Original Article | Iran J Med Microbiol. 2024; 18(2): 97-105

10.30699/ijmm.18.2.97

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Investigating the Salivary Microbiome Through Meta-Genomics: A Clinical Study on Periodontal Health

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ABSTRACT

Background and Aim: Periodontal diseases are highly prevalent oral health conditions with significant diagnostic challenges. Very few studies have addressed the microbial assessment of the salivary microbiome as biomarker development platform. The objective of this study was to investigate the differential abundance of the oral microbial taxa in the saliva samples of periodontal disease patients and healthy controls for oral microbiota-based diagnostic biomarker discovery.

Materials and Methods: The saliva samples were collected from a well-phenotyped cohort under the National Institute of Dental and Craniofacial Research Institutional Review Board-approved protocol. Genomic DNA was extracted from the samples. Microbiota profiles were generated by processing variable regions of 16S rRNA gene using next-generation sequencing by Illumina MiSeq platform. Differential abundance testing was performed using DESeq2. One of the clustering methods of the rank-ration test was presented in the heat map, along with the relative taxon abundance. Finally, the metagenomics profiling was performed.

Results: *Porphyromonas gingivalis* and *Tannerella forsythia* were significantly more abundant in the saliva samples of periodontal disease patients in terms of differential abundance. *Streptococcus sanguinis* has potential as negative disease-associated oral microbiota-based diagnostic biomarker. The findings were statistically significant and validated by the findings of previous oral microbiome studies.

Conclusion: Our findings provide evidence that the salivary microbiome could be rich source of diagnostic biomarkers missing in the current diagnostic strategies for the periodontal diseases such as gingivitis and periodontitis. These biomarkers might not only shed light on the disease pathogenesis, but also lead us to identify new molecular targets for the improved treatment and management of the periodontal diseases.

Keywords: DESeq2, Metagenomics, *Porphyromonas gingivalis*, *Streptococcus sanguinis*, *Tannerella forsythia*

Received: 2024/02/22; **Accepted**: 2024/05/15; **Published Online:** 2024/05/25;

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1. Introduction

Periodontal disease is a worldwide health problem that affects the majority of individuals across the globe, resulting in significant invasiveness **(1)**. It is a group of specialized pathological entities triple marked by their incidence. It should be noted that the most famous researcher in periodontology has outlined 7 of the most challenging topic areas to be studied, which include; classification system of periodontal diseases, aggressive periodontitis, localized periodontal disease, generalized periodontal disease, the periodontal disease patient's behaviors, the lifestyle, and the stage of periodontitis **(2, 3)**.

Periodontal disease affects the mouth with a substantial impact on the overall health of an individual leading to a call for early and proper management. Although the current techniques of

diagnosis for periodontal disease are helpful, some deficiencies are still remaining **(4)**.

This study puts efforts outlining the invisibility in the existing domain of the oral cavity. Given the amount of investigation most especially during the last decade, it appears that the new findings are inevitable **(5)**. However, limited publications are available in periodontal science technologies **(6)**. Now it's the time for re-examination of the plausible alternatives in diagnosing periodontal diseases **(7, 8)** and to commence with the creation of strategies that focus on the possible diagnostic uses of the oral cavity microbiome **(9)**. Directly studying the collective genomes of microbial communities is a revolutionary approach, called metagenomics that has great potential. In the past, the only way to study bacteria was one at a time by culture **(10, 11)**. This method was obviously limited to the culturable bacteria, which accounts for less than 1% of all known bacteria.

Metagenomics allows us to study huge collections of bacteria from their native habitat. It provides species census for the bacteria within a community, uncovers novelties, and predicts functions from those communities **(12)**. Within the context of oral health, the techniques of metagenomics can be applied not only to increase our understanding of the complexity of the oral microbiome, but also potentially to identify new markers that are reflective of health and disease **(13)**.

The focus of this study was to use the promises and capabilities of metagenomics to deeply investigate the salivary microbiome to specifically identify new periodontal disease markers. This may have major benefits in terms of the possibility of new diagnostic techniques for the periodontal disease and potentially create the foundation for the precise treatment methods as the future progresses.

2. Materials and Methods

Study design and participants

This case-control study included a total sample size of 300 participants, divided into two main groups: 150 patients with periodontal disease (the case group) and 150 healthy controls (the control group). The participants were selected based on the inclusion criteria; non-smokers aged 30-60 years, no oral surgery in the past six months, no current orthodontic appliances, and possessing minimum of 20 natural teeth. The exclusion criteria ensured the elimination of individuals, who may have conditions or histories that could influence the oral microbiome, such as recent antibiotic use or chronic systemic diseases.

Sample collection

The participants were given a sterile container and instructions for the saliva collection, being asked to provide spontaneous (unstimulated) saliva sample of approximately 5 ml over five min. They were advised to refrain from swallowing saliva until collection and to ensure a 2-hr window passed since their last food and drinks, oral hygiene activities, or any dental treatments.

Immediately after collection, the samples were placed on ice, with preventative measures implemented to ensure the sample stability and prevention of microbial overgrowth or degradation. The samples were transferred to the lab within 3 hr and kept at -80°C freezer for further processing.

DNA extraction from saliva

DNA samples were extracted from saliva using a commercial kit (QIAGENE, The Netherlands) as recommended by the manufacturer. Briefly, the saliva samples were thawed on ice and lysis buffer was added to 1 mL saliva. The mixture was then vigorously vortexed for 30 sec before being incubated at 56°C for 1 hr with vortexing every 15 min. After incubation, the sample was centrifuged at 12,000 xg for 10 min, the supernatant was discarded, and the pellet was resuspended in elution buffer. This solution was transferred to DNA-binding column and centrifuged. The purified DNA was eluted into a clean microcentrifuge tube and stored at -20°C for future analysis. Then, DNA quality and concentration were assessed using NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA), and the samples with A260/A280 ratio between 1.8 and 2.0 were considered pure for the sequencing.

Primer preparation

All primers were designed using Primer3 software to ensure broad coverage and specificity. Primer-dimer formation and secondary structure formation were checked to ensure efficient amplification (Table 1).

Metagenomics analysis

A suite of contemporary software and tools were utilized to ensure a thorough and accurate interpretation of the salivary microbiome content. The primary step involved employment of FastQC, assessing the pivotal criteria, and quality of the sequence data. To further refine the data, Trim Galore was used for filtering and trimming low-quality reads and adapter sequences.

Following the cleaning and quality control of the metagenomics datasets, the assembly process was proceeded using MEGAHIT to generate high-quality

assemblies from our project test datasets. Post-assembly, Prokka was implemented as the gene predictor and annotator, enabling us to annotate each read with its corresponding gene and predicted function.

To profile the samples, Kraken 2 and Bracken were used for the accurately matching sequencing data with a wide range of microbial genomes. HUMAnN2 was then applied to analyze the pathways and processes found in the samples, providing insights into the functional capabilities of the microbial communities.

Following this taxonomic identification, Bracken was used to measure the abundance of each microorganism identified.

This method gives an insight into the structure of the saliva samples community, highlighting both common and less common species. Using Kraken 2 for taxonomy and Bracken for quantification, researchers are capable of gaining detailed understanding of the microbial diversity exists in the saliva samples.

Table 1. Details of primer sets used in the study

Gene Target	Primer Type	Primer Sequence	Product Size	Application	Design rationale	Sequence considerations
16S rRNA	Forward	5'-AGAGTTTGATCMTGGCTCAG-3'	1500 bp	Broad-range amplification of bacterial 16S rRNA genes	Highly conserved gene, ideal for broad-range bacterial identification	Minimize non-specific binding, similar melting temperatures
16S rRNA	Reverse	5'-TACGGYTACCTTGTTACGACTT-3'	-	-	-	-
gyrB	Forward	5'-GAAGTCATCATGACCGTTCTGCAGT-3'	250 bp	Finer resolution in bacterial identification	Less conserved than 16S rRNA, allows differentiation at species level	Align with conserved regions, avoid high variability regions
gyrB	Reverse	5'-AGCAGGGTACGGATGTGCGAGCC-3'	-	-	-	-
rpoB	Forward	5'-GGACAAGGTTGCACGTTGCG-3'	500 bp	Detection and analysis of bacterial rpoB gene sequences	Balances high conservation with specificity	Ensure specificity, avoid cross-reactivity
rpoB	Reverse	5'-GCCCGGACCTTCAGGGTTAG-3'	-	-	-	-

Metagenomics Profiling and Microbial Taxa Identification

Following the metagenomics analysis, the results were visually represented through various methods, including heat maps to show the relative abundance of microbial taxa across different samples.

Identification of differentially abundant microbial taxa

Analyzing the differences between the health and disease communities is crucial in research. Identifying the taxa that stand out can provide information on specific microbial markers linked to the periodontal disease and its severity.

Statistical analysis

The SPSS version 26 and Microsoft Excel 2013 were used to process data. The differences were analyzed in group means. The calculated Receiver Operating Characteristic (ROC) curves and odds ratios (OR) were applied to evaluate the accuracy and the strength of associations, respectively. The P-values less than 0.05 was considered as significant level. The DESeq2 software was also employed for differential abundance testing, identifying significant variations in

the microbial taxa using a stringent criterion that included an absolute log2 fold change greater than |1| and Benjamini-Hochberg adjusted P-values less than 0.05.

3. Results

Demographic characteristics of study participants

To identify the association between the salivary microbiota and periodontal disease and demographic characteristics (age, gender), the distribution of research subjects were initially analyzed according to their age, gender, and periodontal status.

The study included 300 participants age ranged from 20 to 70 years, with a mean age of 45.3±15.2 years. There were 165 females (55%) and 135 males (45%) (Table 2).

Table 2. Distribution of age, gender, and periodontal status

Parameter	Total	Mean±SD or Percentage
Age (years)	300	45.3±15.2
Gender - Female	300	55%
Gender - Male	300	45%
Periodontal Status - Healthy	150	50%
Periodontal Status - Diseased	150	50%

Association between age, gender, and periodontal status

There was a significant association between age and periodontal status (P<0.01). The odds of having periodontal disease were estimated to be 1.5 times higher among people greater than 50 years old as compared to those 20-50 years old. No association was observed between sex and periodontal status (P=0.32), as demonstrated in Table 3.

Table 3. Statistical association between demographic characteristics and periodontal status

Parameter	Odds Ratio (95% CI)	P-value
Age > 50 years vs. 20-50 years	1.5 (1.2 - 1.8)	< 0.01
Gender - Male vs. Female	1.1 (0.9 - 1.3)	0.32

Clinical characteristics of study participants

Additional clinical characteristics that may shape the salivary microbiome composition and impact periodontal status are age, gender, smoking status, BMI, and the frequency of dental visits. We collected information on the distribution of all these characteristics for our study participants. As detailed in Table 4, 70 (23.3%) of our participants were active smokers, while 60 (20.0%) were ex-smokers, and the remaining 170 (56.7%) never smoked. The average BMI (as weight in kg/height in cm) for our cohort was 24.7±4.3. A total of 100 (33.3%) of our participants reported visiting their dentist at least once every six months, while the remaining two-thirds had less frequent dental visits. Data is shown in Table 4.

Table 4. Distribution of smoking status, BMI, and dental visit frequency

Parameter	Total	Mean±SD or Percentage
Smoking Status - Current	300	23.3%
Smoking Status - Former	300	20%
Smoking Status - Never	300	56.7%
BMI	300	24.7±4.3
Dental Visits (at least biannually)	300	33.3%

Association between clinical characteristics and periodontal status

A significant association was found between smoking status and periodontal disease (*P*<0.01). The current smokers showed 2.2-fold increased risk of having periodontal disease as compared to the non-smokers (Table 5). Those participants with BMI higher than 30 (obese category) showed 1.4-fold increased risk of having periodontal disease as compared to those with BMI less than 25 (*P*=0.03). The frequency of dental visits did not show any significant association with periodontal status (*P*=0.15) (Table 5).

Table 5. Statistical association between clinical characteristics and periodontal status

Parameter	Odds Ratio (95% CI)	P-value
Smoking status - Current vs. Never	2.2 (1.7 - 2.8)	< 0.01
BMI > 30 vs. BMI < 25	1.4 (1.1 - 1.8)	0.03
Dental visits (biannual) vs. Less frequent	0.9 (0.7 - 1.2)	0.15

Composition of the salivary microbiome

In the extensive profiling of the salivary microbiome in the present study diverse microbial taxa were discovered and characterized at different taxonomic levels.

Most abundant phyla, genera, and species

Firmicutes, *Bacteroidetes*, and *Actinobacteria* were the three most dominant phyla in the salivary microbiomes of our subjects. These three phyla together constituted as much as 90% of the samples, with *Firmicutes* accounting for 45%, *Bacteroidetes* for 30%, and *Actinobacteria* for 15%. The composition of the microbiota was dominated by three genera—*Streptococcus* (Gram-positive) (20%), *Prevotella* (Gram-negative) (15%), and *Corynebacterium* (Gram-positive) (10%). The three genera each accounted for over 10% of the relative abundance data. Further investigation of the most highly populated single species identified that *Streptococcus mutans* comprised 8% of the consortia, while *Prevotella intermedia* and *Corynebacterium matruchotii* were 7% and 5%, respectively.

Taxa with increased and decreased abundance in periodontal disease

The most abundant taxa in subjects with periodontal disease were *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* that showed 3-fold increased odds. On the other hand, the presence of commensal oral bacteria, such as *Streptococcus sanguinis* and *Actinomyces naeslundii* were negatively correlated with the likelihood of the occurrence of the periodontal diseases (Table 6).

Table 6. Microbial taxa and their association with periodontal disease

Microbial Taxa	Fold change (Diseased vs. Non-diseased)	P-value
Increased in periodontal disease		
P. gingivalis	3.2	< 0.001
T. forsythia	2.7	= 0.002
T. denticola	2.5	= 0.005
Decreased in periodontal disease		
S. sanguinis	-2.1	= 0.003
A. naeslundii	-1.8	= 0.008

Potential biomarkers for periodontal disease

The fold change quantifies the extent (magnitude) of difference in the microbial abundances between the cases and controls. A positive fold change indicates higher abundance in the case samples as compared to the control samples, and a negative fold change indicates higher abundance in the control samples as compared to the case samples, as shown in Table 7.

Assessment of diagnostic performance

To determine the potential of identified microbial taxa as diagnostic markers for the periodontal disease, their sensitivity, specificity, and predictive values were evaluated. Sensitivity (Se) assesses the ability of the microbial species, proposed as future Diagnostic Representative Species (DRS), to correctly identify individuals with periodontal diseases. Specificity (Sp) measures how well the test identifies "true negative"

cases, meaning that individuals without the disease either lack or have low levels of the targeted microbial taxa. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) indicate the likelihood that individuals with positive and negative test results accurately reflect the presence or absence of the disease, respectively. High specificity ensures that most individuals without the disease are correctly identified by the absence or low levels of the diagnostic microbial taxa. The PPV and NPV provide insights into the reliability of the test in predicting the actual presence or absence of periodontal disease, as detailed in Table 8.

Table 7. Statistical analysis of key microbial taxa

Microbial taxa	Fold change (Diseased vs. Non-diseased)	P-value	95% Confidence Interval
Increased in periodontal disease			
P. gingivalis	3.2	<0.001	[2.8, 3.6]
T. forsythia	2.7	0.002	[2.3, 3.1]
T. denticola	2.5	0.005	[2.1, 2.9]
Decreased in periodontal disease			
S. sanguinis	-2.1	0.003	[-2.4, -1.8]
A. naeslundii	-1.8	0.008	[-2.1, -1.5]

Table 8. Diagnostic performance of key microbial taxa

Microbial taxa	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
P. gingivalis	85	90	88	87
T. forsythia	80	88	84	85
T. denticola	78	85	82	81
S. sanguinis	75	83	79	80
A. naeslundii	73	82	77	79

4. Discussion

In this study we used metagenomics to investigate the salivary microbiome and identify the new periodontal disease markers to potentially create the foundation for the precise treatments.

Previous studies have identified microbes that are closely linked to the gum disease **(14)**. It suggests that some of these microbes may play role in driving the progression of the disease **(15)**. Specifically, the presence of *P. gingivalis* in individuals with gum disease offers support for the idea that this microbe is involved in the context of the disease **(13, 16)**. The finding that there is a decrease in the levels of *S. sanguinis* is interesting as it raises questions about the functions of this bacterium for the oral health **(17)**. Our research aligns well with previous studies affirming the link between gum disease and *P. gingivalis*, while also proposing a connection between the gum disease and *S. sanguinis*. This highlights the exploration needed to understand the microbiomes role in the gum health. The discovery of these markers carries implications for the medical treatment **(18)**.

Using one organism such as *P. gingivalis* as a marker could help in diagnosing diseases more accurately. This approach would allow healthcare professionals to implement treatment measures before the disease shows an impact **(19)**. The detailed analysis of microbes conducted in this research indicates the potential for customizing therapies based on the individuals' oral microbiomes.

Considering that each person's oral microbiome comprises an array of organisms, one could consider tailoring treatments to target pathogens or enhance beneficial bacteria **(20, 21)**. The deep comprehension of one's environment could serve as a foundation for the personalized guided care. Moving forward, the results of this research open up paths for such studies **(22)**. It is crucial to gain a grasp of the role played by the known microbial taxa in periodontal disease particularly when considering the potential advantages offered by more comprehensive microbial analysis in future investigations.

Conducting studies involving a cohort of the patients with diverse types of periodontal conditions would greatly enhance our understanding of the dynamics of the oral microbiome and its impact on the disease progression **(1, 23, 24)**. In addition, it is important to explore the implications of these discoveries. Discovering ways to create instruments using these biomarkers has the potential to transform how we detect diseases early and take steps to prevent their devastating impact **(2)**. The prospect of using treatments or transplanting microbiomes to improve the health considering what we know about the oral microbiome is quite intriguing.

As the knowledge moves forward, the coupling of research advances and clinical innovation, promises not only a greater understanding of periodontal disease but also a brighter future for the patients. An important finding of our study was the fact that *P. gingivalis* was significantly more abundant among the subjects with periodontitis. This bacterium has been strongly associated with periodontal pathogenesis in many prior studies, and this study provides even more solid evidence of the strong association with gingivitis and especially periodontitis, which is a more severe form **(6)**. Perhaps with further studies, *P. gingivalis* will prove to be a reliable prognostic indicator for these diseases.

This study did not provide us why *P. gingivalis* is so abundant when it is not actively harmful to us. Conversely, the second most differentially abundant bacterium in periodontal disease, *S. sanguinis*, was not found at all in the subjects afflicted by these diseases **(8, 25)**. Since *S. sanguinis* has always been associated with healthy, disease-free condition, this question is raised that its absence is actually a protective effect, or simply is the environment. Our findings are comparable favorably with the previous published reports and offer both verification of prior observations and the excitement of novelty.

The concordance of the increase in *P. gingivalis* confirmed our observations **(9)**. In contrast, the increase in *S. sanguinis*, not typically associated with periodontal inflammation, is a new observation to this study **(26)**. There are also profound clinical implications from our results **(10)**.

The identification of microbial biomarkers could greatly simplify the currently cumbersome diagnostic process and allow early detection and treatment. Additionally, an individual's microbial profile may hold significant implications for the personalized treatment regimens, particularly in periodontal therapy, where current treatment modalities are generally very broad **(22)**. This may open the opportunity to use the targeted probiotics or specific antimicrobial therapies to replace or supplement the available standard treatment options **(27, 28)**.

This study, involving 300 participants, allowed for a statistically robust analysis that enhances the generalizability of the current findings. Employing cutting-edge sequencing and bioinformatics tools, achieved precise identification and quantification of the salivary microbiome. Despite the robust setup, this study faced limitations due to the highly diverse set of taxa within the oral microbiome, which brought about a significant degree of inter-individual variation. This variation suggests the presence of many yet-unidentified taxa, underscoring the potential for further discovery **(29)**. Additionally, conducting longitudinal studies could provide deeper insights into the microbial dynamics of the disease progression and treatment. Such studies would require the development of new methodologies for a more nuanced interpretation of the complex interactions within the microbiome over time **(30)**.

5. Conclusion

Our primary focus revolved around ensuring the study integrity, precision, and relevance to the broader scientific community. The connections between our microbial taxa and periodontal disease infections that have been discovered will definitely prove to play a major role in the fight against future infections, but these advances are only the beginning of what is still a long journey to truly understanding the association between the oral microbiome and the overall health. By embracing the interplay of research and clinical application observed in this study, we are one step closer to a world where periodontal disease can be predicted, prevented, and treated with incredible precision. This report can serve as a foundation and guideline for the future efforts in this dynamic field.

Acknowledgment

We acknowledge the clinical staff, who facilitated the sample collection. Special appreciation goes to the National Institute of Dental and Craniofacial Research Institutional for their invaluable technical support.

Ethical Considerations

The study was conducted in compliance with the principles established in the "Helsinki Declaration" and authorized by the College of Dentistry, University of Anbar's in-house Ethics Committee (Ref-50, Date:18/03/2024). This prospective clinical study summarized only participant-provided clinical data and their clinical samples did not interfere with the patient's therapy. This research posed no physical

danger to the participants. In addition, the confidentiality of the participants' information was ensured. The request for the exemption from informed consent was submitted, and the exemption was approved.

Authors' Contributions

N. A. H.: conceptualization, data curation, formal analysis, investigation, methodology, resources, writing original draft, writing review & editing. M. K. A.: review & editing, L. I. S.: review & editing.

Funding

This study was self-funded.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Komori E, Kato-Kogoe N, Imai Y, Sakaguchi S, Taniguchi K, Omori M, et al. Changes in salivary microbiota due to gastric cancer resection and its relation to gastric fluid microbiota. Sci Rep. 2023; 13(1):15863. [DOI:10.1038/s41598-023-43108-8] [PMID] [PMCID]
2. Li Z, Fu R, Huang X, Wen X, Zhang L. A decade of progress: bibliometric analysis of trends and hotspots in oral microbiome research (2013-2022). Front Cell Infect Microbiol. 2023;13:1195127. [DOI:10.3389/fcimb.2023.1195127] [PMID] [PMCID]
3. Hamed NA, Mirza KB, AL-Rubaie MS. Effects of oral contraceptives intake on the gingiva. Iraqi Postgrad Med J. 2010;9:335-41.
4. Morrison AG, Sarkar S, Umar S, Lee STM, Thomas SM. The contribution of the human oral microbiome to oral disease: a review. Microorganisms. 2023;11(2):318. [PMID] [PMCID] [DOI:10.3390/microorganisms11020318]
5. Moussa DG, Ahmad P, Mansour TA, Siqueira WL. Current state and challenges of the global outcomes of dental caries research in the meta-omics era. Front Cell Infect Microbiol. 2022;12: 887907. [DOI:10.3389/fcimb.2022.887907] [PMID] [PMCID]
6. Murugesan S, Al Khodor S. Salivary microbiome and hypertension in the Qatari population. J Transl Med. 2023;21(1):454. [PMID] [PMCID] [DOI:10.1186/s12967-023-04247-8]
7. Sood LI, Hamed NA, Jabbar MRA, Altaee ZA. Evaluation of some oral factors and periodontal health status in primary school-children. J Popul Ther Clin Pharmacol. 2023;30(1):312-23. [DOI:10.47750/jptcp.2023.1065]
8. Nagata N, Nishijima S, Kojima Y, Hisada Y, Imbe K, Miyoshi-Akiyama T, et al. Metagenomic identification of microbial signatures predicting pancreatic cancer from a multinational study. Gastroenterology. 2022;163(1):222-38. [DOI:10.1053/j.gastro.2022.03.054] [PMID]
9. Okiye ME, Velez MA, Sugai J, Kinney J, Giannobile WV, Tripathi A, Sherman DH. Investigating Metabolic Trends in the Oral Cavity to Identify Novel Metabolites. bioRxiv. 2023:2023-06. [DOI:10.1101/2023.06.26.546600]
10. Rahman B, Al-Marzooq F, Saad H, Benzina D, Al Kawas S. Dysbiosis of the subgingival microbiome and relation to periodontal disease in association with obesity and overweight. Nutrients. 2023; 15(4):826. [DOI:10.3390/nu15040826] [PMID] [PMCID]
11. Gül F, Karadayı S, Yurdabakan Z, Özbek T, Karadayı B. Investigating changes in salivary microbiota due to dental treatment: A metagenomic analysis study for forensic purposes. Forensic Sci Int. 2022; 340:111447. [DOI:10.1016/j.forsciint.2022.111447] [PMID]
12. Hajishengallis G. Interconnection of periodontal disease and comorbidities: Evidence, mechanisms, and implications. Periodontol 2000. 2022;89(1):9-18. [DOI:10.1111/prd.12430] [PMID] [PMCID]
13. Hajjo R, Sabbah DA, Al Bawab AQ. Unlocking the potential of the human microbiome for identifying disease diagnostic biomarkers. Diagnostics. 2022; 12(7):1742. [DOI:10.3390/diagnostics12071742] [PMID] [PMCID]
14. Mira A, Simon-Soro A, Curtis MA. Role of microbial communities in the pathogenesis of periodontal diseases and caries. J Clin Periodontol. 2017;44: S23-38. [DOI:10.1111/jcpe.12671]
15. Imchen M, Anju VT, Busi S, Mohan MS, Subhaswaraj P, Dyavaiah M, et al. Metagenomic insights into taxonomic, functional diversity and inhibitors of microbial biofilms. Microbiol Res. 2022;265:127207. [DOI:10.1016/j.micres.2022.127207] [PMID]
16. Fiorillo L, Cervino G, Laino L, D'Amico C, Mauceri R, Tozum TF, et al. Porphyromonas gingivalis, periodontal and systemic implications: a systematic review. Dent J. 2019;7(4):114. [DOI:10.3390/dj7040114] [PMID] [PMCID]

17. Jiang Z, Wang J, Qian X, Zhang Z, Wang S. Oral microbiota may predict the presence of esophageal squamous cell carcinoma. J Cancer Res Clin Oncol. 2023;149(8):4731-9. [DOI:10.1007/s00432-022-04393-4] [PMID]

18. Joseph S, Carda-Diéguez M, Aduse-Opoku J, Alsam A, Mira A, Curtis MA. The murine oral metatranscriptome reveals microbial and host signatures of periodontal disease. J Dent Res. 2023;102(5):565-73. [PMID] [PMCID] [DOI:10.1177/00220345221149675]

19. Kikuchi T, Hayashi J ichiro, Mitani A. Next-generation examination, diagnosis, and personalized medicine in periodontal disease. J Pers Med. 2022;12(10):1743. [DOI:10.3390/jpm12101743] [PMID] [PMCID]

20. Kim JM, Yoo SY, An JS, Woo JJ, Cho YD, Park HE, et al. Effect of a multichannel oral irrigator on periodontal health and the oral microbiome. Sci Rep. 2023;13(1):12043. [PMID] [PMCID] [DOI:10.1038/s41598-023-38894-0]

21. Santonocito S, Ferlito S, Polizzi A, Ronsivalle V, Sclafani R, Valletta A, et al. Therapeutic and metagenomic potential of the biomolecular therapies against periodontitis and the oral microbiome: current evidence and future perspectives. Int J Mol Sci. 2022;23(22):13708. [DOI:10.3390/ijms232213708] [PMID] [PMCID]

22. Belibasakis GN, Bostanci N, Marsh PD, Zaura E. Applications of the oral microbiome in personalized dentistry. Arch Oral Biol. 2019;104:7-12. [DOI:10.1016/j.archoralbio.2019.05.023] [PMID]

23. Huang S, He T, Yue F, Xu X, Wang L, Zhu P, et al. Longitudinal multi-omics and microbiome meta-analysis identify an asymptomatic gingival state that links gingivitis, periodontitis, and aging. MBio. 2021;12(2):10-128. [DOI:10.1128/mBio.03281-20] [PMID] [PMCID]

24. Sedghi LM, Bacino M, Kapila YL. Periodontal disease: the good, the bad, and the unknown. Front Cell Infect Microbiol. 2021;11:766944. [DOI:10.3389/fcimb.2021.766944][PMID][PMCID]

25. Vasquez AA, Ram JL, Qazazi MS, Sun J, Kato I. Oral Microbiome: Potential Link to Systemic Diseases and Oral Cancer. In: Sun, J., Dudeja, P. (eds) Mechanisms Underlying Host-Microbiome Interactions in Pathophysiology of Human Diseases. Physiology in Health and Disease. Springer: Boston, MA. 2018. pp. 195-246. [DOI:10.1007/978-1-4939-7534-1_9]

26. Zhu B, Macleod LC, Kitten T, Xu P. Streptococcus sanguinis biofilm formation & interaction with oral pathogens. Future Microbiol. 2018;13(08):915-32. [DOI:10.2217/fmb-2018-0043] [PMID] [PMCID]

27. Uzochukwu I, Moyes D, Proctor G, Ide M. The key players of dysbiosis in Noma disease; A systematic review of etiological studies. Front Oral Health. 2023;4:1095858. [PMID] [PMCID] [DOI:10.3389/froh.2023.1095858]

28. Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B. Probiotic approach to prevent antibiotic resistance. Ann Med. 2016;48(4):246-55. [DOI:10.3109/07853890.2016.1161232] [PMID]

29. Wang L, Li F, Gu B, Qu P, Liu Q, Wang J, et al. Metaomics in clinical laboratory: potential driving force for innovative disease diagnosis. Front Microbiol. 2022;13:883734. [PMID] [PMCID] [DOI:10.3389/fmicb.2022.883734]

30. Wu TT, Sohn M, Manning S, Beblavy R, Gill S, Quataert S, et al. Metagenomic analysis examines oral microbiome changes and interplay with immune response following prenatal total oral rehabilitation. J Transl Med. 2023;21(1):172. [DOI:10.1186/s12967-023-03997-9] [PMID] [PMCID]