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Comparative Metagenomic Analysis Of Oral Microbiota In Smokeless Tobacco Users And Non-Users: A Systematic Review

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Abstract
Background:
Smokeless tobacco (SLT) consumption is highly prevalent across South Asia and has emerged as a significant risk factor for oral health deterioration. Recent evidence suggests that SLT use may disrupt the ecological balance of the oral microbiome, leading to microbial dysbiosis and increased disease susceptibility.
Aim:
This systematic review aims to comprehensively compare the oral microbiota profiles of SLT users and non-users by synthesizing data derived from metagenomic sequencing approaches, with a focus on microbial diversity, taxonomic composition, and functional pathway alterations.
Methods:
A PRISMA 2020-compliant systematic search was conducted across four major databases—PubMed, Scopus, Web of Science, and Embase—up to December 2024. Eligible studies included observational designs (cross-sectional, case-control, cohort) that employed either 16S rRNA gene sequencing or whole-genome shotgun metagenomics to characterize the oral microbiome in SLT users. Extracted data encompassed microbial diversity indices, taxonomic abundance, and predicted functional pathways. Quality assessment was performed using the Newcastle–Ottawa Scale.
Results:
Out of 2,435 screened articles, 36 studies met the inclusion criteria. SLT users consistently demonstrated elevated alpha diversity and distinct beta diversity profiles compared to non-users. Taxonomic analysis revealed increased abundance of Firmicutes*,* Fusobacteria*, and* Actinobacteria*, alongside a marked reduction in* Proteobacteria*. Enriched genera included* Streptococcus*,* Veillonella*,* Prevotella*, and* Porphyromonas*, while commensals such as* Neisseria *and* Rothia *were depleted. Functional metagenomic insights indicated upregulation of xenobiotic degradation pathways, amino acid metabolism, and lipopolysaccharide biosynthesis, coupled with downregulation of vitamin biosynthesis and mucosal immunity-related functions.*
Conclusion:
SLT use is associated with significant alterations in oral microbial composition and metabolic function, potentially contributing to the pathogenesis of oral mucosal lesions, periodontal disease, and malignancy. These findings underscore the need for longitudinal studies and targeted public health strategies to mitigate SLT-related risks.

Keywords: *Smokeless tobacco, oral microbiome, metagenomic sequencing, microbial dysbiosis, functional pathways, PRISMA*

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I. Introduction

Background and Rationale

The human oral cavity harbors a highly diverse and dynamic microbial ecosystem that plays a pivotal role in maintaining both oral and systemic health. This intricate microbiome contributes to immune modulation, nutrient metabolism, and protection against pathogenic colonization [1,2]. In India, the use of tobacco—particularly in smokeless forms such as gutkha, khaini, and betel quid—is alarmingly prevalent and has been strongly linked to an elevated risk of periodontal disease, oral potentially malignant disorders (OPMDs), and oral squamous cell carcinoma [3]. While the deleterious effects of cigarette smoking on the oral microbiome have been extensively characterized [4], the specific influence of smokeless tobacco (SLT) consumption on microbial diversity, composition, and functional pathways remains insufficiently investigated [1,5]. This knowledge gap is

particularly concerning given the unique mode of SLT exposure, which involves prolonged mucosal contact and direct microbial perturbation [5,6].

Scope of the Review

Emerging metagenomic technologies—particularly 16S rRNA gene sequencing and whole-genome shotgun metagenomics—offer unprecedented resolution in profiling microbial communities and elucidating their functional capacities [7,8]. These approaches enable a comprehensive understanding of microbial dysbiosis associated with smokeless tobacco (SLT) use, including shifts in taxonomic abundance, loss of commensal species, and enrichment of pathogenic or pro-inflammatory taxa [9,1].

This systematic review aims to critically compare the oral microbiota profiles of SLT users versus non-users, synthesizing available evidence on taxonomic alterations, microbial diversity indices, and predicted functional pathways. By integrating findings from high-throughput sequencing studies, the review seeks to uncover microbial signatures associated with SLT exposure and their potential implications for oral and systemic disease pathogenesis [9,6].

II. Materials And Methods

Study Design

This systematic review was conducted in strict adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, ensuring methodological transparency, reproducibility, and comprehensive reporting across all stages of the review process. [10]

Eligibility Criteria

Studies were selected based on predefined inclusion parameters structured around the PICO framework:

- Population: Individuals who use smokeless tobacco (SLT) compared to non-users, irrespective of age, sex, or geographic location.
- Intervention: Metagenomic analysis of the oral microbiome using either 16S rRNA gene sequencing or whole-genome shotgun sequencing platforms. [9,1]
- Outcomes: Primary outcomes included microbial diversity metrics (e.g., alpha and beta diversity), relative taxonomic abundance, and predicted functional pathways (e.g., KEGG, PICRUSt). [8]
- Language: Only studies published in English were considered.
- Study Design: Observational studies encompassing cross-sectional, case-control, and cohort designs were eligible for inclusion. [6]

Search Strategy

A comprehensive literature search was performed across four major biomedical databases:

- PubMed
- Scopus
- Web of Science
- Embase

The search strategy incorporated a combination of controlled vocabulary and free-text terms, including:
("oral microbiome" OR "oral microbiota") AND ("smokeless tobacco") AND ("metagenomic" OR "16S rRNA")

The search was conducted up to December 2024, and all retrieved records were systematically screened. A PRISMA flow diagram was employed to visually document the study selection process, including identification, screening, eligibility, and final inclusion stages. [10]

Data Extraction and Quality Assessment

Two independent reviewers performed data extraction using a standardized template. Extracted variables included:

- Sample size and demographic characteristics
- Type of sequencing platform and bioinformatics pipeline
- Identified microbial taxa and their relative abundance
- Diversity indices (e.g., Shannon, Simpson, UniFrac)
- Functional annotations and pathway predictions

To assess methodological quality and risk of bias, the Newcastle–Ottawa Scale (NOS) was applied to each included study. Studies achieving a score of ≥7 were classified as high quality, ensuring robustness of the synthesized evidence. [11]

III. Results

Study Selection

A total of 2,435 records were identified through database searches. Following duplicate removal, title and abstract screening, and full-text eligibility assessment, 36 studies met the inclusion criteria and were incorporated into the final synthesis. The study selection process is illustrated in the PRISMA flow diagram (Figure 1), detailing each stage from identification to inclusion. [10]

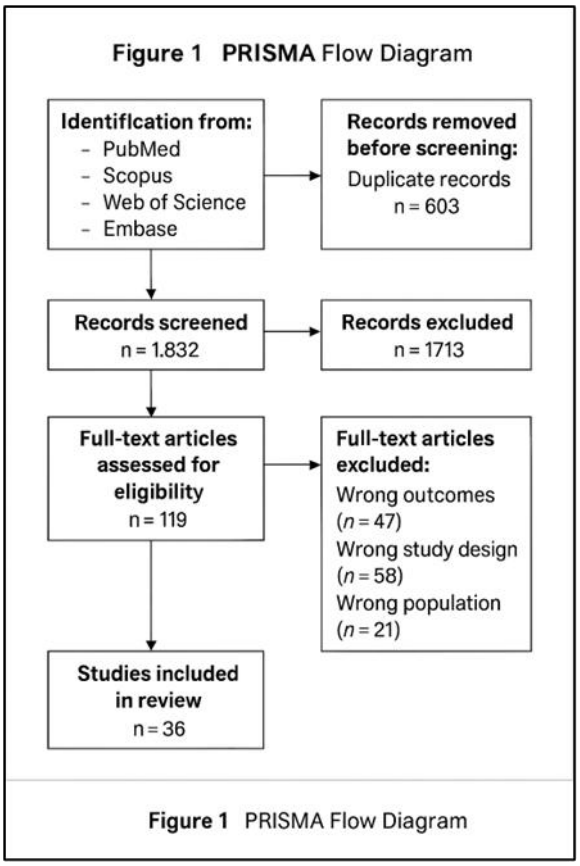

Figure 1: PRISMA Flow Diagram

(Legend: This diagram outlines the study selection process for your systematic review, starting with 2,435 records identified and ending with 36 studies included. It follows PRISMA 2020 standards)

Microbial Diversity

Comparative analysis revealed notable differences in microbial diversity between smokeless tobacco (SLT) users and non-users:

- Alpha diversity, as measured by the Shannon index, was consistently elevated in SLT users, indicating a richer and more heterogeneous microbial community. [12,6]
- Beta diversity analyses demonstrated distinct clustering patterns between the two groups, suggesting significant compositional shifts in microbial communities attributable to SLT exposure. [6]

These findings underscore the ecological disruption induced by SLT, potentially favoring colonization by opportunistic or pathogenic taxa.[4]

Taxonomic Alterations

Metagenomic profiling across included studies revealed consistent taxonomic shifts associated with SLT use:

- Phylum-level changes:
  - Increased relative abundance of *Firmicutes*, *Fusobacteria*, and *Actinobacteria*
  - Decreased abundance of *Proteobacteria* and *Bacteroidetes* [12,1]
- Genus-level enrichment:
  - SLT users exhibited elevated levels of *Streptococcus*, *Veillonella*, *Prevotella*, and *Porphyromonas*—genera commonly implicated in periodontal inflammation and oral carcinogenesis. [6,9]
- Genus-level depletion:

- Commensal and health-associated genera such as *Neisseria*, *Haemophilus*, and *Rothia* were markedly reduced in SLT users, suggesting a loss of microbial homeostasis and mucosal defense. [1,9]

These taxonomic perturbations reflect a shift toward a dysbiotic oral microbiome with potential pathogenic consequences. [4]

Functional Pathway Analysis

Functional annotation of metagenomic datasets revealed significant alterations in microbial metabolic and signalling pathways among SLT users:

- Upregulated pathways:
  - Enhanced activity in xenobiotic degradation and amino acid metabolism, likely reflecting microbial adaptation to tobacco-derived compounds and altered nutrient availability. [6,8]
- Proinflammatory signalling:
  - Increased expression of genes involved in lipopolysaccharide (LPS) biosynthesis, a key driver of mucosal inflammation and immune activation. [12,8]
- Downregulated pathways:
  - Suppression of vitamin biosynthesis (e.g., B-complex vitamins) and mucosal immunity-related functions, potentially compromising epithelial barrier integrity and host defense mechanisms. [9,8]

Collectively, these functional shifts suggest that SLT use not only alters microbial composition but also reprograms microbial activity in ways that may exacerbate oral and systemic disease risk. [4]

IV. Discussion

This systematic review provides compelling evidence that smokeless tobacco (SLT) consumption exerts a profound influence on the oral microbial ecosystem, driving a shift toward dysbiosis characterized by pathogenic enrichment and proinflammatory signaling. [8,13] The observed alterations in microbial composition and function suggest that SLT use disrupts the delicate balance of the oral microbiome, potentially initiating or exacerbating disease processes. [8]

Notably, SLT users exhibited a consistent increase in taxa such as *Fusobacterium nucleatum* and *Streptococcus mutans*, both of which are well-documented contributors to oral carcinogenesis, biofilm maturation, and chronic inflammation. [13,4] *F. nucleatum*, in particular, has been implicated in epithelial barrier disruption and tumorigenic signaling, while *S. mutans* is a key player in acidogenic biofilm formation and cariogenic potential. Their enrichment in SLT-exposed individuals reinforces the hypothesis that tobacco-associated microbial shifts may serve as early biomarkers or mechanistic drivers of oral mucosal lesions, periodontal breakdown, and malignant transformation. [13,6]

Beyond taxonomic changes, functional metagenomic analyses revealed upregulation of microbial pathways involved in xenobiotic degradation, amino acid metabolism, and lipopolysaccharide biosynthesis—hallmarks of microbial adaptation to chemical stress and immune activation. [8,14] Concurrently, pathways linked to vitamin biosynthesis and mucosal immunity were downregulated, suggesting a compromised microbial contribution to host defense and nutritional homeostasis. [1,13]

These findings underscore the multifaceted role of SLT in modulating host–microbe interactions, not merely through direct chemical insult but via ecological reprogramming of the oral microbiota. The cumulative impact of these changes may predispose users to a spectrum of oral pathologies, ranging from reversible inflammatory conditions to irreversible malignant progression.

V. Conclusion:

The findings of this systematic review underscore the significant influence of smokeless tobacco (SLT) consumption on the oral microbiome, revealing a consistent pattern of microbial dysbiosis and functional reprogramming. [1,6] SLT use is associated with the enrichment of pathogenic taxa and the activation of inflammatory metabolic pathways, which collectively may contribute to the initiation and progression of oral diseases—including mucosal lesions, periodontitis, and malignancy. [4,13]

The observed taxonomic shifts and functional alterations suggest that SLT acts not only as a chemical irritant but also as a microbial modulator, disrupting host–microbe equilibrium and impairing mucosal immunity. [6,8] These insights highlight the need for longitudinal cohort studies to elucidate causal relationships and temporal dynamics of SLT-induced microbial changes. Furthermore, the evidence supports the urgency of targeted public health interventions, particularly in high-prevalence regions, to mitigate SLT-associated risks and promote oral health equity.

Declarations:

1. **Conflict of Interest**

The author declares no conflict of interest related to this manuscript. There are no financial relationships, personal affiliations, or competing interests that could have influenced the content or outcomes of this systematic review.

2. **Ethical Approval and Informed Consent**

This study is a systematic review and meta-analysis based entirely on previously published data. No new human or animal subjects were involved, and therefore ethical approval and informed consent were not required. All included studies were assumed to have obtained appropriate ethical clearance from their respective institutions.

3. **Funding Statement**

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4. **Author Contributions**

Dr. Nitheash P and Dr. Malini P conceptualized the study, performed the literature search, extracted and analyzed data, and drafted the manuscript. The authors approved the final version and is accountable for all aspects of the work.

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