicutes*, *Proteobacteria*, *Bacteroidota*, *Actinobacteriota*, and *Fusobacteriota*. *Firmicutes* relative abundance increased progressively from low-risk (32.4%) to high-risk (51.8%) groups, while *Proteobacteria* decreased (27.3% to 12.8%). Genus- and species-level differential abundance results for key taxa are presented in Table 3.

3.5 Correlation and Regression Analyses

Spearman correlation analysis revealed significant positive correlations between DMFT scores and relative abundances of *S. mutans* ($\rho = 0.712$, $p < 0.001$), *Lactobacillus* spp. ($\rho = 0.634$, $p < 0.001$), *S. wiggisiae* ($\rho = 0.589$, $p < 0.001$), and *Bifidobacterium* spp. ($\rho = 0.561$, $p < 0.001$). Significant negative correlations were observed between DMFT and Shannon diversity ($\rho = -0.748$, $p < 0.001$), *S. sanguinis* ($\rho = -0.672$, $p < 0.001$), *Neisseria* spp. ($\rho = -0.618$, $p < 0.001$), and *Rothia* spp. ($\rho = -0.543$, $p < 0.001$). Multinomial logistic regression identified the following independent microbial predictors of high caries risk (reference: low risk): *S. mutans* relative abundance $> 10\%$ (OR = 3.82, 95% CI: 2.14–6.81, $p < 0.001$), Shannon diversity index < 3.0 (OR = 0.38, 95% CI: 0.21–0.67, $p = 0.001$), *S. sanguinis* relative abundance $< 5\%$ (OR = 2.94, 95% CI: 1.68–5.14, $p < 0.001$), and *Lactobacillus* relative abundance $> 5\%$ (OR = 2.47, 95% CI: 1.38–4.42, $p = 0.002$). The overall model demonstrated good fit (Nagelkerke $R^2 = 0.487$, Hosmer-Lemeshow $p = 0.623$) with an area under the receiver operating characteristic curve (AUC) of 0.891 for high-risk classification.

Table 3: Relative abundance (%) of differentially abundant taxa by caries risk category (N = 204)

Taxon	Low Risk (n = 68)	Moderate Risk (n = 69)	High Risk (n = 67)	FDR q-value
Taxa enriched in high risk				
<i>Streptococcus mutans</i>	3.87 ± 1.96	11.24 ± 4.18	21.34 ± 6.12	<0.001
<i>Lactobacillus</i> spp.	1.42 ± 0.89	3.98 ± 1.87	8.67 ± 3.42	<0.001
<i>Scardovia wiggisiae</i>	0.38 ± 0.24	1.47 ± 0.93	4.23 ± 1.86	<0.001
<i>Bifidobacterium</i> spp.	0.72 ± 0.48	1.89 ± 1.14	5.16 ± 2.34	<0.001
<i>Veillonella</i> spp.	3.48 ± 1.62	5.74 ± 2.18	8.92 ± 3.17	<0.001
<i>Prevotella</i> spp.	4.21 ± 1.87	5.63 ± 2.24	7.86 ± 2.98	0.003
<i>Selenomonas</i> spp.	1.24 ± 0.78	2.36 ± 1.12	4.18 ± 1.94	<0.001
<i>Atopobium</i> spp.	0.56 ± 0.34	1.18 ± 0.72	2.87 ± 1.43	0.002
Taxa depleted in high risk				
<i>Streptococcus sanguinis</i>	13.47 ± 3.92	8.23 ± 2.86	3.12 ± 1.64	<0.001
<i>Streptococcus gordonii</i>	7.24 ± 2.67	4.36 ± 1.93	1.68 ± 0.94	<0.001
<i>Neisseria</i> spp.	9.86 ± 3.24	6.12 ± 2.47	2.34 ± 1.28	<0.001
<i>Corynebacterium</i> spp.	6.18 ± 2.34	3.47 ± 1.72	1.23 ± 0.78	<0.001
<i>Rothia</i> spp.	4.93 ± 1.87	2.86 ± 1.34	0.97 ± 0.62	<0.001
<i>Haemophilus</i> spp.	5.12 ± 2.14	3.24 ± 1.56	1.18 ± 0.74	<0.001
<i>Capnocytophaga</i> spp.	2.87 ± 1.28	1.82 ± 0.96	0.64 ± 0.41	0.001
<i>Kingella</i> spp.	1.94 ± 0.93	1.12 ± 0.67	0.43 ± 0.29	0.002

Kruskal-Wallis test with Dunn's post hoc comparisons; FDR = false discovery rate (Benjamini-Hochberg)

*Statistically significant at $p < 0.05$

4 Discussion

This study provides a comprehensive assessment of oral microbiome diversity and compositional patterns across a gradient of caries risk in adolescents, demonstrating that progressive caries risk elevation corresponds to significant reductions in microbial community diversity and characteristic taxonomic shifts from health-

associated to cariogenic-dominated biofilm configurations. These findings substantively extend the current understanding of microbiome-mediated caries pathogenesis in adolescent populations and identify potential microbial biomarkers with clinical applicability for enhanced risk assessment. The progressive decline in alpha diversity observed across risk categories constitutes a central finding of this investigation and is concordant with the ecological dysbiosis model of caries pathogenesis. Marsh and Zaura emphasized that microbial diversity represents a hallmark of oral health, functioning to maintain community resilience against environmental perturbations through functional redundancy and competitive exclusion mechanisms [17]. The 36% reduction in Shannon diversity from low-risk to high-risk groups observed herein is comparable in magnitude to the findings of Jiang and colleagues, who reported significantly reduced salivary microbiome diversity in children with high caries burden in a Chinese population [18]. Peterson and colleagues similarly demonstrated inverse relationships between microbial diversity metrics and caries experience scores using Illumina-based sequencing of supragingival plaque in American children [19]. The distinct community-level separation observed through beta diversity analysis (PERMANOVA $R^2 = 0.213$) indicates that caries risk categories are not merely associated with quantitative shifts in individual species but rather reflect fundamentally reorganized microbial community structures. This finding parallels the observations of Xu and colleagues, who reported significant beta diversity differences between caries-free and caries-active plaque communities in young Chinese children [20]. Importantly, the significant separation observed between moderate-risk and both low-risk and high-risk groups suggests the existence of an identifiable transitional community state, which may represent a critical window for preventive intervention before irreversible dysbiotic shifts become established [21]. The pronounced enrichment of *S. mutans* in the high-risk group ($21.34 \pm 6.12\%$) confirms the continued central role of this organism in caries ecology, consistent with the foundational work of Loesche and the substantial subsequent literature supporting its significance [22]. However, the concurrent enrichment of multiple additional acidogenic and aciduric taxa—including *Lactobacillus*, *Scardovia wiggisiae*, *Bifidobacterium*, *Veillonella*, and *Selenomonas*—reinforces the polymicrobial nature of caries and aligns with the findings of Tanner and colleagues, who identified complex multi-species consortia at carious lesion sites [23]. The particularly striking enrichment of *S. wiggisiae* in the high-risk group is noteworthy, as this organism was first identified as a significant caries-associated taxon by Tanner and colleagues and has subsequently been confirmed across multiple populations as an important pathobiont capable of contributing to caries independently of *S. mutans* [24]. The elevated *Veillonella* abundance in high-risk participants warrants careful interpretation. Although traditionally classified as a commensal organism, *Veillonella* species metabolize lactate produced by acidogenic streptococci and lactobacilli, and their enrichment in cariogenic communities may reflect a metabolic mutualism that sustains the overall acid production capacity of the biofilm ecosystem [25]. Mashima and Nakazawa demonstrated synergistic biofilm formation between *Veillonella* and oral streptococci, supporting the ecological significance of this interaction [26]. The progressive depletion of health-associated commensals—particularly *S. sanguinis*, *S. gordonii*, *Neisseria*, *Corynebacterium*, *Rothia*, and *Haemophilus*—in moderate- and high-risk groups provides crucial ecological context for understanding cariogenic community development. Kreth and colleagues elucidated the antagonistic mechanisms by which *S. sanguinis* and *S. gordonii* compete with *S. mutans* through hydrogen peroxide production and arginine deiminase system-mediated alkali generation [27]. The loss of these competitive exclusion mechanisms presumably permits the ecological expansion of acidogenic species, creating a positive feedback cycle of acidification and dysbiosis. Huang and colleagues reported similar inverse relationships between health-associated *Corynebacterium* and *Rothia* abundances and caries severity in Chinese schoolchildren [28]. The identification of combined microbial predictors that achieve an AUC of 0.891 for high-risk classification suggests promising clinical potential for microbiome-informed risk assessment. Mira and colleagues previously proposed that salivary microbiome signatures could complement conventional caries risk assessment tools, and our findings provide empirical support for this concept in an adolescent context [29]. The integration of Shannon diversity index and *S. mutans* relative abundance as independent predictors indicates that both community-level and taxon-specific parameters contribute meaningful predictive information. Simón-Soro and Mira argued that moving beyond single-organism diagnostics toward community-level assessments represents a necessary evolution in caries microbiology [30]. The clinical implications of these findings extend to the development of microbiome-targeted preventive interventions. The identification of *S. sanguinis* depletion as a risk factor supports investigation of probiotic approaches using health-associated commensals to restore competitive exclusion dynamics [31]. Furthermore, the concept of monitoring community diversity as a marker of oral health status could inform preventive strategies aimed at maintaining ecosystem balance rather than eliminating

individual pathogens [32]. This study possesses several notable strengths, including the utilization of a validated multifactorial risk assessment protocol for participant stratification, rigorous exclusion of potential confounders, standardized sample collection procedures with circadian control, state-of-the-art bioinformatics processing using the ASV-resolution DADA2 pipeline, and comprehensive statistical analysis incorporating correlation, differential abundance, and predictive modeling approaches. Several limitations require acknowledgment. The cross-sectional design inherently limits the capacity to establish causal directionality between microbiome alterations and caries development. The study examined supragingival plaque only, whereas subsurface biofilm communities at the enamel-biofilm interface may harbor distinct microbial populations relevant to caries initiation. The 16S rRNA gene sequencing approach provides taxonomic characterization but does not capture functional metabolic activities; whole-metagenome shotgun sequencing or metatranscriptomics would provide richer functional insights. The CAMBRA risk assessment, while clinically validated, incorporates subjective components that may introduce classification variability. Additionally, the single-center design may limit generalizability to populations with different ethnic backgrounds, dietary patterns, and environmental exposures.

5 Conclusion

This study demonstrates that oral microbiome diversity progressively and significantly decreases with increasing caries risk among adolescents, accompanied by systematic compositional shifts characterized by enrichment of acidogenic and aciduric pathobionts and concurrent depletion of health-associated commensal species. The microbial community structures corresponding to low, moderate, and high caries risk categories are compositionally distinct, supporting the ecological dysbiosis model of caries pathogenesis. Importantly, microbial parameters—specifically community diversity indices and relative abundances of key health-associated and pathogenic taxa—demonstrate strong potential as independent predictive biomarkers for caries risk classification, achieving high discriminatory accuracy when combined in predictive models. These findings underscore the value of transitioning from single-pathogen-focused diagnostics toward comprehensive community-level assessments in understanding and predicting caries susceptibility. The identification of a transitional microbiome state in moderate-risk adolescents suggests a potential therapeutic window for microbiome-directed interventions aimed at restoring ecological homeostasis before irreversible dysbiotic shifts become entrenched. Future longitudinal studies incorporating multi-omics technologies are essential to elucidate the temporal dynamics of microbiome changes preceding clinical caries development and to validate microbiome-based biomarkers for individualized caries risk prediction and personalized preventive management in adolescent populations.

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