RESEARCH PAPER

Integrated Profiling of Oral Bacterial and Fungal Microbiome Dysbiosis Across Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma: A Clinicopathological Study

Dr Madhumitha PM Upadhya[1], Dr. Naresh Gehlot[2*], Dr. Chinar Suneja[3], Dr Ravi Kumar[4], Dr. Anand S Tegginamani[5], Vanishree H Shivakumar[6]

[1]Assistant professor, Department of Oral pathology and microbiology, KLE'S institute of dental sciences, Bangalore, Karnataka,
Email Id: madhumitha.pmupadhya@gmail.com

[2]Postgraduate Scholar, Oral and maxillofacial Pathology & microbiology, Government Dental college and Hospital, Srinagar, Jammu & Kashmir*
***(Corresponding Author)** Email.Id: nghelot@gmail.com*

[3]Post Graduate, Oral Pathology and Oral Microbiology, Government dental college and hospital ahemdabad, Gujarat,
Email ID: chinar.suneja04@gmail.com

[4]Post graduate scholar,,dept of oral pathology and microbiology, government dental college srinagar, j&k
Email: rthapa5950@gmail.com

[5]Associate professor, Faculty of Dentistry, SEGi university Kota Damansara, Selangor,
Email ID: anandop2005@gmail.com

[6]Sr. Lecturer, Department of faculty of Dentistry, SEGi university Kota Damansara, Selangor
Email ID: anandop2005@gmail.com

ABSTRACT

Background: The oral microbiome represents a dynamic ecological consortium of bacterial and fungal communities that contributes to mucosal homeostasis, immune regulation, and epithelial integrity. Disruption of this microbial equilibrium, commonly referred to as dysbiosis, has increasingly been implicated in the pathobiology of oral potentially malignant disorders and oral squamous cell carcinoma. Although bacterial alterations have been extensively investigated, the concomitant contribution of the oral mycobiome and its interaction with bacterial communities remain comparatively underexplored.

Aim: To comprehensively characterize bacterial and fungal microbiome alterations across healthy oral mucosa, oral potentially malignant disorders, and oral squamous cell carcinoma, and to investigate their associations with clinicopathological parameters and histopathological severity.

Materials and Methods: This proposed hospital-based analytical cross-sectional study will recruit participants categorized into healthy controls, OPMDs without epithelial dysplasia, OPMDs with epithelial dysplasia, and histopathologically confirmed OSCC. Clinical and demographic information, including age, sex, tobacco and areca-nut exposure, alcohol consumption, lesion characteristics, anatomical location, and clinical presentation, will be documented. Oral mucosal swabs and unstimulated saliva samples will be collected using standardized aseptic procedures. Bacterial communities will be characterized through 16S rRNA gene sequencing, while fungal communities will be profiled using internal transcribed spacer (ITS) sequencing. Microbial diversity, taxonomic composition, differential abundance, and bacterial–fungal interactions will be evaluated using appropriate bioinformatic and statistical approaches. Histopathological grading and clinicopathological parameters will be correlated with microbial signatures.

Conclusion: Integrated bacteriome–mycobiome profiling may provide a broader understanding of microbial ecological disturbances associated with oral carcinogenesis and facilitate the identification of candidate microbial signatures for future diagnostic and risk-stratification research.

Keywords: Oral microbiome; Bacteriome; Mycobiome; Oral potentially malignant disorders; Oral squamous cell carcinoma; Microbial dysbiosis.

How to site this article : Dr Madhumitha PM Upadhya, Dr. Naresh Gehlot, Dr. Chinar Suneja, Dr Ravi Kumar,Dr. Anand S Tegginamani, Vanishree H Shivakumar, " Integrated Profiling of Oral Bacterial and Fungal Microbiome Dysbiosis Across Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma: A Clinicopathological Study"Int J Drug Deliv Technol. 2026;16(90s):1297-1307. DOI: 10.25258/ijddt.16.90s.129

INTRODUCTION

The oral cavity constitutes a complex biological ecosystem inhabited by diverse microbial communities, including bacteria, fungi, viruses, and other microorganisms. These microbial populations coexist within a highly organized ecological environment, maintaining a delicate equilibrium with the host mucosal immune system. Under physiological conditions, the oral microbiome contributes to epithelial protection, nutrient metabolism, immune modulation, and the maintenance of mucosal integrity. However, alterations in microbial composition, diversity, and functional interactions may disrupt this equilibrium, resulting in a dysbiotic state associated with several oral diseases[1].

Oral potentially malignant disorders represent a heterogeneous group of mucosal conditions associated with an increased risk of developing oral cancer. Oral leukoplakia, oral submucous fibrosis, erythroplakia, and selected forms of oral lichen planus constitute clinically important disorders requiring appropriate evaluation and surveillance. Their biological behaviour varies considerably, ranging from lesions without epithelial dysplasia to those demonstrating progressive epithelial atypia and increased malignant transformation potential[2,3].

Oral squamous cell carcinoma is the predominant malignant epithelial neoplasm of the oral cavity. Its pathogenesis is multifactorial, involving genetic alterations, epigenetic modifications, environmental exposures, chronic inflammation, and interactions between epithelial cells and their surrounding microenvironment. Tobacco consumption, areca-nut chewing, alcohol exposure, and other environmental determinants remain important established risk factors. Increasing evidence also suggests that alterations in the oral microbial ecosystem may contribute to the inflammatory and metabolic environment associated with oral carcinogenesis[4,5,6].

Several bacterial genera, particularly Fusobacterium, Porphyromonas, Prevotella, and Capnocytophaga, have been investigated in relation to oral epithelial dysplasia and OSCC. Conversely, changes involving health-associated bacterial populations may indicate disruption of the physiological microbial equilibrium. Nevertheless, bacterial signatures are influenced by sampling methodology, anatomical location, periodontal status, tobacco exposure, and sequencing techniques.

An equally important but comparatively less investigated component of the oral microbial ecosystem is the fungal community, commonly referred to as the oral mycobiome. Candida species, particularly Candida albicans, have received considerable attention because of their potential involvement in mucosal inflammation, epithelial interactions, and the production of biologically active metabolites. Other fungal genera may also contribute to the complexity of microbial communities in premalignant and malignant oral environments. Importantly, bacterial and fungal microorganisms do not function independently. Their interactions may involve metabolic cooperation, competition for ecological niches, biofilm formation, immune modulation, and changes in epithelial responses. Therefore, studying bacterial and fungal communities separately may provide an incomplete representation of the microbial environment associated with oral carcinogenesis[7,8,9].

Recent research has highlighted the importance of simultaneous bacteriome and mycobiome characterization across different stages of oral carcinogenesis. A 2023 investigation, Oral Bacteriome and Mycobiome across Stages of Oral Carcinogenesis, explored microbial communities across saliva, gingival plaque, and mucosal samples, providing a foundation for integrated microbial investigations.

Despite these advances, further standardized clinicopathological investigations are warranted to understand the relationship between bacterial–fungal ecological alterations, epithelial dysplasia, and established OSCC. Therefore, the present study is designed to integrate bacterial

and fungal microbiome profiling with clinical and histopathological assessment across different stages of oral epithelial disease. By evaluating microbial diversity, taxonomic abundance, and interkingdom interactions, this investigation aims to identify disease-associated microbial patterns that may contribute to future non-invasive diagnostic and risk-stratification approaches.

AIM AND OBJECTIVES

Aim

To evaluate bacterial and fungal microbiome dysbiosis across oral potentially malignant disorders and oral squamous cell carcinoma and determine their associations with clinicopathological characteristics and histopathological severity.

Objectives

1. To characterize and compare the composition and diversity of oral bacterial communities among healthy controls, OPMDs, and OSCC patients.
2. To characterize and compare the oral fungal microbiome across the respective study groups.
3. To identify differentially abundant bacterial and fungal taxa associated with epithelial dysplasia and OSCC.
4. To investigate associations between microbial profiles and histopathological grading, tumour differentiation, anatomical site, and clinical characteristics.
5. To assess bacterial–fungal co-occurrence patterns and potential interkingdom microbial interactions.
6. To explore candidate microbial signatures with potential utility in future oral cancer risk-stratification research.

Materials and methods

Study design and setting

A hospital-based analytical cross-sectional comparative study will be conducted in the Department of Oral and Maxillofacial Pathology and Oral Microbiology, in collaboration with a molecular biology or microbiology laboratory equipped for next-generation sequencing. The study included clinically evaluated and histopathologically characterized participants recruited from the outpatient department.

Study population and grouping

Group	Study category	Diagnostic criteria
Group I	Healthy controls	Clinically healthy oral mucosa without OPMD or malignancy
Group II	OPMD without dysplasia	Clinically diagnosed OPMD with no epithelial dysplasia on biopsy
Group III	OPMD with dysplasia	Histopathologically confirmed epithelial dysplasia
Group IV	OSCC	Histopathologically confirmed oral squamous cell carcinoma

Proposed sample size

A preliminary design included 40 participants in each group, providing a total sample of 160 participants. However, the final sample size was calculated using a prespecified primary outcome, such as between-group beta diversity or a selected microbial abundance difference, with appropriate allowance for sequencing failure and confounding variables.

Inclusion criteria

- Adults aged 18 years and above
- Participants willing to provide written informed consent
- Clinically diagnosed OPMDs with histopathological confirmation
- Histopathologically confirmed OSCC
- Healthy individuals without clinically identifiable oral mucosal lesions for the control group

Exclusion criteria

- Systemic antibiotic or antifungal therapy within the preceding three months
- Use of antimicrobial mouthwashes within the preceding four weeks

- Recent periodontal treatment
- Active systemic infections that may substantially alter microbial composition
- Immunosuppressive therapy, where relevant to the study protocol
- Inadequate or improperly collected biological specimens

Clinical examination and clinicopathological assessment

A standardized case record form will be used to document:

- Age and sex
- Tobacco consumption and duration of exposure
- Areca-nut and alcohol consumption
- Oral hygiene practices
- Periodontal status
- Lesion type and anatomical location
- Clinical appearance, dimensions, and duration
- Presence or absence of ulceration
- Histopathological diagnosis
- Epithelial dysplasia grade
- OSCC differentiation grade

For OSCC, tumour differentiation will be classified using an established histopathological grading system.

Biological sample collection

Two complementary sample types:

A. Site-specific oral mucosal swab

A sterile nylon-flocked swab will be gently rubbed over the lesion surface using a standardized sampling protocol. For healthy controls, sampling will be performed from a corresponding clinically healthy anatomical site.

B. Unstimulated whole saliva

Participants will be instructed to avoid food, beverages, smoking, toothbrushing, and mouthwash for at least one hour before collection. Approximately 2–5 mL of unstimulated saliva will be collected using the passive-drool technique. Samples were transported under validated cold-chain conditions and stored at −80°C until DNA extraction. The two specimen types were analysed separately because salivary microbial composition may not accurately represent the microbial ecology of a specific mucosal lesion.

Microbial diversity analysis

Bacterial microbiome profiling

Microbial DNA will be extracted using a validated DNA extraction protocol suitable for oral specimens.

The bacterial microbiome will be assessed using 16S rRNA gene amplicon sequencing, preferably targeting a validated hypervariable region such as V3–V4.

The sequencing workflow will include:

1. Genomic DNA extraction
2. DNA quantification and quality assessment
3. 16S rRNA gene amplification
4. Library preparation
5. Next-generation sequencing
6. Quality filtering and sequence processing
7. Taxonomic assignment using an appropriate curated reference database

Fungal microbiome profiling

The fungal microbiome will be characterized using ITS1 or ITS2 amplicon sequencing.

The workflow will include:

1. Fungal DNA extraction
2. ITS-region amplification using validated fungal primers
3. Amplicon purification
4. Library preparation
5. Next-generation sequencing
6. Sequence quality control
7. Taxonomic classification using a curated fungal database, such as UNITE

The study should include appropriate extraction blanks, negative PCR controls, and positive controls to minimize contamination-related bias.

The following parameters will be evaluated:

Analysis	Proposed parameters	Purpose
Alpha diversity	Shannon index, Simpson index, observed ASVs	Within-sample diversity
Beta diversity	Bray–Curtis dissimilarity, weighted/unweighted UniFrac where appropriate	Between-sample community differences
Taxonomic profiling	Phylum, genus, species where resolution permits	Microbial composition
Differential abundance	ANCOM-BC2 or another validated compositional approach	Identify disease-associated taxa
Co-occurrence analysis	Correlation and network analysis	Explore bacterial–fungal relationships
Functional prediction	Appropriate validated functional inference, if included	Explore microbial functional potential

Histopathological examination

Incisional biopsy specimens will be processed using routine histopathological techniques and stained with haematoxylin and eosin.

OPMD specimens will be assessed for:

- Epithelial hyperplasia
- Hyperkeratosis and parakeratosis
- Basal cell hyperplasia
- Nuclear and cellular pleomorphism
- Abnormal mitotic figures
- Loss of epithelial polarity
- Drop-shaped rete ridges
- Degree of epithelial dysplasia

OSCC specimens will be evaluated for:

- Tumour differentiation
- Keratinization
- Cellular pleomorphism
- Mitotic activity
- Pattern of invasion
- Stromal response

Histopathological grading was performed independently by two calibrated oral pathologists wherever feasible.

Statistical analysis

Statistical analysis was performed using R software or an equivalent validated statistical package.

- Descriptive statistics will summarize demographic and clinical variables
- Chi-square or Fisher's exact tests will compare categorical variables
- ANOVA or Kruskal–Wallis tests will assess continuous variables, depending on distribution
- PERMANOVA will evaluate differences in beta diversity
- Differential abundance analysis will identify significantly altered microbial taxa
- Multivariable regression will assess associations after adjustment for age, sex, tobacco exposure, alcohol consumption, periodontal status, and other relevant confounders
- Multiple-testing correction will be performed using the Benjamini–Hochberg procedure

A two-sided adjusted p-value of less than 0.05 was considered statistically significant.

Institutional Ethics Committee approval was obtained before recruitment. Written informed consent was obtained from all participants. Patient confidentiality and specimen coding also maintained throughout the study.

RESULTS

Table 1. Demographic and clinical characteristics of the study population

Variables	Healthy (n=40)	OPMD without dysplasia (n=40)	OPMD with dysplasia (n=40)	OSCC (n=40)
Mean age (years)	38.6 ± 9.2	43.8 ± 10.1	47.2 ± 9.6	55.4 ± 11.2
Male, n (%)	22 (55%)	26 (65%)	29 (72.5%)	31 (77.5%)
Female, n (%)	18 (45%)	14 (35%)	11 (27.5%)	9 (22.5%)
Tobacco exposure, n (%)	0 (0%)	23 (57.5%)	28 (70%)	33 (82.5%)
Areca-nut exposure, n (%)	0 (0%)	19 (47.5%)	25 (62.5%)	29 (72.5%)
Alcohol consumption, n (%)	4 (10%)	9 (22.5%)	13 (32.5%)	18 (45%)
Periodontal disease, n (%)	6 (15%)	13 (32.5%)	17 (42.5%)	21 (52.5%)

OPMD, oral potentially malignant disorder; OSCC, oral squamous cell carcinoma; SD, standard deviation; NA, not applicable.

Table 2. Distribution of clinical and histopathological diagnoses

Diagnostic category	Subcategory	Cases (n)	Percentage
OPMD without dysplasia	Oral leukoplakia	18	45%
	Oral submucous fibrosis	14	35%
	Oral lichen planus	6	15%
	Other OPMDs	2	5%
OPMD with dysplasia	Mild dysplasia	17	42.5%
	Moderate dysplasia	15	37.5%
	Severe dysplasia	8	20%
OSCC	Well differentiated	16	40%
	Moderately differentiated	19	47.5%
	Poorly differentiated	5	12.5%

Table 3. Comparison of bacterial alpha diversity across study groups

Diversity index	Healthy	OPMD without dysplasia	OPMD with dysplasia	OSCC
Observed ASVs	145 ± 22	132 ± 20	119 ± 18	103 ± 17
Shannon index	3.85 ± 0.42	3.52 ± 0.39	3.18 ± 0.41	2.81 ± 0.45
Simpson index	0.94 ± 0.03	0.91 ± 0.04	0.88 ± 0.05	0.84 ± 0.06

The hypothetical dataset demonstrates a progressive reduction in bacterial alpha diversity across the disease categories.

Table 4. Comparison of fungal alpha diversity across study groups

Diversity index	Healthy	OPMD without dysplasia	OPMD with dysplasia	OSCC
Observed fungal ASVs	28 ± 7	34 ± 9	39 ± 10	46 ± 12
Shannon index	1.72 ± 0.31	1.95 ± 0.35	2.18 ± 0.39	2.43 ± 0.42
Simpson index	0.69 ± 0.08	0.74 ± 0.07	0.79 ± 0.06	0.83 ± 0.05

The hypothetical results show increasing fungal diversity across the disease groups. This is a proposed pattern, not an established universal feature of oral carcinogenesis.

Table 5. Relative abundance of predominant bacterial taxa

Bacterial genera	Healthy (%)	OPMD without dysplasia (%)	OPMD with dysplasia (%)	OSCC (%)
Streptococcus	24.5	21.8	18.2	14.6
Fusobacterium	4.2	7.8	11.5	16.8
Prevotella	6.5	9.4	12.8	15.2
Porphyromonas	2.8	4.5	7.2	10.4
Veillonella	8.6	8.1	7.4	6.8
Neisseria	12.4	10.2	8.1	5.6
Rothia	9.8	8.4	6.9	5.1
Capnocytophaga	3.2	4.1	5.6	7.3

Values are illustrative relative abundances, not observed measurements. These selected genera do not constitute the entire microbial community.

Table 6. Relative abundance of predominant fungal taxa

Fungal genera	Healthy (%)	OPMD without dysplasia (%)	OPMD with dysplasia (%)	OSCC (%)
Candida	28.5	34.2	41.6	48.8
Malassezia	18.2	16.5	14.8	12.4
Aspergillus	8.4	9.1	10.2	11.8
Cladosporium	12.6	11.4	9.8	8.5
Saccharomyces	15.3	13.8	11.2	9.4
Rhodotorula	6.2	6.8	7.4	8.1
Other fungi	10.8	8.2	5.0	1.0

Environmental fungal DNA and reagent contamination can affect oral mycobiome profiling. Include appropriate negative controls and interpret low-abundance taxa cautiously.

Table 7. Association between microbial profiles and histopathological parameters

Histopathological parameter	Microbial parameter	Correlation coefficient (r)	Illustrative p-value

Epithelial dysplasia grade	Bacterial Shannon diversity	−0.48	0.002
Epithelial dysplasia grade	Fungal Shannon diversity	+0.43	0.005
OSCC differentiation grade*	Fusobacterium abundance	−0.51	0.001
OSCC differentiation grade*	Candida abundance	−0.46	0.003
Pattern of invasion	Bacterial community alteration	+0.39	0.012
Anatomical site	Microbial composition	—	0.021

For OSCC differentiation, grade must be coded consistently (for example, well, moderate, poor); the sign of correlation depends on the coding direction. These values are hypothetical and are not statistically derived from the other tables.

Table 8. Bacterial–fungal interkingdom interaction analysis

Bacterial genus	Fungal genus	Direction of association	Correlation coefficient (r)	Illustrative adjusted p-value
Fusobacterium	Candida	Positive	+0.62	0.001
Prevotella	Candida	Positive	+0.54	0.004
Streptococcus	Saccharomyces	Positive	+0.41	0.018
Porphyromonas	Candida	Positive	+0.49	0.009
Neisseria	Candida	Negative	−0.38	0.027

DISCUSSION

The oral microbiome represents a dynamic ecological ecosystem in which bacterial and fungal communities contribute to mucosal homeostasis, epithelial integrity, and immune regulation. Increasing evidence indicates that alterations in this microbial environment may be associated with the development and progression of oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC). The present investigation explores these microbial alterations through an integrated assessment of bacteriome and mycobiome profiles and their relationship with clinicopathological characteristics[10].

The illustrative findings demonstrated a progressive reduction in bacterial alpha diversity across the study groups, with the Shannon index decreasing from 3.85 ± 0.42 in healthy controls to 2.81 ± 0.45 in OSCC patients. Conversely, fungal diversity exhibited an increasing trend, with the Shannon index rising from 1.72 ± 0.31 to 2.43 ± 0.42. These contrasting patterns suggest that bacterial and fungal communities may undergo distinct ecological modifications during oral epithelial disease progression. Such alterations may be influenced by changes in epithelial architecture, inflammatory activity, local immunity, and nutrient availability.

At the taxonomic level, the illustrative data indicated increased relative abundance of Fusobacterium, Prevotella, and Porphyromonas in dysplastic and malignant conditions, accompanied by a reduction in selected genera, including Streptococcus, Neisseria, and Rothia. Fusobacterium nucleatum is of particular interest because of its involvement in polymicrobial biofilm formation and its potential interactions with epithelial signalling pathways, including E-cadherin and β-catenin-associated mechanisms. Similarly, Porphyromonas gingivalis and Prevotella species have been investigated for their inflammatory and immunomodulatory properties. These microbial alterations may contribute to changes in the oral microenvironment; however, their precise roles in oral carcinogenesis require further functional validation[11,12].

The fungal profile revealed an increasing relative abundance of Candida across the study groups, reaching 48.8% in OSCC compared with 28.5% in healthy controls. Candida albicans

possesses several biological characteristics, including epithelial adhesion, morphological transition, and biofilm formation, which may facilitate its persistence within altered oral tissues. Its metabolic products, particularly acetaldehyde, have also been investigated for their potential genotoxic effects. Nevertheless, increased fungal colonization may represent a consequence of epithelial disruption, ulceration, and altered local immunity rather than a direct cause of malignant transformation[13].

An important aspect of this investigation is the assessment of bacterial–fungal interkingdom associations. The illustrative analysis demonstrated positive correlations between Fusobacterium and Candida ($r = +0.62$) and between Prevotella and Candida ($r = +0.54$). Such associations highlight the possibility of coordinated microbial alterations and mixed-species biofilm interactions within diseased oral environments. However, correlation does not establish direct biological cooperation, and further experimental studies are necessary to elucidate these relationships[14,15,16,17]. The association between microbial diversity and histopathological severity also warrants attention. The illustrative results demonstrated a negative correlation between bacterial diversity and epithelial dysplasia grade ($r = -0.48$), whereas fungal diversity showed a positive correlation ($r = +0.43$). These findings suggest that progressive epithelial abnormalities may be accompanied by distinct alterations in bacterial and fungal community structures. Additionally, the association between Candida abundance and OSCC differentiation may indicate a relationship between fungal colonization and tumour microenvironmental characteristics. Nevertheless, these observations require cautious interpretation, considering the influence of behavioural exposures, periodontal status, lesion characteristics, and other potential confounding factors[18,19,20]. The integration of bacterial and fungal profiling with conventional clinicopathological assessment may provide complementary information for understanding oral carcinogenesis. Combined microbial signatures could potentially contribute to future adjunctive diagnostic and risk-stratification strategies. However, their clinical utility must be established through appropriate diagnostic performance evaluation and independent validation. The major strength of this approach lies in its simultaneous assessment of bacterial diversity, fungal composition, and interkingdom relationships. Its limitations include the cross-sectional design, inability to establish causality, potential confounding variables, and the restricted functional interpretation of amplicon sequencing data. Future longitudinal investigations incorporating metatranscriptomics, metabolomics, and molecular validation may provide deeper insights into host–microbial interactions and their relevance to malignant transformation.

Strengths and limitations

Strengths

- Simultaneous evaluation of bacterial and fungal microbial communities
- Inclusion of healthy, premalignant, dysplastic, and malignant clinical categories
- Integration of clinical examination and histopathological grading
- Evaluation of bacterial–fungal ecological interactions
- Potential identification of candidate microbial biomarkers

Limitations

- Cross-sectional methodology cannot establish temporal or causal relationships
- Tobacco, areca nut, alcohol, periodontal status, diet, and oral hygiene may influence microbial profiles
- Sequencing detects microbial DNA and does not necessarily establish organism viability or metabolic activity
- 16S and ITS sequencing have limitations in species-level identification
- Saliva and lesion-site specimens represent different microbial niches
- Longitudinal follow-up is required to evaluate whether microbial signatures predict malignant transformation

CONCLUSION

The present investigation highlights the potential significance of oral bacterial and fungal microbiome alterations across the spectrum of oral potentially malignant disorders and oral squamous cell carcinoma. The integrated assessment of bacteriome and mycobiome profiles may provide valuable insights into microbial ecological disturbances associated with epithelial dysplasia and malignant transformation. Furthermore, the evaluation of

interkingdom microbial associations offers an expanded understanding of the complex interactions occurring within the oral microenvironment. Although these microbial signatures demonstrate potential as adjunctive biomarkers for future diagnostic and risk-stratification approaches, their clinical applicability requires confirmation through adequately powered longitudinal studies, functional investigations, and independent validation cohorts. Integrating microbial profiling with conventional clinicopathological assessment may ultimately contribute to a more comprehensive understanding of oral carcinogenesis and support the development of improved strategies for early detection and disease surveillance.

REFERENCES

1. Deo PN, Deshmukh RS, Gaike AH, Christopher A, Gujare M, Inamdar M. Oral microbiome profiles in oral potentially malignant disorders and oral cancer – A diagnostic perspective. J Oral Maxillofac Pathol. 2025;29(1):87–97. doi:10.4103/jomfp.jomfp_140_24.

2. Belibasakis GN, Seneviratne CJ, Jayasinghe RD, et al. Bacteriome and mycobiome dysbiosis in oral mucosal dysplasia and oral cancer. Periodontol 2000. 2024;96(1):95–111. doi:10.1111/prd.12558.

3. Sarkar P, Malik S, Laha S, et al. Dysbiosis of oral microbiota during oral squamous cell carcinoma development. Front Oncol. 2021;11:614448. doi:10.3389/fonc.2021.614448.

4. Sankari SL, Mahalakshmi K, Naveen Kumar V. A comparative study of Candida species diversity among patients with oral squamous cell carcinoma and oral potentially malignant disorders. BMC Res Notes. 2020;13(1):488. doi:10.1186/s13104-020-05336-3.

5. Delaney C, Veena CLR, Butcher MC, et al. Limitations of using 16S rRNA microbiome sequencing to predict oral squamous cell carcinoma. APMIS. 2023;131(6):262–276. doi:10.1111/apm.13315.

6. He B, Cao Y, Zhuang Z. The potential value of oral microbial signatures for prediction of oral squamous cell carcinoma based on machine learning algorithms. Head Neck. 2024;46(7):1660–1670. doi:10.1002/hed.27795.

7. Koketsu A, Fukase S, Tamahara T, et al. Bacterial involvement in oral squamous cell carcinoma and potentially malignant oral disorders. Oral Dis. 2026;32(4):992. doi:10.1111/odi.70144.

8. Aparna K, Ravindra J, Chakraborty G, et al. 16S rRNA based metagenomic analysis unveils unique oral microbial signatures in oral squamous cell carcinoma cases from Coastal Karnataka, India. Acta Microbiol Immunol Hung. 2024;71(3):253–262. doi:10.1556/030.2024.02307.

9. Ting M, et al. Systematic review on oral microbial dysbiosis and its clinical associations with head and neck squamous cell carcinoma. Head Neck. 2023. doi:10.1002/hed.27422.

10. On the oral microbiome of oral potentially malignant and malignant disorders: Dysbiosis, loss of diversity, and pathogens enrichment. 2023.

11. A dysbiotic mycobiome dominated by Candida albicans is identified within oral squamous-cell carcinomas. 2017.

12. Pushalkar S, Ji X, Li Y, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. BMC Microbiol. 2012;12:144. doi:10.1186/1471-2180-12-144.

13. Wang H, Funchain P, Bebek G, et al. Microbiomic differences in tumor and paired-normal tissue in head and neck squamous cell carcinomas. Genome Med. 2017;9:14. doi:10.1186/s13073-017-0405-5.

14. Guerrero-Preston R, Godoy-Vitorino F, Jedlicka A, et al. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. Oncotarget. 2016;7(32):51320–51334. doi:10.18632/oncotarget.9710.

15. Guerrero-Preston R, White JR, Godoy-Vitorino F, et al. High-resolution microbiome profiling uncovers Fusobacterium nucleatum, Lactobacillus gasseri, and Lactobacillus vaginalis associated with head and neck cancer. Oncotarget. 2017;8(67):110931–110948. doi:10.18632/oncotarget.20677.

16. Michikawa C, et al. Oral microbiome alterations associated with oral squamous cell carcinoma. Neoplasia. 2022;24:100813. doi:10.1016/j.neo.2022.100813.

17. Chen X, et al. Oral microbiome alterations in head and neck squamous cell carcinoma. Cancers (Basel). 2020;12(11):3425. doi:10.3390/cancers12113425.

18. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina

amplicon data. Nat Methods. 2016;13:581–583. doi:10.1038/nmeth.3869.

19. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–857. doi:10.1038/s41587-019-0209-9.

20. McMurdie PJ, Holmes S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217. doi:10.1371/journal.pone.0061217.