DEVELOPMENT OF MULTI-MICROBIAL PROFILES OF THE ORAL CAVITY AS A BASIS FOR PRECISION DENTISTRY: INTEGRATION OF TRADITIONAL AND MOLECULAR APPROACHES

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Abstract: *This study addresses the development of multi-microbial oral profiles across various age groups in Uzbekistan. In modern clinical practice, traditional diagnostic methods require supplementation with molecular genetic data. The goal of this research is to justify the use of microbial "fingerprints" for the early prediction of dental caries and periodontal pathologies. The authors propose a synergistic model integrating modern molecular technologies (mNGS, Real-Time PCR) with preventive strategies to maintain oral health.*

Keywords*: oral microbiome, dental caries, periodontitis, PCR diagnostics, precision medicine, Uzbekistan.*

INTRODUCTION

In recent years, dentistry has been rapidly evolving toward the principles of precision medicine, which emphasize individualized approaches to diagnosis, prevention, and treatment based on patient-specific biological characteristics. One of the most significant factors influencing oral health is the composition and dynamics of the oral microbiota—a complex and highly diverse ecosystem comprising bacteria, fungi, viruses, and protozoa. The balance of this microbial community plays a crucial role in maintaining oral homeostasis, while its disruption (dysbiosis) is associated with the development of common dental diseases such as caries, gingivitis, and periodontitis.Traditional microbiological methods, primarily based on culturing techniques, have long been used to identify and quantify oral microorganisms. However, these approaches are limited by their inability to detect non-cultivable or fastidious species, which constitute a substantial portion of the oral microbiome. The emergence of molecular techniques, including polymerase chain reaction (PCR), next-generation sequencing (NGS), and 16S rRNA gene analysis, has significantly expanded our ability to characterize microbial communities with high sensitivity and specificity. The concept of developing multi-microbial profiles of the oral cavity represents a promising advancement in precision dentistry. Such profiles integrate quantitative and qualitative data on microbial composition, enabling a comprehensive understanding of microbial interactions, pathogenic potential, and individual variability. By combining traditional microbiological approaches with modern molecular tools, it becomes possible to generate detailed and clinically relevant microbial signatures for each patient.

This integrative approach not only enhances diagnostic accuracy but also facilitates early detection of dysbiotic shifts, risk assessment of oral diseases, and the development of personalized therapeutic strategies. Therefore, the study of multi-microbial profiling in the

oral cavity is of great scientific and practical importance, contributing to the advancement of precision dentistry and improving overall oral healthcare outcomes.

Materials and Methods

The study was conducted at Zarmed University, analyzing saliva and dental plaque samples from 300 volunteers (aged 6–45). Methodology integrated traditional clinical examinations with advanced molecular genetic profiling. We utilized Real-Time PCR and metagenomic sequencing (mNGS) to identify both cultivable and non-cultivable pathobionts [5]. This approach allows for a comprehensive mapping of the microbial landscape, building upon our previous findings regarding microbial associations in complex parasitic-bacterial systems [6].

Study design and population: A prospective comparative study was conducted involving participants with different oral health statuses. The study population included systemically healthy individuals divided into groups: clinically healthy subjects and patients diagnosed with dental caries, gingivitis, and periodontitis. Inclusion and exclusion criteria were defined to ensure homogeneity of the study groups. All participants provided informed consent prior to sample collection.

Sample collection: Biological samples were obtained from the oral cavity under sterile conditions, including:

- supragingival and subgingival dental plaque;
- unstimulated saliva;
- periodontal pocket contents (in patients with periodontal disease).

Samples were collected using sterile swabs and curettes, transferred into transport media, and delivered to the laboratory within 2–4 hours at +4 °C for further analysis.

Clinical assessment: Comprehensive стоматологическое обследование was performed using standard indices:

- DMFT (Decayed, Missing, Filled Teeth) index;
- Oral Hygiene Index-Simplified (OHI-S);
- Papillary-Marginal-Attached (PMA) index;
- Community Periodontal Index (CPI).

These parameters were used to correlate microbial findings with clinical status.

Microbiological analysis (culture-based methods):

- Samples were inoculated onto selective and non-selective media (blood agar, Mueller–Hinton agar, Sabouraud agar);
- Incubation was carried out at 37 °C under aerobic and anaerobic conditions for 24–72 hours;
- Pure cultures were isolated and identified based on colony morphology, Gram staining, and biochemical tests (e.g., catalase, coagulase tests);
- Quantitative analysis was performed by calculating colony-forming units (CFU/ml).

Molecular methods:

- Genomic DNA was extracted from samples using commercial extraction kits;
- Polymerase chain reaction (PCR) was applied to detect specific oral pathogens;
- 16S rRNA gene sequencing was performed to determine the taxonomic composition of the microbiota;
- Next-generation sequencing (NGS) technologies were used for comprehensive microbial profiling;
- Detection of virulence and antibiotic resistance genes was also performed.

Bioinformatics analysis: Sequencing data were processed using bioinformatics tools such as QIIME and BLAST. Taxonomic classification, microbial diversity (alpha and beta diversity), and relative abundance of taxa were analyzed. Individual multi-microbial profiles were constructed based on the obtained data.

Statistical analysis: Statistical processing was carried out using software packages such as SPSS and Microsoft Excel. Mean values, standard deviations, and confidence intervals were calculated. Student's t-test and chi-square (χ^2) test were used to determine statistical significance. Correlation analysis was performed to assess relationships between microbiological and clinical parameters.

Ethical considerations: The study was conducted in accordance with ethical standards and guidelines. All participants provided informed consent, and confidentiality of personal data was maintained.The integrated application of clinical, microbiological, and molecular methods enabled a comprehensive assessment of the oral microbiome and the development of individualized multi-microbial profiles, forming the basis for precision dentistry.

Results and Discussion

The research revealed that the oral biofilm structure in the Samarkand region is age-specific. In children (ages 6–12), aggressive strains of Streptococcus mutans and Lactobacillus spp. dominate. However, their concentration alone does not always trigger immediate decay; the balance between pathogens and "protective" strains, such as Streptococcus sanguinis, is the decisive factor [2].

In adults, environmental factors and water composition in the Zarafshan Valley affect the mineralizing function of saliva, altering its pH and promoting the colonization of anaerobic Gram-negative bacteria [10]. Our developed multi-microbial profiles demonstrated that molecular shifts occur 6–12 months before clinical enamel destruction becomes visible [3]. This confirms that precision monitoring of the microbiome [4] is a superior predictive tool compared to traditional visual-tactile methods [7].

CONCLUSION

The integration of multi-microbial profiling into clinical dental protocols enables a transition from "treating consequences" to "managing risks." The development of localized diagnostic panels is a priority for import substitution and improving the quality of life in the

Republic of Uzbekistan, aligning with national healthcare strategies [9]. The study demonstrates that the development of multi-microbial profiles of the oral cavity provides a powerful tool for understanding the complexity and variability of the oral microbiota in individuals with different dental conditions. Analysis revealed that microbial communities are highly individualized and closely associated with the presence of caries, gingivitis, and periodontitis, highlighting the need for personalized approaches in oral healthcare.

Integration of traditional culture-based microbiological methods with modern molecular techniques, such as PCR and 16S rRNA sequencing, significantly enhances the accuracy and depth of microbial identification, including hard-to-culture and uncultivable species. This approach allows the early detection of dysbiotic shifts, assessment of pathogenic potential, and identification of microbial patterns associated with oral diseases. The creation of individualized multi-microbial profiles enables clinicians to implement precision diagnostics, tailor preventive strategies, and optimize therapeutic interventions for each patient. Overall, the combination of traditional and molecular methods in multi-microbial profiling represents a promising advancement in precision dentistry, improving disease prediction, prevention, and treatment outcomes.

REFERENCES:

1. Belibasakis, G. N., & Bostanci, N. (2021). The Oral Microbiome in Health and Disease. Frontiers in Cellular and Infection Microbiology.

2. Kilene, J., & Mashburn-Warren, L. (2023). Synergistic and Antagonistic Interactions in Oral Biofilms. Molecular Oral Microbiology.

3. Vakhidova, A. M., & Khudoyarova, G. N. (2024). Molecular Synergism of Microbial Associations in Biofilm-Related Dental Diseases. Journal of Clinical Stomatology.

4. Rosier, B. T., et al. (2022). Resilience of the Oral Microbiome. Microbiology and Molecular Biology Reviews.

5. Zhang, Y., et al. (2024). Advances in mNGS Technologies for Dental Diagnosis. Dental Research Journal.

6. Vakhidova, A. M. (2023). Morphofunctional state of the echinococcal bladder and the role of microbial associations. Journal of Theoretical and Clinical Medicine.

7. Khudoyarova, G. N. (2023). Features of the oral microbiome in children of the Samarkand region. Problems of Biology and Medicine.

8. Rasulov, M. M. (2022). Dental health of the population of Uzbekistan under anthropogenic load. Medical Journal of Uzbekistan.

9. Inoyatov, A. Sh. (2021). Modern strategies for the prevention of caries and periodontal diseases. Tashkent.

10. Akhmedov, A. A. (2025). The role of environmental factors in the biochemical composition of saliva in arid zones. Infection, Immunity, and Pharmacology.