



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Review article

Oral pathogens in peri-implant disease: Insights from 16S rRNA gene sequencing

Ehab Qasim Talib^{1*}, Ghada Ibrahim Taha¹, Dhuha Mahmood Ali¹, Israa Amer Hasan²

1- Department of Basic Sciences, College of Dentistry, University of Baghdad, Baghdad, Iraq.

2- Institute of Genetic Engineering, University of Baghdad, Baghdad, Iraq.

ARTICLE INFO

Article history:

Received 28 November 2023

Received in revised form 15 December 2023

Accepted 20 December 2023

Keywords:

Oral microbiome

Peri-implantitis

Streptococcus mutans

Lactobacillus

Porphyromonas gingivalis

ABSTRACT

Background: Oral health depends on the complicated oral microbial environment. This variable oral microbiome includes microbial populations on teeth, gums, and tongue. Genetics, food, oral hygiene, and health conditions all influence these microbial ecosystems. This ecology is sensitive, and interruption may cause oral dysbiosis. Oral mucositis and peri-implant infections frequently result from this imbalance. This extensive analysis examines oral pathogens such as *Streptococcus*, *Lactobacillus*, and *Porphyromonas*. 16S rRNA gene sequencing is used to study how these bacteria cause oral illnesses. This powerful molecular method illuminated oral microbial dynamics. The study illuminated disease development by examining the complex interactions between these pathogenic microorganisms and oral health. The essay also emphasized the need to use cutting-edge scientific methods to treat oral illnesses. It described how these tools have transformed oral pathology research. The findings from this study are crucial for improving treatment and prevention methods. The review stresses oral hygiene and encourages additional study. Dental treatment might become more personalized and targeted, improving oral health management.

Introduction

The oral microbiome is a complex and dynamic ecosystem that plays a pivotal role in maintaining oral health. It comprises a diverse array of microbial communities that exist in various microenvironments within the oral cavity, including nonshedding dental surfaces which facilitate the development of polymicrobial biofilm communities [1]. These communities are characterized not only by the distinct microbes they comprise but also by their cumulative activities. The composition of the oral microbiome is influenced throughout life by a variety of factors including host genetics, environmental factors such as diet and oral hygiene practices, and systemic factors. This intricate

balance can be disturbed, leading to oral dysbiosis and a range of dental and periodontal diseases [2].

Oral mucositis, a particularly debilitating condition often secondary to head and neck chemoradiation, exemplifies the complex interplay between the oral microbiome and disease [2]. It involves a multifaceted molecular pathogenesis that include epithelial and microvascular injury, the release of proinflammatory cytokines, and intricate host microbiome communications. This cascade results in oxidative stress and the emergence of erosions and ulcers in the oral mucosa, leading to malnutrition, psychological distress, and a heightened risk of opportunistic infections. These complications can severely impact the quality of life

and may even interrupt vital anti-neoplastic therapies [3].

Similarly, peri-implant diseases, such as peri-implant mucositis and peri-implantitis are significant concerns in dentistry, especially with the increasing use of osseointegrated dental implants. Peri-implant mucositis is a reversible inflammatory lesion confined to the peri-implant soft tissue, while peri-implantitis extends to involve the bone supporting the implant, characterized by loss of osseointegration and potential bone destruction. Both conditions are primarily instigated by the colonization of pathogenic bacteria on implant surfaces, leading to plaque-induced inflammation and tissue destruction. Effective management of these conditions hinges on early detection and regular cleaning to minimize bacterial load [4].

The genetic makeup of oral bacteria influences their pathogenicity and interaction with the host. Studies have shown that the oral microbiome is heritable, and genetic factors play a role in shaping the composition and stability of the oral microbiome. Certain strains of *S. mutans* and *P. gingivalis* have specific genetic factors that enable them to cause mucositis and peri-implantitis more effectively than other strains.

The interaction between *Streptococcus mutans*, *Lactobacillus* spp., and *Porphyromonas gingivalis* in the oral cavity can have a combined impact on the progression of mucositis and peri-implantitis. *S. mutans* and *P. gingivalis* can form biofilms on dental surfaces and oral mucosal membranes, which can serve as a reservoir for the bacterium and facilitate its adherence to the host. *Lactobacillus* spp. can produce lactic acid, which can lower the oral pH and create a more acidic environment, favoring the growth of other acid-tolerant bacteria, including those that cause mucositis and peri-implantitis.

In conclusion, the oral microbiome is a complex and dynamic ecosystem that plays a crucial role in maintaining oral health (**Figure 1**) [5]. Dysbiosis of the oral microbiome can lead to a range of dental and periodontal diseases, including mucositis and peri-implantitis. Understanding the genetic makeup and interactions of oral bacteria can inform treatment strategies and improve disease management. Early detection and regular cleaning are essential for effective management of these conditions [4,5].

Characteristics of *Staphylococcus mutans*

Streptococcus mutans is a significant and frequent isolate in the oral cavity and perioral region. It is known for its ability to cause a variety of infections and is present in both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) forms. The common specimen types from which MSSA and MRSA are isolated include oral rinses and tongue swabs, respectively. *S. mutans* in the oral cavity should be considered as a potential source of cross-infection and dissemination to other body sites [6].

Role and mechanisms in oral diseases: Mucositis and peri-implantitis

In the context of peri-implantitis, *S. mutans* has been identified as part of a cluster of bacteria associated with the condition. It is found in higher counts in implants affected by peri-implantitis suggesting a significant role in the development of this disease.

Regarding mucositis, while specific studies directly linking *S. aureus* to oral mucositis were not accessible within the constraints of this search, it is known that oral mucositis involves a complex interplay of microbial factors, including pathogenic bacteria, which may include *S. mutans*. The role of *S. mutans* in this condition would likely involve interactions with the host's immune response and the disruption of the oral mucosal barrier [6,7].

Overview of *Lactobacillus* spp. in the oral cavity

Lactobacillus species appear in the oral cavity during the early years of a child's life and their presence is influenced by various factors, including ecological niches like natural anfractuositities of the teeth. There is a strong correlation established between the saliva *Lactobacillus* count and dental caries. Among children, the presence of lactobacilli in coronal caries is significant, and among adults, these bacteria are found in root caries. Most species of oral lactobacilli belong to the *Lactobacillus casei* group. Understanding the specific correlation between certain lactobacilli species and caries can lead to the development of new prevention tools [8].

Dual role in oral health and disease processes

In the context of peri-implant diseases, the role of *Lactobacillus* spp. is explored in terms of their potential therapeutic benefits. A systematic review and meta-analysis evaluated the adjunctive effect of probiotic *Lactobacillus* in the nonsurgical management of peri-implant diseases, including

peri-implant mucositis and peri-implantitis. However, the study concluded that *Lactobacillus* provided limited benefits in these conditions, suggesting a complex interaction with peri-implant diseases [9].

Regarding oral mucositis, a study on *Lactobacillus brevis* CD2 lozenges showed promising results in preventing oral mucositis in patients undergoing high-dose chemotherapy followed by haematopoietic stem cell transplantation. The study encouraged the use of *L. brevis* CD2 lozenges as a supportive care treatment option for oral mucositis, although further research with larger, randomized trials is warranted [10].

***Porphyromonas gingivalis*: A key player in oral pathology**

Pathogenicity of *Porphyromonas gingivalis*

Porphyromonas gingivalis is acknowledged as a critical bacterium in the etiology and pathogenesis of periodontal diseases. It is part of the "red complex" bacteria group, known for its association with severe periodontitis. *P. gingivalis* can initiate periodontal diseases even at low levels within biofilm, but requires the presence of other bacteria to cause disease. This Gram-negative anaerobe alters the entire microbial community, promoting the development of "pathobiont" species and triggering bacterial dysbiosis.

P. gingivalis adapts to changing nutritional environments and is inflammophilic and asaccharolytic. It meets its metabolic and nutritional needs by digesting peptide accretions from tissue-damaging metabolites, enzymes, and proinflammatory cytokines produced during host inflammation. The organism maintains an inflammatory, nutrient-rich environment for its survival, showcasing its adaptability (Figure 3) [11,12].

Contributions to mucositis and peri-implantitis

While specific references to the contributions of *P. gingivalis* in the development and progression of mucositis and peri-implantitis were not immediately accessible, it's understood that its pathogenic mechanisms, particularly in creating an inflammatory environment and disrupting microbial balance, likely play a significant role in these conditions. Its ability to manipulate the host immune response and alter the microbial community suggests a potential impact on the onset and progression of both mucositis and peri-implantitis.

Further research would be necessary to elucidate these specific contributions [12].

Genetic insights into oral pathogens

The genetic makeup of oral bacteria influences their pathogenicity and interaction with the host. Studies have shown that the oral microbiome is heritable, and genetic factors play a role in shaping the composition and stability of the oral microbiome [13,14]. For example, certain strains of *S. mutans* and *P. gingivalis* have specific genetic factors that enable them to cause mucositis and peri-implantitis more effectively than other strains [14].

The genetic makeup of oral bacteria can influence their pathogenicity and interaction with the host in several ways: [14]

1. Encoding the production of toxic factors and enzymes that can damage host tissues and induce inflammation.
2. Regulating the formation of biofilms and the adherence of bacteria to dental surfaces and oral mucosal membranes.
3. Modulating the host's immune response, which can lead to chronic inflammation and tissue destruction.

16S rRNA gene sequencing

16S rRNA gene sequencing is a key method in microbiology for identifying and classifying bacteria in complex environments. Its main advantage lies in its ability to analyze bacterial diversity without the need for culture, identifying organisms down to genus or species level [15,16]. Widely used for bacterial taxonomy, 16S rRNA sequencing is preferred for its robustness, reproducibility, and accuracy, surpassing traditional phenotypic methods. While it excels in taxonomy, its resolution at the species level is limited. Modern studies often sequence only part of the gene, although the full gene offers better resolution [17].

The role of gene expression in disease development

Gene expression in bacteria like *Streptococcus*, *Lactobacillus*, and *Porphyromonas* identified through 16S rRNA gene sequencing, plays a significant role in disease development. The 16S rRNA gene is crucial for bacterial identification and analysis. The chosen sub-regions of the gene, however, have an impact on the ability of 16S rRNA gene sequencing to reflect true bacterial diversity and species identification [18]. Not all sub-regions

capture sufficient sequence variation to discriminate between closely related taxa. Also, many bacterial genomes contain multiple polymorphic copies of the 16S gene, and distinguishing between legitimate and artefactual sequence variation is essential [15,16]. New sequencing technologies, such as Circular Consensus Sequencing (CCS), have made it easier to find single-nucleotide differences in the 16S gene. These differences can show differences between strains of the same species. This ability is critical for understanding how gene expression differences among strains of the same species contribute to disease [16]. Variations in the 16S sequences within species' genome can be significant for identifying specific strains that may have different pathogenic potentials [17]. Understanding how gene expression affects the development of diseases brought on by these bacteria depends on these insights. The ability to accurately identify and differentiate between bacterial strains through advanced gene sequencing

techniques is vital for developing targeted treatments and understanding the pathogenic mechanisms of these bacteria [18].

Interaction among oral pathogens in disease states

The interaction between *Streptococcus mutans*, *Lactobacillus* spp., and *Porphyromonas gingivalis* in the oral cavity can have a combined impact on the progression of mucositis and peri-implantitis [14,15]. *S. mutans* and *P. gingivalis* can form a synergistic relationship, where *S. mutans* provides *P. gingivalis* with a favorable environment for growth and *P. gingivalis* produces proteases that can degrade host tissues, allowing *S. mutans* [19,20].

Figure 1. Oral microbiome composition. Created based on information from references in the literature [5].

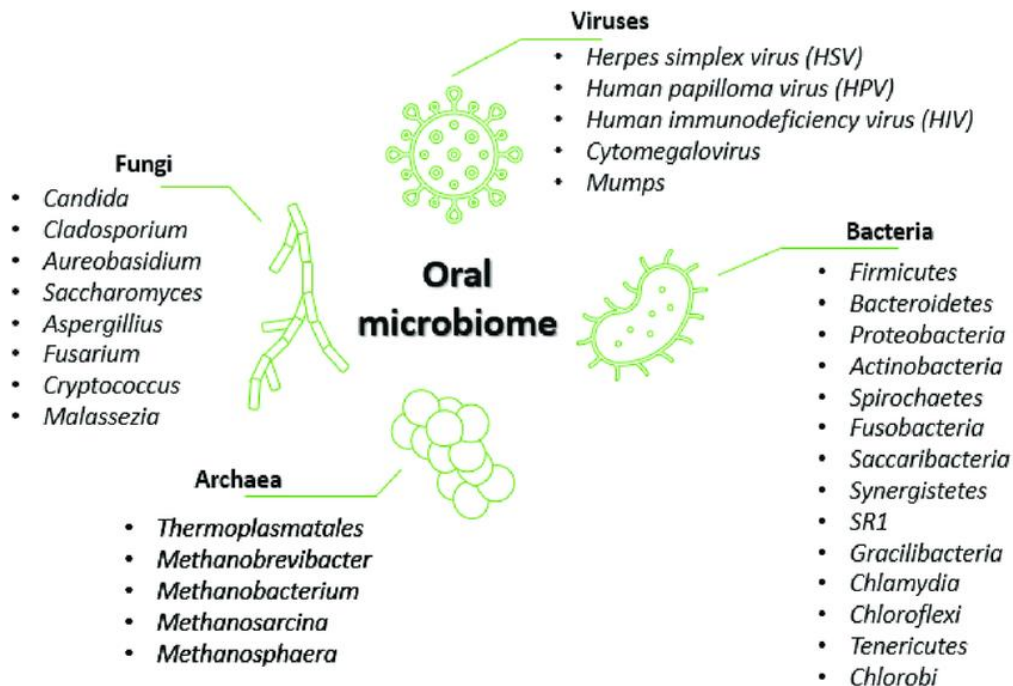


Figure 2. Inflammatory pathway in peri-implantitis [6].

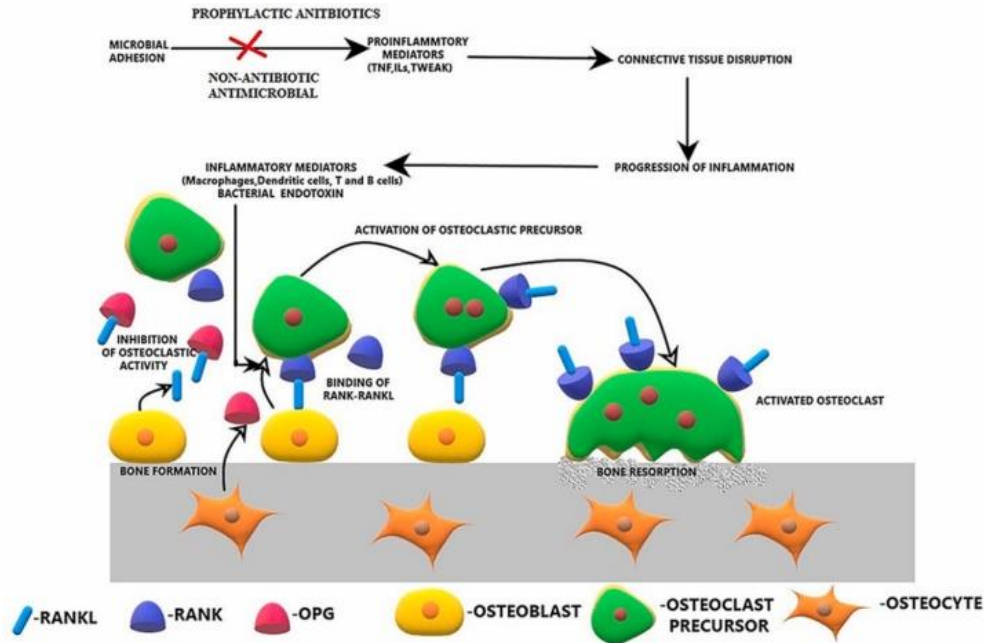
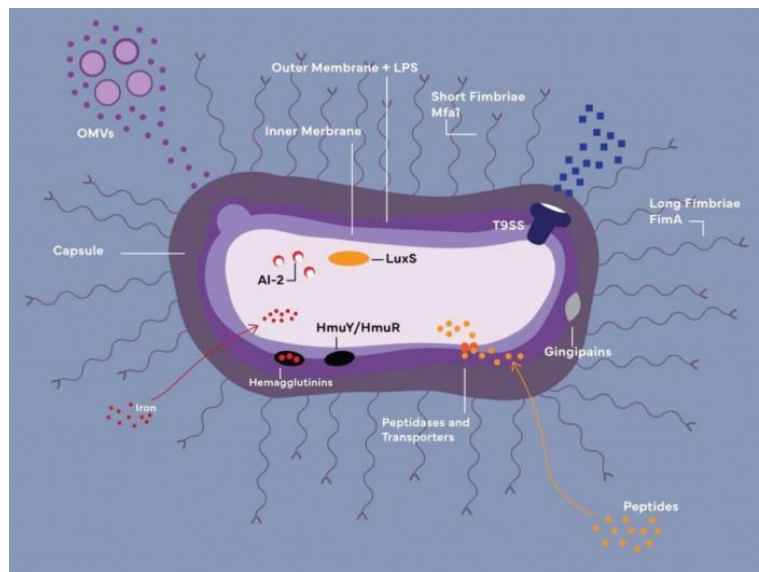


Figure 3. *Porphyromonas gingivalis* structure and major virulence factors [11].



OMVs: Outer membrane vesicles, LPS: Lipopolysaccharide, HmuY/HmuR: Iron-acquiring transporters (function together with hemagglutinins and gingipains), Al-2/LuxS: Components of quorum-sensing system, Credit: Cheyenne Syrek.

Conclusion

The genetic makeup and interactions of oral bacteria, such as *Streptococcus mutans*, *Lactobacillus* spp., and *Porphyromonas gingivalis*, significantly contribute to mucositis and peri-implantitis. Their ability to form biofilms, produce harmful substances, and interact with the host's immune system underscores the importance of early detection and regular oral hygiene in managing these diseases. Utilizing 16S rRNA gene sequencing, the study highlights the intricate interplay between these pathogens and their

contributions to oral health. It emphasizes the importance of molecular techniques for a deeper understanding and effective management of oral diseases, paving the way for more targeted and efficient treatment strategies in dentistry.

Conflict of interest

The authors declared no conflict of interest.

Funding

No funding sources.

References

- 1- **Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL.** The oral microbiome: Role of key organisms and complex networks in oral health and disease. *Periodontol* 2000. 2021;87(1):107-131.
- 2- **Doty RL.** Treatments for smell and taste disorders: A critical review. *Handb Clin Neurol* 2019;164:455-479.
- 3- **Shetty SS, Maruthi M, Dhara V, de Arruda JAA, Abreu LG, Mesquita RA, et al.** Oral mucositis: Current knowledge and future directions. *Dis Mon.*2022;68(5):101300.
- 4- **Klinge B, Klinge A, Bertl K, Stavropoulos A.** Peri-implant diseases. *Eur J Oral Sci* 2018;126:88-94.
- 5- **Alves CH, Russi KL, Rocha NC, Bastos F, Darrieux M, Parisotto TM, et al.** Host-microbiome interactions regarding peri-implantitis and dental implant loss. *J Transl Med* 2022;20(1):1-11.
- 6- **Vijayaragavalu S, Kandasamy R, Krishnaswami V.** Microbiome and the inflammatory pathway in peri-implant health and disease with an updated review on treatment strategies. *J Oral Biol Craniofac Res* 2022.
- 7- **Salvi GE, Cosgarea R, Sculean A.** Prevalence and mechanisms of peri-implant diseases. *J Dent Res* 2017;96(1):31-37.
- 8- **Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S.** Oral lactobacilli and dental caries: a model for niche adaptation in humans. *J Dent Res* 2015;94(9_suppl):110S-118S.
- 9- **Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, et al.** Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. *J Clin Periodontol* 2017;44:S5-S11.
- 10- **Sharma A, Tilak T, Bakhshi S, Raina V, Kumar L, Chaudhary S, et al.** *Lactobacillus brevis* CD2 lozenges prevent oral mucositis in patients undergoing high dose chemotherapy followed by haematopoietic stem cell transplantation. *ESMO Open* 2017;1(6):e000138.
- 11- **Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T, et al.** *Porphyromonas gingivalis*: major periodontopathic pathogen overview. *J Immunol Res.* 2014;2014.
- 12- **Reyes L.** *Porphyromonas gingivalis*. *Trends Microbiol* 2021;29(4):376-377.
- 13- **Esberg A, Haworth S, Kuja-Halkola R, Magnusson PKE, Johansson I.** Heritability of Oral Microbiota and Immune Responses to Oral Bacteria. *Microorganisms* 2020;8(8):1126.
- 14- **Sharma N, Bhatia S, Sodhi AS, Batra N.** Oral microbiome and health. *AIMS Microbiol* 2018;4(1):42–66.
- 15- **Yadav P, Korpole S, Prasad GS, Sahni G, Maharjan J, Sreerama L, et al.** Morphological, enzymatic screening, and phylogenetic analysis of thermophilic bacilli isolated from five hot springs of Myagdi, Nepal. *J Appl Biol Biotechnol* 2018;6(3):1-8.
- 16- **Muhamad Rizal NS, Neoh HM, Ramli R, ALK Periyasamy PR, Hanafiah A, Abdul Samat MN, et al.** Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. *Diagnostics* 2020;10(10):816.
- 17- **Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, et al.**

Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* 2019;10(1):5029.

18-**Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S.** Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. *Clin Microbiol Rev* 2020;33(4):10-1128.

19-**Deo PN, Deshmukh R.** Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol* 2019;23(1):122-128.

20-**Sa'ad MA, Kavitha R, Fuloria NK, Fuloria S, Ravichandran M, Lalitha P.** The virulence system of *Porphyromonas gingivalis*: Genes, mechanism and potential role of gingipains inhibitors. *Malaysian J Microbiol* 2021;17(2).

Talib E Q, Taha G I, Ali D M, Hasan I A. Oral pathogens in peri-implant disease: Insights from 16S rRNA gene sequencing. *Microbes Infect Dis* 2024; Article-In-Press, **DOI:** 10.21608/mid.2023.251782.1680.