The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(3): 547-552, 2025 www.thebioscan.com

Microbial Profiling of Dental Biofilms in Cavitated and Non-Cavitated Carious Lesions

Dr. P.R. SANJAYA M.D.S.,

Assistant Professor,Oral Pathology & Microbiology,Basic Dental & Medical Sciences,College of Dentistry, University of Hail, Uoh Id: 7130272, Hail province, Kingdom of Saudi Arabia

DOI: 10.63001/tbs.2025.v20.i03.pp547-552

KEYWORDS
Dental Biofilms, Cavitated Lesions, Non-Cavitated Caries, Microbial Profiling, Oral Microbiome

Received on:

30-06-2025

Accepted on:

31-07-2025

Published on:

02-09-2025

ABSTRACT

Dental caries remains one of the most prevalent chronic oral diseases worldwide, arising from complex interactions between host factors, diet, and microbial communities within dental biofilms. Recent advances in molecular microbiology have revealed that caries is not the outcome of a single pathogen, but rather the result of a dynamic shift in the balance of diverse microbial populations inhabiting the oral cavity. This study focuses on microbial profiling of biofilms associated with cavitated and non-cavitated carious lesions, aiming to delineate the distinct microbial signatures that contribute to the initiation and progression of caries. Clinical samples were collected from patients presenting with early non-cavitated white spot lesions as well as advanced cavitated dentinal caries. High-throughput sequencing techniques, complemented with culture-based assays, were employed to characterize the bacterial composition. Comparative analyses highlighted a clear microbial gradient from non-cavitated to cavitated lesions. Non-cavitated lesions were predominantly enriched with early colonizers such as *Streptococcus sanguinis*, *Streptococcus gordonii*, and *Actinomyces spp.*, which are typically associated with initial plaque biofilm formation. These organisms contributed to biofilm stability but exhibited moderate acidogenic potential. In contrast, cavitated lesions demonstrated a significant dominance of acidogenic and aciduric species, including *Streptococcus mutans*, *Lactobacillus spp.*, and *Bifidobacterium dentium*. The metabolic activities of these microorganisms, particularly their capacity for sustained acid production under low pH conditions, correlated strongly with demineralization and lesion advancement. Additionally, metagenomic profiling revealed a greater prevalence of anaerobic taxa such as *Veillonella* and *Prevotella*, suggesting a microbial shift towards a more complex, pathogenic community as cavitation progresses. Functional annotation of microbial genes further indicated enhanced carbohydrate metabolism and acid tolerance pathways in cavitated lesions compared with non-cavitated sites. The study underscores the significance of ecological succession within dental biofilms, illustrating how subtle microbial imbalances can transition a biofilm from a relatively stable state to a highly cariogenic consortium. These findings emphasize the need for preventive strategies targeting early microbial changes before cavitation occurs. Moreover, microbial profiling may serve as a diagnostic adjunct to identify high-risk patients and guide personalized caries management. By bridging clinical presentation with microbial ecology, this research contributes to a deeper understanding of caries pathogenesis and highlights novel avenues for preventive and therapeutic interventions.

INTRODUCTION

Dental caries has long been recognized as one of the most widespread and persistent oral health challenges worldwide. Despite decades of research and preventive initiatives, caries continues to affect billions of individuals across all age groups, imposing a considerable burden on global health systems. Caries is no longer regarded as a mere consequence of sugar consumption and poor oral hygiene but rather as a multifactorial disease influenced by host susceptibility, dietary patterns, salivary properties, genetic predisposition, and most importantly, the dynamic microbial communities that colonize tooth surfaces. The contemporary view of caries pathogenesis emphasizes the pivotal role of the oral microbiome and its ecological shifts, which drive the progression from a healthy biofilm to a cariogenic one. The oral cavity harbors one of the most complex microbial ecosystems in the human body, consisting of bacteria, fungi, archaea, and viruses that interact in intricate ways. Within this environment, dental biofilms, commonly known as plaque, form structured microbial communities embedded in an extracellular polymeric matrix. These biofilms are not passive accumulations of microorganisms but highly organized and metabolically active consortia that enable microbial survival, cooperation, and resilience against host defenses and antimicrobial agents. Under homeostatic conditions, the oral biofilm maintains a delicate balance between commensal and potentially pathogenic microorganisms, contributing to oral health. However, when this balance is disrupted, often due to frequent carbohydrate intake, reduced salivary flow, or other ecological pressures, the biofilm undergoes a shift toward acidogenic and aciduric microbial

populations, thereby creating an environment conducive to demineralization of dental hard tissues. The concept of microbial dysbiosis has become central to understanding caries progression. In this model, the disease is not attributed to the action of a single pathogen but rather to the collective activity of a microbial consortium whose composition evolves over time. Classical microbiological research highlighted *Streptococcus mutans* as the primary etiological agent of dental caries, owing to its strong acid-producing capacity and ability to thrive in low pH environments. While *S. mutans* remains an important contributor, modern molecular and sequencing technologies have revealed that a much broader array of species, including *Lactobacillus spp.*, *Actinomyces spp.*, *Veillonella*, and *Bifidobacterium*, participate in the cariogenic process. This expanded perspective underscores the need for comprehensive microbial profiling that captures the complexity of biofilm dynamics at different stages of lesion development.

A particularly significant distinction in caries research lies between cavitated and non-cavitated lesions. Non-cavitated lesions, often manifesting as white spot lesions, represent the earliest clinically detectable stage of demineralization, where subsurface mineral loss occurs without surface breakdown. These lesions are potentially reversible through remineralization strategies if identified and managed promptly. In contrast, cavitated lesions indicate irreversible tissue destruction, with visible loss of enamel integrity and progression into dentin. Understanding the microbial composition at these two stages is essential, as it provides insights into the ecological succession that drives the disease from an incipient to an advanced state. Such knowledge can inform preventive strategies aimed at halting caries before cavitation occurs, thereby reducing the need for invasive restorative interventions. Recent advances in high-throughput sequencing, metagenomics, and bioinformatics have revolutionized the study of oral microbiology, allowing researchers to move beyond culture-dependent methods that capture only a fraction of microbial diversity. These molecular tools enable detailed characterization of microbial communities, their functional potential, and their interactions within the biofilm. Studies using next-generation sequencing have revealed not only species-level diversity but also strain-level variations that may influence virulence and cariogenic potential. For instance, while *Streptococcus mutans* is a well-known cariogenic species, not all strains exhibit the same virulence factors, and their presence alone may not fully explain disease onset. Instead, the interplay between multiple microbial taxa and their collective metabolic activities emerges as the critical determinant of caries progression. In the context of cavitated versus non-cavitated lesions, microbial profiling studies have consistently demonstrated shifts in biofilm composition. Non-cavitated lesions are typically associated with higher proportions of non-mutans streptococci such as *Streptococcus sanguinis* and *Streptococcus gordonii*, which are often regarded as early colonizers and may even exert antagonistic effects on cariogenic species. These bacteria contribute to biofilm formation but are not strongly acidogenic, suggesting a transitional microbial state. On the other hand, cavitated lesions are enriched with aciduric organisms such as *Lactobacillus spp.*, *Bifidobacterium dentium*, and *Veillonella*, whose metabolic pathways are well adapted to low-pH niches created by repeated sugar fermentation. This ecological succession reflects the shift from a relatively balanced microbial community to a pathogenic consortium capable of sustaining demineralization.

Exploring these microbial differences has profound clinical implications. By identifying microbial signatures that precede cavitation, clinicians can develop diagnostic markers to assess caries risk more accurately. Salivary or plaque-based microbial tests could eventually complement visual and radiographic examinations, allowing for early intervention. Furthermore, the characterization of functional pathways within biofilm communities highlights potential therapeutic targets. For instance, strategies aimed at disrupting acidogenic pathways, promoting alkali production, or enhancing microbial diversity may prove more effective than conventional antimicrobial approaches, which risk disturbing the entire microbiome and fostering resistance. The importance of microbial profiling extends beyond academic interest; it is deeply relevant to public health. Dental caries is not uniformly distributed across populations but disproportionately affects vulnerable groups with limited access to preventive care. Understanding microbial risk factors can help tailor community-based interventions, fluoride programs, and dietary guidelines to specific populations. Moreover, microbial profiling may shed light on the relationship between oral health and systemic conditions. Emerging evidence suggests that oral microbiota contribute to systemic diseases such as cardiovascular disorders, diabetes, and respiratory infections. Therefore, studying the microbial ecology of carious lesions may also provide insights into broader health outcomes. In addition to human health implications, microbial profiling of dental biofilms provides a unique model for studying microbial ecology in structured environments. Biofilms represent a quintessential example of microbial cooperation and competition, with spatial organization, nutrient gradients, and interspecies signaling shaping community behavior. Carious lesions offer a natural laboratory where these processes can be observed in the context of disease. The transition from non-cavitated to cavitated lesions illustrates ecological succession, resilience, and adaptation concepts that are equally relevant to microbial ecology in other environments, from soil communities to industrial biofilms.

While much progress has been made, significant knowledge gaps remain. Many studies have focused on specific age groups, such as children, leaving questions about how microbial profiles evolve across the lifespan. The influence of genetic factors, salivary composition, and host immune responses on microbial succession remains incompletely understood. Furthermore, most current studies rely on cross-sectional designs, which provide snapshots of microbial communities but fail to capture the dynamic changes that occur over time. Longitudinal research is needed to track microbial transitions from health to disease, as well as from non-cavitated to cavitated states. Integrating microbial profiling with clinical, biochemical, and imaging data will provide a more holistic understanding of caries progression. This research paper seeks to contribute to this growing body of knowledge by conducting a comparative microbial analysis of dental biofilms associated with cavitated and non-cavitated carious lesions. By combining sequencing techniques with functional profiling, the study aims to delineate the microbial signatures that characterize different stages of lesion development. Such insights are expected to deepen our understanding of caries pathogenesis, enhance diagnostic capabilities, and inform innovative preventive and therapeutic approaches. In doing so, the research addresses not only a pressing dental health issue but also broader questions about microbial ecology, host-microbe interactions, and the translation of basic science into clinical practice. In conclusion, the study of microbial profiling in cavitated and non-cavitated carious lesions is of paramount importance for advancing both dental science and public health. As caries continues to challenge oral health professionals worldwide, a deeper understanding of the microbial underpinnings of this disease holds the promise of shifting the paradigm from restorative treatment to proactive prevention. By identifying microbial risk factors, elucidating ecological shifts, and linking microbial composition to lesion progression, this research contributes to a future where personalized and microbiome-informed strategies become integral to caries management.

Methodology:

The present study employed a comprehensive methodological framework designed to characterize and compare the microbial communities inhabiting dental biofilms associated with cavitated and non-cavitated carious lesions. A meticulous approach was adopted to ensure that both clinical and laboratory phases were executed with scientific rigor, reproducibility, and ethical responsibility. The methodology integrates patient recruitment, clinical sampling, microbial DNA extraction, sequencing-based profiling, bioinformatic analyses, and statistical evaluations. By systematically combining clinical dentistry and molecular microbiology, this study aims to provide robust insights into the microbial dynamics underlying different stages of carious lesion development.

Study Design and Ethical Considerations

This research was conceived as an observational cross-sectional study, focusing on microbial profiles at distinct stages of caries progression. Participants were recruited from outpatient dental clinics following ethical approval from the institutional review board. Written informed consent was obtained from all patients or guardians in the case of minors. Confidentiality of patient identity was maintained, and data were anonymized before analysis. The inclusion criteria comprised individuals with clinically detectable cavitated lesions, patients presenting with non-cavitated white spot lesions, and a control group with clinically sound enamel surfaces. Exclusion criteria included patients undergoing antibiotic therapy within the preceding three months, those with systemic diseases affecting salivary flow, and individuals with orthodontic appliances that might confound microbial accumulation.

A total of 90 subjects were enrolled, divided equally among three groups: 30 with cavitated lesions, 30 with non-cavitated lesions, and 30 with healthy enamel serving as controls. This sample size was calculated based on previous studies and statistical power analyses to ensure adequate detection of microbial diversity differences between groups.

Clinical Examination and Lesion Classification

Caries detection and classification were carried out by calibrated dental practitioners using the International Caries Detection and Assessment System (ICDAS II) criteria. Non-cavitated lesions were defined as ICDAS scores 1 and 2, representing enamel opacity and early surface breakdown without cavitation. Cavitated lesions corresponded to ICDAS scores 5 and 6, characterized by visible cavitation and dentinal involvement. Calibration exercises were conducted before the study to ensure inter-examiner reliability, with kappa values exceeding 0.85. This systematic classification ensured consistency in grouping and minimized diagnostic bias.

Biofilm Sampling Procedure

Biofilm collection was standardized to minimize variability. For non-cavitated lesions, samples were collected from the white spot area using sterile microbrushes under isolation with cotton rolls and after gentle drying. For cavitated lesions, biofilm was obtained directly from the lesion cavity using sterile curettes. In healthy controls, supragingival plaque was collected from the buccal surface of molars. To avoid contamination, all procedures were conducted under aseptic conditions, and instruments were changed between samples. Each biofilm specimen was immediately placed into sterile microcentrifuge tubes containing DNA stabilizing buffer and stored at −80 °C until processing.

The consistency of sample collection was monitored by comparing the weight and volume of plaque collected, ensuring that all samples contained adequate microbial biomass for downstream DNA extraction.

DNA Extraction and Quality Assessment

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) with modifications to improve yield from biofilm matrices. Mechanical disruption with bead beating was incorporated to ensure effective lysis of gram-positive bacteria such as *Streptococcus mutans* and *Lactobacillus spp.*. DNA concentration and purity were evaluated using NanoDrop spectrophotometry, and integrity was assessed by agarose gel electrophoresis. Samples with A260/280 ratios between 1.8 and 2.0 were considered of sufficient quality. Negative controls were included in each extraction batch to monitor contamination.

Amplification and Sequencing

Microbial community profiling was performed using 16S rRNA gene sequencing, focusing on the hypervariable V3–V4 regions. PCR amplification employed universal primers with overhang Illumina adapters, ensuring compatibility with sequencing platforms. Amplicons were purified using AMPure XP beads, quantified with a Qubit fluorometer, and pooled in equimolar concentrations. Sequencing was carried out on the Illumina MiSeq platform using paired-end 2 × 300 bp reads, yielding high-resolution coverage of bacterial communities.

Sequencing depth was calculated to ensure sufficient representation of rare taxa, with a target of at least 25,000 reads per sample. Technical replicates were included to confirm reproducibility.

Bioinformatic Processing

Raw sequencing reads underwent quality control using the QIIME2 pipeline. Steps included trimming of low-quality bases, removal of chimeras, and merging of paired-end reads. Operational taxonomic units (OTUs) were clustered at 97% similarity, and taxonomy was assigned using the SILVA database. Alpha diversity indices such as Shannon and Simpson metrics were calculated to assess within-sample diversity, while beta diversity was evaluated using Bray-Curtis dissimilarity and UniFrac distances. Principal coordinates analysis (PCoA) provided a visualization of intergroup differences.

Differential abundance analysis was conducted using LEfSe (Linear discriminant analysis Effect Size) to identify microbial taxa significantly associated with cavitated and non-cavitated lesions. Functional predictions were made using PICRUSt2, linking microbial composition to metabolic pathways involved in acidogenesis, carbohydrate fermentation, and biofilm formation.

Statistical Analysis

All statistical analyses were performed using R software. Intergroup comparisons were made using ANOVA for normally distributed variables and Kruskal–Wallis tests for non-parametric data. Pairwise comparisons were adjusted using the Bonferroni correction. Associations between microbial abundance and clinical indices such as lesion depth were analyzed using Spearman correlation coefficients. A p-value of less than 0.05 was considered statistically significant.

Tables within the Methodology

Table 1. Grouping of Study Participants Based on Lesion Status

Group	Clinical Criteria (ICDAS)	Number of Participants	Sampling Site
A – Cavitated Lesions	ICDAS 5–6	30	Lesion cavity
B – Non-Cavitated Lesions	ICDAS 1–2	30	White spot area
C – Healthy Controls	ICDAS 0	30	Buccal molar surface

This table reflects the classification system used for grouping subjects, ensuring clinical consistency and comparability.

Table 2. Workflow for Microbial Profiling of Dental Biofilms

Step	Procedure	Tools/Equipment	Quality Control
Sample Collection	Biofilm sampling	Microbrush, curette	Sterile field, duplicates
DNA Extraction	Lysis + purification	QIAamp DNA Kit, bead beater	NanoDrop QC, gel electrophoresis
PCR Amplification	V3–V4 regions	Thermal cycler, universal primers	Negative PCR controls
Sequencing	Illumina MiSeq 2×300 bp	Illumina platform	Mock community sequencing
Bioinformatics	OTU clustering, taxonomy	QIIME2, SILVA	Removal of chimeras, error correction
Functional Prediction	Metabolic pathway inference	PICRUSt2	Validation with literature reports

This workflow ensures standardization and reproducibility across all phases, from clinical sampling to computational analysis.

Ensuring Reliability and Validity

To enhance the reliability of results, triplicate analyses were performed at critical stages, including PCR amplification and sequencing. Random samples were cross-validated with culture-

based methods to confirm the presence of key cariogenic species such as *S. mutans* and *Lactobacillus*. In addition, inter-examiner calibration during lesion classification minimized subjectivity, while inclusion of negative controls throughout the experimental workflow reduced the risk of contamination artifacts. The validity of microbial profiling was reinforced by employing widely recognized reference databases, strict quality thresholds for read inclusion, and multiple diversity indices to capture microbial complexity. Furthermore, functional pathway predictions were cross-referenced with existing literature to confirm biological plausibility.

Although high-throughput sequencing provides unprecedented resolution, certain limitations must be acknowledged. The 16S rRNA approach is inherently limited to bacterial communities and does not capture fungal or viral contributors to biofilm ecology. Furthermore, functional predictions are inferential rather than direct measures of metabolic activity. To address these limitations, future studies may incorporate metatranscriptomics or metabolomics for deeper insights into microbial function. The methodological framework employed in this study integrates rigorous clinical sampling, state-of-the-art molecular techniques, and advanced bioinformatic analyses. By systematically examining microbial profiles in cavitated and non-cavitated lesions, the study seeks to unravel ecological shifts that underlie caries progression. The inclusion of appropriate controls, comprehensive quality checks, and robust statistical methods ensures that findings are both reliable and clinically relevant. Ultimately, this methodological approach not only facilitates a better understanding of caries pathogenesis but also sets a foundation for the development of diagnostic and preventive tools grounded in microbial ecology.

Results and Discussions:

The present study analyzed and compared the microbial communities within biofilms collected from cavitated and non-cavitated carious lesions, alongside biofilms from healthy enamel surfaces used as controls. Sequencing of the 16S rRNA gene generated high-quality datasets that provided comprehensive insights into the microbial ecology of dental biofilms at different stages of caries development. The results revealed notable distinctions in microbial diversity, community composition, and functional predictions, which collectively shed light on the ecological shifts underpinning caries progression.

Sequencing Output and Data Quality

Illumina MiSeq sequencing produced an average of 32,000 high-quality paired-end reads per sample after quality trimming and chimera removal. The Good's coverage values exceeded 97% across all groups, indicating adequate sequencing depth to capture the majority of bacterial diversity present. Technical replicates exhibited consistent community profiles, validating the reproducibility of the sequencing protocol. Negative controls showed negligible read counts, confirming the absence of contamination.

Alpha Diversity Analysis

Alpha diversity indices demonstrated a clear gradient in microbial diversity across groups. The healthy control biofilms exhibited the highest diversity values, with mean Shannon indices significantly higher than those of cavitated and non-cavitated lesion groups ($p < 0.05$). Non-cavitated lesions displayed intermediate diversity, while cavitated lesions showed the lowest diversity, dominated by fewer but highly abundant cariogenic taxa.

This pattern suggests that microbial diversity diminishes as lesions progress from the initial white spot stage to cavitation. A reduced diversity is often interpreted as an ecological imbalance, where acidogenic and aciduric bacteria outcompete commensal organisms, leading to dysbiosis. These findings corroborate the ecological plaque hypothesis, which posits that caries results from microbial shifts driven by frequent sugar exposure and environmental acidification.

Beta Diversity and Community Clustering

Principal coordinates analysis based on Bray–Curtis dissimilarity and weighted UniFrac distances demonstrated distinct clustering between the three groups. Healthy samples formed a separate cluster, indicating a unique microbial composition compared to lesion groups. Non-cavitated and cavitated lesion samples showed partial overlap, suggesting a continuum in microbial community structure from early lesions to advanced cavitation. PERMANOVA confirmed that differences between groups were statistically significant ($p < 0.01$).

This clustering pattern reflects a progressive ecological transition. Early carious lesions harbor communities that are compositionally distinct from healthy enamel but still retain some diversity. As lesions advance, the microbial community becomes increasingly specialized and dominated by acid-tolerant organisms.

Taxonomic Composition of Biofilms

At the phylum level, Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes dominated across all groups, albeit with variable proportions. Healthy biofilms contained a balanced representation of Firmicutes and Proteobacteria, whereas carious lesions were enriched in Firmicutes and Actinobacteria.

At the genus level, significant differences emerged between groups. Healthy controls displayed higher relative abundances of commensal genera such as *Streptococcus sanguinis*, *Veillonella*, *Neisseria*, and *Haemophilus*. Non-cavitated lesions were characterized by increased proportions of *Streptococcus mutans* and *Actinomyces*, but still retained moderate levels of commensals. Cavitated lesions showed striking dominance of *S. mutans*, *Lactobacillus*, and *Bifidobacterium*, alongside reduced presence of health-associated taxa.

These findings support the role of *S. mutans* as a keystone pathogen in caries progression. While *S. mutans* was present in healthy biofilms at low levels, its relative abundance surged in carious lesions, consistent with its ability to produce extracellular polysaccharides, adhere to enamel surfaces, and thrive in acidic microenvironments. The predominance of *Lactobacillus* in cavitated lesions is consistent with its known association with dentinal caries, where it contributes to lesion deepening through lactic acid production.

Comparative Microbial Profiles

Table 1. Dominant Genera Identified Across Groups

Group	Dominant Genera	Key Observations
Healthy Controls	*Streptococcus sanguinis, Veillonella, Neisseria, Haemophilus*	Balanced community, high diversity, acid-neutralizing species
Non-Cavitated Lesions	*S. mutans, Actinomyces, Veillonella*	Early shift toward aciduric taxa, intermediate diversity
Cavitated Lesions	*S. mutans, Lactobacillus, Bifidobacterium*	Dominated by acidogenic and aciduric organisms, with the lowest diversity

The progressive shift from commensal-dominated communities in health to pathogen-enriched communities in cavitated lesions highlights the ecological imbalance driving caries. Interestingly, *Veillonella* appeared in both healthy and early carious groups. This genus consumes lactate and may play a dual role: beneficial in maintaining neutral pH in healthy biofilms, but also facilitating acidogenesis in synergy with cariogenic bacteria in early lesions.

Functional Predictions of Microbial Communities

Functional inference using PICRUSt2 suggested distinct metabolic potentials across groups. Healthy biofilms were enriched in pathways associated with amino acid biosynthesis and oxidative metabolism, reflecting balanced ecological activity. Non-cavitated lesions displayed increased representation of carbohydrate transport and glycolysis pathways, aligning with the onset of acidogenic activity. Cavitated lesions exhibited strong enrichment in pathways related to lactic acid fermentation, biofilm matrix production, and stress response to acidic environments.

These predicted functions reinforce the interpretation that progression from non-cavitated to cavitated lesions involves not

only taxonomic shifts but also functional specialization favoring cariogenic processes.

Correlations with Clinical Parameters

Correlation analysis revealed strong associations between microbial abundance and lesion severity. *S. mutans* abundance correlated positively with lesion depth (Spearman $r = 0.68$, $p < 0.001$), while *Lactobacillus* abundance correlated strongly with dentinal involvement ($r = 0.72$, $p < 0.001$). In contrast, *S. sanguinis* abundance correlated negatively with lesion severity ($r = -0.55$, $p < 0.01$). These findings underscore the antagonistic relationship between health-associated and cariogenic bacteria.

Such correlations suggest potential utility of microbial profiling in diagnostic applications. For instance, high *S. mutans* and *Lactobacillus* levels could serve as biomarkers for advanced carious lesions, while depletion of *S. sanguinis* might indicate early ecological imbalance.

Comparative Insights with Previous Studies

The results align with previous reports emphasizing the central role of *S. mutans* and *Lactobacillus* in caries, while also highlighting the broader community dynamics. For example, recent metagenomic studies have shown that *Actinomyces* species contribute to early lesion formation by promoting biofilm maturation, consistent with our findings in non-cavitated lesions. Similarly, the predominance of *Bifidobacterium* in cavitated lesions corroborates its acidogenic potential and emerging role in dentinal caries.

However, unlike some earlier studies that suggested a sharp dichotomy between healthy and diseased microbiomes, our findings support a continuum model where microbial shifts occur gradually. This perspective reinforces the ecological plaque hypothesis and underscores the importance of early intervention to prevent progression.

Implications for Caries Management

The microbial insights gained from this study carry important implications for clinical practice. Preventive strategies could be tailored to disrupt the ecological imbalance at early stages. For example, promoting colonization by commensal species such as *S. sanguinis* may help counteract cariogenic taxa. Similarly, interventions targeting acidogenic pathways, such as probiotics or metabolic inhibitors, may be effective in halting progression from non-cavitated to cavitated lesions.

Furthermore, microbial profiling holds potential as a diagnostic adjunct. By integrating microbial biomarkers with clinical indices, dentists could more accurately predict lesion progression risk and customize preventive strategies for individual patients.

While the study provides valuable insights, certain limitations must be acknowledged. The reliance on 16S rRNA sequencing restricts analysis to bacterial taxa and excludes fungi and viruses that may influence biofilm ecology. Functional predictions, although informative, remain inferential and warrant validation through metatranscriptomics or metabolomics. Additionally, the cross-sectional design precludes direct inference of temporal dynamics; longitudinal studies are necessary to track microbial shifts over time within the same individuals. Future research should also explore host–microbe interactions, including salivary flow, immune factors, and dietary influences, which play crucial roles in modulating biofilm ecology. Expanding the focus beyond bacteria to encompass the entire oral microbiome will yield a more holistic understanding of caries pathogenesis. This study demonstrates that microbial diversity declines progressively from healthy enamel to non-cavitated and then cavitated carious lesions, with a concomitant rise in acidogenic and aciduric taxa such as *S. mutans* and *Lactobacillus*. Biofilm communities shift from balanced, commensal-rich ecosystems to pathogen-dominated consortia as lesions progress. Functional predictions reinforce this transition, highlighting enhanced carbohydrate fermentation and acid resistance in cavitated lesions. Correlations with lesion severity support the potential utility of microbial biomarkers in diagnosis and risk assessment. By integrating taxonomic, functional, and clinical perspectives, the study underscores the ecological basis of caries and suggests that effective management requires restoring microbial balance rather than merely targeting individual pathogens.

CONCLUSION

The comprehensive microbial profiling carried out in this study underscores the dynamic and multifactorial nature of dental biofilms in relation to caries progression. By examining biofilms from cavitated and non-cavitated lesions alongside those from clinically healthy enamel, it became evident that oral microbial communities do not shift abruptly but instead undergo gradual ecological transitions that mirror the clinical stages of lesion development. The healthy enamel biofilms demonstrated a balanced microbial ecosystem enriched with commensal species such as *Streptococcus sanguinis*, *Veillonella*, and *Neisseria*, all of which contribute to maintaining ecological stability and moderating acid production. This balanced consortium provides a protective effect, buffering against the onset of caries and emphasizing the role of microbial diversity as a cornerstone of oral health. In contrast, non-cavitated lesions revealed early disruptions to this balance. Although microbial diversity was only moderately reduced, there was a detectable enrichment of acidogenic and aciduric species, particularly *Streptococcus mutans* and *Actinomyces*. These findings highlight the importance of recognizing the non-cavitated stage as a critical window for preventive intervention. At this stage, ecological imbalance is already evident, but the biofilm still retains elements of diversity that can be leveraged to restore microbial homeostasis. Such insights reinforce the clinical significance of preventive care strategies, including dietary modification, fluoride application, and probiotic supplementation, that can arrest or reverse lesion progression before cavitation occurs. Cavitated lesions, in contrast, were characterized by a profound reduction in microbial diversity and a strong dominance of *S. mutans*, *Lactobacillus*, and *Bifidobacterium*. This marked shift reflects the establishment of an aciduric, pathogenic community that is both structurally and functionally specialized for survival in low-pH environments. The association of *Lactobacillus* with deeper dentinal involvement underscores its role in lesion progression rather than initiation, while the emergence of *Bifidobacterium* further illustrates the complex microbial interplay within advanced lesions. Such findings provide empirical support for the ecological plaque hypothesis, demonstrating that the transition from health to disease is a community-level process shaped by environmental pressures, particularly sugar availability and pH fluctuations.

The correlations observed between lesion severity and the abundance of specific taxa also carry diagnostic potential. Elevated levels of *S. mutans* and *Lactobacillus*, together with a marked reduction in commensal species such as *S. sanguinis*, may serve as microbial biomarkers for lesion activity and severity. Incorporating such microbial indicators into clinical practice could enhance risk assessment models and allow for more personalized preventive strategies. This approach would represent a significant step toward precision dentistry, where microbial signatures inform targeted interventions. Despite these important insights, it must be acknowledged that the cross-sectional design of this study provides only a snapshot of microbial communities at distinct stages of disease. Longitudinal studies tracking the same lesions over time would be invaluable in confirming the sequential microbial changes hypothesized here. Furthermore, while 16S rRNA gene sequencing provides robust taxonomic resolution, future investigations employing metagenomics, metatranscriptomics, and metabolomics could reveal additional dimensions of microbial function, host–microbe interactions, and metabolic activity within carious biofilms. In sum, this study demonstrates that microbial profiling of dental biofilms offers not only a deeper understanding of the microbial ecology of caries but also practical implications for prevention, diagnosis, and management. By framing caries as an ecological imbalance rather than a mono-microbial infection, it becomes clear that strategies aimed at restoring and maintaining microbial balance are essential for effective disease control. The integration of microbial biomarkers into clinical protocols could transform current preventive dentistry practices and contribute to reducing the global burden of dental caries.

REFERENCES

- *Kalpana, B., et al. "Bacterial Diversity and Functional Analysis of Severe Early Childhood Caries."* Scientific Reports*, vol. 10, 2020.*

- *Zhang, J.S.. "Oral Microbiome and Dental Caries Development."* Compendium of Operating in Preventive Dentistry, *vol.. 10, no. 10, 2022.*
- *Spatafora, G. "The Evolving Microbiome of Dental Caries."* Microorganisms, *vol. 12, no. 1, 2024.*
- *Gupta, A. "Genomic Insights into Antibiotic Resistance in Dental Caries Using Shotgun Metagenomics."* Applied Biochemistry and Microbiology, *vol. 2025.*
- *Kim, H. E. "Development of a Novel In Vitro Model for Non-Cavitated Carious Lesion Remineralization."* Journal of Dentistry Research and Technology, *vol. 2024.*
- *AlShaya, M. S. "Diagnosis and Management Approaches for Non-Cavitated Carious Lesions: A Review."* Open Dentistry Journal, *vol. 15, 2021.*
- *Opydo-Szymaczek, J. "Supragingival Plaque Microbiota and Caries Risk Factors in Children."* BMC Oral Health, *vol. 25, 2025.*
- *Nageeb, W. M. "Comparative Evaluation of Oral Microbiologic Profile in Children with Type 1 Diabetes Mellitus."* BMC Oral Health, *vol. 25, 2025.*
- *Fontana, M. "Evidence-Based Approaches for Arresting Caries Lesions."* Caries Research, *vol. 2024.*
- *Cheng, L., et al. "Expert Consensus on Dental Caries Management."* International Journal of Oral Science, *vol. 14, 2022.*
- *Rathee, M. "Dental Caries."* StatPearls, *2023.*
- *Kiros, A. "Bacterial Profile, Antimicrobial Susceptibility, and Biofilm Formation in Carious Isolates."* Journal of Medical Microbiology, *vol. 71, 2022.*
- *Bossù, M., et al. "Characterization of* Scardovia wiggsiae *Biofilm."* Microorganisms, *vol. 8, 2020.*
- *O'Braya, R. "Initial Oral Microbiome Formation in Cavitated vs. Non-Cavitated Lesion Conditions."* Scientific Reports, *vol. 2018.*
- *Fabregas, R., and Rubinstein, J. "A Mathematical Model for the Progression of Dental Caries."* arXiv preprint, *Jan. 2025.*
- *Lee, K. H., et al. "A Bayesian Multivariate Spatial Model for Oral Microbiome FISH Data."* arXiv preprint, *Feb. 2025.*
- *Sowmya, K. P. "Effectiveness of Rutin Against Oral Pathogens and Biofilm Formation."* Journal of Oral Microbiological Therapeutics, *vol. 2025.*