REVIEW ARTICLE

Metagenomics as an Effective Diagnostic Approach for Exploring Oral Microbial Diversity and Dental Diseases: A Narrative Review

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Abstract

Aim and background: The oral cavity harbors a diverse microbiota that significantly influences oral health and disease. Conventional microbiological techniques have limitations in detecting the full range of microbial species, particularly those that are uncultivable. Metagenomics, through culture-independent, high-throughput sequencing methods, offers a comprehensive approach to studying oral microbial diversity. This narrative review aims to evaluate the role of metagenomics in exploring the oral microbiome and its association with dental diseases.

Methods: This review systematically synthesized current literature and research on metagenomic technologies, including 16S ribosomal RNA (rRNA) sequencing, shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics. It highlighted their principles, diagnostic capabilities, and limitations in analyzing microbial communities in caries, endodontic infections, and periodontitis. It also reviewed auxiliary tools such as quantitative polymerase chain reaction (qPCR), microarrays, fluorescence *in situ* hybridization (FISH), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and discussed the integration of artificial intelligence (AI) in metagenomic data interpretation.

Results: Metagenomic studies have expanded the scope of known microbial species involved in dental caries beyond *Streptococcus mutans*, highlighting the contributions of *Lactobacillus*, *Veillonella*, *Actinomyces*, and *Candida albicans*. In endodontics, resistant species such as *Enterococcus faecalis*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum* are implicated in persistent infections. In periodontitis, a dysbiotic microbial shift has been associated with the presence of complex microbial consortia, including red and orange complex bacteria.

Conclusion: Metagenomics is a powerful diagnostic tool that provides an in-depth characterization of the complex microbial ecosystem of the oral cavity. It offers diagnostic potential through early and accurate detection of pathogenic shifts, promotes personalized treatment planning, and opens avenues for the development of potential biomarkers of disease progression.

Clinical significance: The integration of metagenomics into dental practice can revolutionize caries risk assessment, treatment precision, and disease prevention strategies. Although challenges such as high cost, data complexity, and lack of standardization remain, ongoing advancements in sequencing technologies and bioinformatics are expected to enhance its accessibility and clinical relevance.

Keywords: Dental diseases, Metagenomics, Microbial diversity, Microbiome analysis, Oral diagnostics, Oral microbiome.

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Introduction

For decades, the understanding of the oral cavity has centered on the teeth and their immediate surrounding tissues, and has been limited to what we could see with our eyes. However, a hidden universe of microscopic life exists within the oral biofilm (plaque).[1] This is a structured community of microorganisms that adhere to the surfaces of the teeth and gums. It is composed of bacteria, their metabolic byproducts, proteins from saliva, and food particles. This complex community creates an ecosystem within the oral cavity, the oral microbiome, which consists primarily of bacteria but also encompasses fungi, viruses, and archaea, and plays a critical role in maintaining oral health.[2] The oral microbiota is a major human microbiome. In fact, it is considered the second-most diverse microbiome in the human body, after the gut.[3]

Metagenomics is the genetic analysis of DNA obtained from a community of microbes. Recent advancements in metagenomics, a powerful technique for analyzing the collective genetic material of these microbes, have revolutionized the understanding of the intricate relationship between the oral microbiome and dental diseases.[4] Traditional culture methods can only grow a small fraction of oral bacteria.[5] Many microorganisms are difficult or impossible to culture in laboratory conditions, leading to an incomplete

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understanding of the microbial community and may not accurately identify microorganisms to the species level. Metagenomics directly analyzes genetic material, revealing the entire microbial landscape, including uncultivable bacteria. It also provides detailed information on the diversity, abundance, and functional potential of oral bacteria, offering a more complete picture of the microbiome's role in health and disease.[6]

By identifying specific microbial signatures associated with dental diseases such as caries and periodontitis, metagenomics can potentially improve early diagnosis and personalized risk assessment. Metagenomic studies play a crucial role in elucidating the potential contributions of oral bacteria to the initiation and establishment of dental plaque.[7] Traditionally, *Streptococcus mutans* has been recognized as the principal pathogen in caries formation due to its acidogenic and aciduric properties.[8] However, recent advancements in metagenomics have revolutionized our understanding of the oral microbiome, revealing a far more complex and diverse microbial ecosystem associated with caries.[9]

Understanding the functional potential of the microbiome opens doors for developing novel treatment strategies, such as probiotic therapies or antimicrobial agents that target specific pathogens without disrupting beneficial bacteria. Metagenomics can also track changes in the microbiome following dental interventions, allowing for personalized monitoring, and optimizing treatment regimens. It has the potential to improve dental care by enabling more precise diagnostics and personalized treatment strategies.[10]

By providing a comprehensive profile of the oral microbiome, metagenomics offers valuable insights into an individual's susceptibility to dental diseases.[7] This information can pave the way for the development of personalized preventive and therapeutic strategies, ultimately promoting better oral health outcomes. This paper will review the potential applications of metagenomics to explore oral microbial diversity and its application in diagnosing dental diseases and identify therapeutic targets to modulate the oral microbiome in favor of health.

Bacterial Metagenome/Oral Microbiome in Dental Caries

Dental caries is primarily a biofilm-mediated process, where the microbial composition of the oral cavity plays a critical role.[11] The microbial communities present in the bacterial metagenome are crucial in maintaining oral health, but imbalances within them can lead to the development of dental caries.[2] The intricate interactions between the collective genome of all the microbial species and the host's environment shape the onset and progression of caries.[12]

The bacterial metagenome offers a comprehensive view of the microbial landscape in dental caries, highlighting the complexity and diversity of the oral microbiome.[13] This expanded knowledge challenges the traditional monomicrobial paradigm and underscores the need for a holistic approach in caries management. Metagenomic studies have revealed that *S. mutans*, long considered the primary pathogen in dental caries, is not present in all patients with the disease.[14] Instead, other streptococci, such as *Streptococcus salivarius*, *Streptococcus sobrinus*, and *Streptococcus parasanguinis*, along with strains of *Veillonella* spp., are predominant in certain individuals.[15] This variation in microbial composition underscores the complexity of the oral microbiome and suggests that a detailed metagenomic analysis could lead to modifications in current treatment protocols and the development of more effective prophylactic measures.[4]

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The metagenomic approach also allows for the identification of microbial metabolic pathways linked to caries progression, including those involved in carbohydrate metabolism, acid production, and biofilm formation.[16] Understanding these pathways provides insight into the functional dynamics of the microbial community and its interaction with the host, which is critical for developing targeted preventive and therapeutic strategies.

By employing high-throughput sequencing technologies, researchers have been able to map the vast array of microbial communities and their functional capabilities.[17] This approach has uncovered numerous previously undetected oral microbial diversity that are either associated with or protective against dental caries.[18] For instance, species such as *Lactobacillus*, *Veillonella*, and *Actinomyces* have been found to contribute to the cariogenic process by producing acids or facilitating the colonization of caries-associated species.[19]

Microbial Species in Dental Caries

Bacteria are the most extensively studied component of the oral microbiome, with several species directly implicated in the development of dental caries.[20] *S. mutans* has been considered the primary cariogenic bacterium due to its acidogenic and aciduric properties. It ferments dietary carbohydrates, producing lactic acid, which demineralizes tooth enamel.[8] Often found alongside *S. mutans*, *S. sobrinus* is another significant contributor to caries. It shares similar virulence factors, including acid production and biofilm formation.[21] *Lactobacillus species* are associated with the progression of deep carious lesions. *Lactobacilli* thrive in low pH environments and further acidify the dental biofilm, exacerbating enamel and dentin demineralization.[22] *Veillonella* species, while not directly acidogenic, *Veillonella* spp. play a role in caries by metabolizing lactic acid produced by other bacteria into weaker acids, which can modulate the pH and influence the microbial composition of the biofilm.[23] Other non-*mutans Streptococci*, such as *S. salivarius*, *Streptococcus gordonii*, and *S. parasanguinis*, are also present in the oral cavity. Some of these species can be involved in the early stages of biofilm formation and influence the overall cariogenic potential of the microbial community.[24] Fungi, particularly *Candida albicans*, are increasingly recognized as contributors to dental caries.[25] *C. albicans* can colonize the oral cavity and is often found in carious lesions.[26] This yeast can adhere to dental surfaces and interact with cariogenic bacteria, such as *S. mutans*, enhancing biofilm formation and increasing the cariogenic potential of the biofilm. *C. albicans* also produce acids from carbohydrates, contributing to enamel demineralization.[27] The role of viruses in dental caries is an emerging area of research. Viruses, particularly bacteriophages, influence the oral microbiome by infecting and lysing specific bacterial hosts, which can alter the composition and behavior of the bacterial community. Bacteriophages target specific bacteria within the oral microbiome.[28]

Bacterial Metagenome/Oral Microbiome in Endodontics

Endodontic infections typically originate from the invasion of the dental pulp by microorganisms, primarily bacteria, following

carious lesions and traumatic injuries.[29,30] These infections can lead to pulpitis, pulp necrosis, and ultimately periapical diseases, such as apical periodontitis and abscess formation.[31] The root canal system, once infected, harbors a diverse microbial community, often forming complex biofilms that are resistant to host defenses and antimicrobial treatments. The course of disease appears to depend on the interaction between the microbial flora and the host's immune system.[30]

Bacterial Diversity in Endodontics

Enterococcus faecalis: *E. faecalis* is one of the most frequently detected species in persistent endodontic infections, particularly in cases of failed root canal treatments. It is highly resistant to harsh environmental conditions, including the antimicrobial agents used during root canal therapy, and can survive in the root canal system even after extensive treatment.[32]

Porphyromonas endodontalis: This Gram-negative anaerobic bacterium is often found in primary endodontic infections. It is associated with apical periodontitis and can produce virulent factors that contribute to tissue destruction and inflammation.[33]

Fusobacterium nucleatum: Another Gram-negative anaerobe, *F. nucleatum* is involved in both periodontal and endodontic infections. It plays a role in bridging other bacterial species in biofilms, facilitating the formation of complex microbial communities within the root canal system.[34]

Streptococcus and *Lactobacillus* species: Facultative anaerobes such as *Streptococcus* and *Lactobacillus* species are frequently found in endodontic infections, particularly in cases where the infection has spread from a carious lesion. These bacteria can contribute to the acidic environment within the root canal, further complicating the infection.[35]

Role of Fungi and Viruses in Endodontics

These microorganisms also play significant roles in the complex microbial ecosystem of the root canal system. These organisms can influence the progression of endodontic diseases, impact treatment outcomes, and contribute to the persistence of infections, especially in cases of failed root canal treatments.

Fungi: *C. albicans* has been increasingly recognized in endodontic infections, especially in persistent and secondary infections where traditional bacterial treatments have failed. It can survive in the harsh conditions of the root canal, including high pH and low oxygen environments, which often follow endodontic treatments.[36] The biofilms formed by *C. albicans* are particularly resilient, making them difficult to eradicate with standard endodontic antimicrobial treatments.

Viruses: Human herpesviruses have been implicated in the pathogenesis of endodontic diseases and are detected particularly in periapical lesions associated with chronic apical periodontitis. Their presence in the periapical tissues and root canal system can modulate the host immune response and influence the course of the infection.[37]

The bacterial metagenome, encompassing the collective genomes of bacteria within the oral cavity, is central to understanding the etiology and progression of periodontitis.[2] Recent advances in metagenomic analysis have provided deeper insights into the microbial communities involved in periodontitis, revealing the diverse and dynamic nature of the oral microbiome in health and disease.[38] Periodontitis develops because of a dysbiotic shift in the oral microbiome, where the balance between commensal and pathogenic microorganisms is disrupted, leading to an overgrowth of pathogenic species. This microbial imbalance triggers an inflammatory response in the periodontal tissues, resulting in the destruction of the alveolar bone and periodontal ligament.[39]

Metagenomic approaches have transformed our understanding of the oral microbiome in periodontitis. By analyzing the complete genetic material of all microorganisms present in periodontal pockets, researchers have uncovered the full diversity of the microbial community, including unculturable and previously unrecognized species. Metagenomic studies have also highlighted the functional capabilities of the periodontal microbiome, revealing metabolic pathways that contribute to disease progression.[38] For example, pathways involved in amino acid and protein catabolism, sulfur metabolism, and the production of short-chain fatty acids have been linked to the pathogenicity of the oral microbiome in periodontitis.[40] These insights have significant implications for the diagnosis and treatment of periodontitis. By understanding the specific microbial and functional profiles associated with disease, clinicians can develop more targeted therapeutic strategies, such as the use of specific antimicrobials, probiotics, or personalized oral hygiene regimens aimed at restoring a healthy microbial balance.[2]

Bacterial Diversity in Periodontitis

Several bacterial species have been strongly associated with periodontitis. These bacteria are often referred to as members of the "red complex" and "orange complex," which are groups of microorganisms linked to severe periodontal disease.[41]

Porphyromonas gingivalis: *P. gingivalis* is a keystone pathogen in periodontitis and a member of the red complex. It produces numerous virulence factors, such as gingipains (proteolytic enzymes), which degrade host tissues and modulate the immune response. *P. gingivalis* can also interfere with the host's inflammatory pathways, promoting a chronic inflammatory environment conducive to disease progression.[42]

Tannerella forsythia: Another red complex bacterium, *T. forsythia* is associated with advanced periodontitis. It produces virulence factors such as proteases and glycosidases, which contribute to tissue destruction and biofilm formation.[43]

Treponema denticola: This spirochete is also part of the red complex and is frequently found in deep periodontal pockets. *T. denticola* is motile, allowing it to penetrate periodontal tissues and interact synergistically with other pathogens such as *P. gingivalis*, enhancing the pathogenic potential of the microbial community.[44]

Prevotella intermedia: A member of the orange complex, *P. intermedia*, is associated with both gingivitis and periodontitis. It can proliferate in the inflamed periodontal environment and contribute to the progression of the disease by producing proteolytic enzymes that degrade host tissues.[42]

Fusobacterium nucleatum: *F. nucleatum* is a key player in the formation of periodontal biofilms. It acts as a bridging organism, facilitating the coaggregation of different bacterial species and promoting the maturation of the biofilm.[34]

Role of Fungi and Viruses in Periodontitis

While bacteria are the primary contributors to periodontitis, other microorganisms, such as fungi, viruses, and archaea, also play important roles in the disease process.[25] Fungi: *C. albicans* is the most detected fungal species in periodontal pockets. Fungi can contribute to the complexity of the biofilm and interact with bacteria, potentially exacerbating the inflammatory response in periodontal tissues.[26,27] Viruses: Human viruses, particularly herpesviruses such as Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV), have been implicated in periodontitis.

These viruses can infect periodontal cells and modulate the immune response, leading to an environment that favors bacterial colonization and persistence.[45]

Available Methods to Determine the Composition of the Oral Microbiome

Several methods can be employed to determine the composition of the oral microbiome, each with its strengths and limitations. The choice of method often depends on the research or diagnostic goals, the depth of analysis required, and the available resources. Following are the commonly used methods for studying the oral microbiome:

- Metagenomics technologies: They can identify pathogens associated with periodontal disease, caries, peri-implantitis, and endodontic infections even before symptoms appear. These methods also detect antibiotic-resistant bacteria, providing a comprehensive picture of the oral microbiome, including uncultivable bacteria, fungi, and even viruses.[7]
  - 16S ribosomal RNA (rRNA) gene sequencing: This method targets the 16S rRNA gene, which is present in all bacteria. It allows for the identification of bacterial taxa and their relative abundance in a sample. While 16S rRNA sequencing provides information about the overall bacterial composition, it may not offer species-level resolution.[46]
  - Shotgun metagenomic sequencing: Shotgun metagenomic sequencing involves the sequencing of all genetic material in a sample, including the genomes of bacteria, viruses, fungi, and archaea. This method provides a more comprehensive view of the microbial community, allowing for the identification of species, functional genes, and potential pathogens.[47]
- Quantitative polymerase chain reaction (qPCR): qPCR is a molecular biology technique that quantifies the abundance of specific DNA sequences in a sample. It is often used to quantify the relative abundance of specific microbial taxa or to measure the expression of target genes associated with oral health or disease.[7]
- Microbiome microarrays: Microarrays designed for microbiome analysis contain probes that hybridize with specific DNA sequences from known microbial species. This method allows for the simultaneous detection and quantification of multiple species in a sample.[48]
- Fluorescence *in situ* hybridization (FISH): FISH is a microscopy-based technique that uses fluorescently labeled DNA probes to bind specifically to microbial cells. It allows for the visualization and quantification of specific microbial groups within a sample.[49]
- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS): MALDI-TOF MS is a rapid and high-throughput method for identifying microbial species based on their unique mass spectra. It is commonly used in clinical microbiology laboratories for bacterial identification, including those in the oral microbiome.[50]
- Cultivation-based techniques: Traditional cultivation-based techniques involve isolating and growing microbial colonies on specific media. While this method may not capture the entire diversity of the oral microbiome, it allows for the isolation and characterization of individual strains.[38]
- Nucleic acid hybridization: Hybridization techniques involve the use of labeled nucleic acid probes to detect specific DNA or RNA sequences within a sample. This method can be used to identify and quantify target microorganisms.[17]

The choice of the best method depends on the specific research or diagnostic objectives, the level of resolution required, and the available resources. Often, a combination of techniques is used to obtain a more comprehensive understanding of the oral microbiome. Advances in high-throughput sequencing technologies, such as next-generation sequencing, have significantly contributed to the depth and accuracy of oral microbiome studies[51] (Table 1).

Methods of Metagenomic Analyses[46,47,50,52,53]

Applications of Metagenomics in Diagnosing Dental Diseases[7,38,54,55]

Early Dental Disease Detection

Use of microbial signatures and biomarkers is identified through metagenomics to diagnose dental diseases at an early stage before clinical symptoms become apparent.

Distinctive Treatment Plans

Development of personalized dental care plans is based on an individual's unique microbial profile to target specific pathogens more effectively.

Noninvasive Diagnostic Tools

Creation of noninvasive diagnostic tests, such as saliva-based assays, to monitor oral health and detect diseases without the need for invasive procedures.

Risk Assessment

Assessment of an individual's risk for developing dental diseases based on their microbial composition and dynamics.

Therapeutic Targets

Identification of new therapeutic targets and developing novel treatments, such as probiotics or antimicrobials, to modulate the oral microbiome in favor of health.

Monitoring Disease

Continuous monitoring of microbial changes to track the progression of dental diseases and the effectiveness of treatments.

Public Health Surveillance

Use of metagenomic data to track the prevalence and spread of dental diseases within populations, informing public health strategies and policies.

Challenges in Use of Metagenomics in Dentistry

Handling and interpreting large-scale metagenomic data require advanced bioinformatics tools and expertise for data management. This requires significant financial investment in technology, equipment, and reagents. While metagenomics has advanced in recent years, it is still a developing field. High-throughput sequencing technologies are improving, but they are not yet widely accessible or affordable in all dental research settings. Moreover, there are challenges related to the resolution and accuracy of microbial community identification, particularly in complex

Table 1: Metagenomic analyses provide insights into the genetic diversity, functional potential, and dynamics of the oral microbiome

Method	*Principle*	*Application*	*Benefits*	*Limitations*
16S rRNA sequencing	Targets and sequences the 16S ribosomal RNA gene to identify bacterial taxa	• Oral microbiome profiling • Detection of specific oral pathogens • Monitoring microbiome changes in oral diseases	• Cost-effective • High-throughput sequencing capability • Well-established protocol	• Limited to bacterial identification, cannot detect viruses or fungi • Low resolution at species/strain level • Bias due to PCR amplification
Whole metagenome shotgun sequencing (WMS)	Sequences all DNA in a sample, providing a comprehensive view of all microorganisms and their genes	• Comprehensive oral microbiome profiling • Functional analysis of microbial communities • Discovery of novel pathogens and antibiotic resistance genes	• Provides information on all microorganisms (bacteria, viruses, fungi) • Enables strain-level resolution • Offers insights into microbial functions and metabolic pathways	• High cost and complex data analysis • Requires large computational resources • Risk of contamination affecting results
Metatranscriptomics	Sequences RNA to analyze gene expression of microorganisms in a sample	• Understanding active microbial functions in the oral environment • Identifying microbial responses to oral health interventions	• Reveals functional activities and gene expression profiles • Can differentiate between active and dormant microbes	• Requires high-quality RNA and is technically challenging • Data interpretation is complex and may not correlate directly with protein activity
Metaproteomics	Analyzes proteins expressed by the microbial community to determine their function	• Functional profiling of oral microbiomes • Identification of specific proteins linked to oral diseases	• Direct assessment of microbial function through protein analysis • Can detect post-translational modifications	• Requires advanced proteomic techniques and is costly • Limited by protein extraction efficiency and database completeness
Metabolomics	Studies metabolites produced by the microbial community to infer metabolic processes	• Studying metabolic changes associated with oral diseases • Identifying biomarkers for oral health and disease	• Provides insights into microbial metabolic activities • Can identify potential biomarkers for early disease detection	• Metabolite data can be difficult to link directly to specific microorganisms • Requires sophisticated analytical techniques and data analysis

environments such as the oral cavity, where microbial diversity is vast.[56] Lack of standardized protocols for sample collection, DNA extraction, sequencing, and data interpretation is a significant barrier. Variability in these protocols can lead to inconsistencies in results, making it difficult to compare studies and establish reliable conclusions.[57] Ethical issues related to privacy, data security, and the proper use of genetic information must be addressed. Metagenomics is still in the early stages of being integrated into clinical practice in dentistry. Making metagenomic technologies affordable and accessible to dental practitioners and patients is crucial for widespread adoption.[58]

Future Prospects of Metagenomics in Dentistry

Figure 1 illustrates the future of metagenomics in dentistry. Metagenomics offers an innovative approach to understanding the complex microbial communities in the oral cavity, paving the way for improved diagnostic tools, personalized treatments, and preventative strategies in dental care.[7,54] Traditionally, identifying oral pathogens relied on culturing methods, missing a significant portion of unculturable bacteria. Metagenomics will enable comprehensive profiling of the oral microbiome, allowing

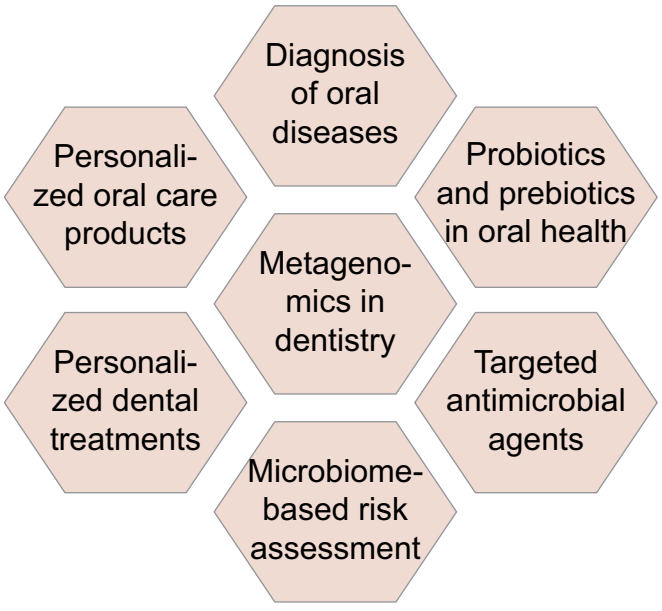

Fig. 1: Future applications of metagenomics in dentistry

dentists to diagnose dental diseases with greater accuracy.[7,38] By understanding the specific bacterial makeup of a patient's mouth, clinicians can tailor and plan personalized treatment strategies to target the most relevant pathogens. Metagenomics might identify subtle shifts in the microbiome that signal the beginning of a dental disease, enabling intervention before symptoms arise.[7] Metagenomics can pave the way for the development of personalized probiotics and bacteriophages that can modulate the oral microbiome. Introduction of beneficial bacteria can promote a healthy oral environment and potentially prevent dental diseases. It can also help in the elimination of specific pathogenic bacteria involved in oro-dental diseases. Understanding the interaction between the oral microbiome and various oral care products can lead to the development of more effective toothpastes, mouthwashes, and other hygiene products that specifically target the beneficial bacteria.[56]

Artificial Intelligence (AI) in metagenomics

Artificial intelligence can significantly enhance metagenomic data analysis in dentistry, aiding in various applications. By interrogating metagenomic data using AI, dentists can gain deeper insights into the oral microbiome, leading to more accurate diagnostics, personalized treatment plans, and improved disease prevention.[59] Metagenomics followed by AI data evaluation can change the precision of the diagnosis in healthcare.[60] While some research has explored various human microbiome pathogens, there is a clear need for more AI-driven metagenomics studies specifically causing dental diseases.

Conclusion

The future of metagenomics in dentistry is promising, with the potential to transform how dental diseases are diagnosed, treated, and prevented. Despite its potential, the integration of metagenomics into routine dental practice faces challenges, including the need for standardized methodologies, high-quality reference databases, and overcoming barriers in data interpretation. Future research should focus on improving metagenomic data analysis pipelines, integrating AI for predictive modeling, and exploring the microbiome's dynamic role in dental disease prevention. Additionally, advances in point-of-care sequencing could bring metagenomics into mainstream dental diagnostics. As metagenomics continues to evolve, it holds the potential to revolutionize dentistry, enabling more accurate diagnostics, targeted therapies, and a deeper understanding of the oral microbiome's role in overall health. As technologies advance and our understanding of the oral microbiome deepens, personalized and effective dental care strategies will become increasingly feasible, leading to improved oral and overall health outcomes.

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