



Role of oral microbiome in forensic odontology

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ABSTRACT-

Oral bacteria form a dynamic biofilm community that is constantly challenged by the host and the environment. Human physiological activities such as immunity, metabolism, and nutrition are closely correlated with the resident microbial consortia found in the mouth, gut, skin, nasal cavity and urogenital tract. The oral cavity serves as the main entry point for the human digestive tract and the oral biofilm contains one of the body's most diverse locations for microorganisms.¹ Hard, non-shedding tooth surfaces in the mouth promote microbial colonization and the development of the dental plaque biofilm. The mouth has a number of habitats other than the teeth like the tongue, gingival sulcus, cheek, hard and soft palates which add to its enormous biological complexity. These locations develop niches that are impacted by changes in the amount of oxygen and nutrients available. Mechanical stress and salivary flow, work in concert to support the colonization and maintenance of various populations.³ In forensic and criminal investigations, saliva is the preferred bodily fluid due to its accessibility, non-invasiveness and cost-effective collection techniques.⁴ Advances in DNA technology have increased the accuracy and enhanced the separation of human DNA in dried saliva stains derived from bite marks and lip prints. The study of the human microbiome, specifically the microbial DNA of bacteria, fungi, microeukarya and viruses in salivary secretion can be related to this relatively new field of salivary research. When combined with well-established salivary biomarkers, this data can help forensic investigators better understand the suspect's lifestyle, cohabitation and health.⁵

Keywords: Oral microbiome, saliva, forensic, crime

Introduction:

More than 2000 bacterial species, including a significant number of opportunistic pathogens, make up the human oral microbiome. The oral microbiome is significant in that, it contains microbial community markers that vary between individuals, indicating the possibility that it could be highly individualized and even unique to each person.⁶ In both criminal and civil situations, the oral microbial remains, which when found at the crime scenes may therefore provide leads for further investigation. It alludes to the analysis of the quantities of different microorganisms found in saliva that may be helpful in identifying a certain person.

In the salivary microbiome, eight genera—Streptococcus, Neisseria, Prevotella, Haemophilus, Veillonella, Porphyromonas, Rothia and Fusobacterium—typically account for more than 70% of the overall bacterial population⁵

Based on a person's age, circadian rhythms, lifestyle, eating habits, cohabitants, presence of pets, smoking habits, interactions like kissing, general health state and the makeup of their salivary microbiome can change.^{7,8,9}

As a result, the components of saliva collected from the

crime scene can be matched to the suspect's close friends and previous dental records.

The exclusivity of the salivary microbiome profile over skin, as opposed to human DNA, which would be the same in both skin and saliva, is another benefit of salivary bacterial DNA recovered from sexual assault locations.¹⁰

Salivary proteomic and genomic biomarkers can be used to diagnose oral malignancies and malignant tumors, as well as conditions of underlying systemic disorders. Oral candida indicates leukemia or iron deficiency anemia. Candida with glucose and crevicular fluid indicates diabetes mellitus.

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Hence, detection of these organisms in saliva may be suggestive of systemic conditions associated to suspects under consideration.¹⁰

The mouth serves as a point of entry for the digestive and respiratory systems; being heavily vascularized it implicates the oral microbiome in other systemic disorders. In fact, an increasing number of studies have found links between variations in the oral microbiota and other disorders. As a result, it is possible that oral microbiota could offer biomarkers that could be used to diagnose some systemic disorders and hence the evolution of metagenomic studies and its association between the oral microbiome and oral diseases.^{13,14,15} This can further help us in identifying individuals in mass disaster by comparing or analyzing the human microbiome.

To ascertain if the microbiome in the first hours of death which can be associated with the host's health before death, Pechal et al. provided a thorough analysis of the postmortem human microbiome¹¹

Samples were taken with postmortem intervals ranging from less than 24 hours to more than 73 hours. The study's findings demonstrate that the microbiome found in various anatomical locations differ significantly from one another and a microbial sequence may be seen which relates to the predicted period after death. The authors of this study also contend that ante-mortem microbial communities may continue to exist for several hours after a person has passed away and may be used to reveal the condition of their health along with individual identification after comparing with the previous records.

Leake et al has observed that samples from the same person group together regardless of time of sampling. The results indicate that it is possible to distinguish two people using the bacterial microbiota present in their saliva.³

Discussion

To analyze the oral microbiome there are various stages to be followed:

- Stage 1: Sample collection - saliva is collected from bite marks on the victim's skin or from things like cell phones or computer keyboards. If necessary, they can also be compared to environmental microbiome DNA using samples taken from soil, air or the surfaces of classrooms. Cohabiting partners or families can exchange microbial DNA signatures.
- Stage 2: To determine the taxonomic distribution of bacterial species, guided (16S rRNA sequences) and

undirected whole genome shotgun [WGS] sequencing are performed. The Human Microbiome Project, which examined the microbiomes of 15–18 body sites in 300 individuals, concluded that bacterial compositions varied less between individuals when they were located in the specific body site.

- Stage 3: WGS is created to target all gene sequencing but has not yet been applied as a forensic technique and analysis of 16S rRNA sequences is made to compare with the published dataset of salivary microbiome.

Hence, it is necessary to conduct oral microbiome examination in all individuals and the dataset stored in a microbiome lab for further matching, correlation and ultimately identification of a victimized individual in a crime scenario.

Salivary enzymes prevent the bacterial DNA from being broken down. Oral streptococcus species are also individual to each person. Therefore, by isolating bacteria from bite marks, oral streptococcal genotype profile analysis could be suggested as a strategy for forensic personal identification in criminal cases.¹²

Another significant application of oral microbiome in forensic odontology is its application in bioterrorism. Bioterrorism is terrorism involving the intentional release or dissemination of biological agents. These agents include bacteria, viruses, insects, fungi, and/or toxins, and may be in a naturally occurring or a human-modified form, in much the same way as in biological warfare¹³ Unpredictable biological terror attacks are a possibility, and they could have negative long-term implications.¹⁴

By creating potent virulence factors and then spreading to crucial organs, bacteria can get around the human immune system. By interacting with host cells through receptors, the bacterial toxins can cause erratic immunological reactions.¹⁵ Polymorphonuclear leukocytes (PMNs) will be stimulated by endotoxins that enter the submucosal layer to release proinflammatory cytokines through chemotaxis activity.¹⁶

On the other hand, this offers the chance to gather biological markers from people to determine whether they were exposed to an outside agent that had an impact on their bodies prior to passing away.¹⁷

Contaminating the food and water sources that humans use, giving infectious agents to animals and insects, or releasing aerosols containing infective agents are some of the methods used to carry out bioterrorism.



The systematic review conducted on bioterrorism showed that microorganisms have a significant role in forensic identification.

Bioterrorism agents are categorized into 3 categories. Category A include organisms that can be easily disseminated and transmitted and hence are the highest risk to the national security examples are *C. botulinum*, Hanta, Marburg, Plague whereas Category B include *E. coli* O157:H7, Hepatitis A, Salmonella, Typhus, Ricin toxin, yellow fever which are moderately easy to disseminate and require enhanced diagnostic capacity and disease surveillance. Category C include emerging pathogens like Nipah, Rabies, Prions, Tick borne Encephalitis that could be engineered for mass dissemination and have potential for high morbidity and mortality rates and major health impact.

The disorders that affect the oral cavity are known to be accompanied by the bacteria.¹² Toxins made by bacteria come in three different varieties. Superantigens (SAGs), such as type I toxin, are made by the streptococci *S. aureus* and *S. pyogenes*. Hemolysin and phospholipase are examples of type II toxins that can damage host cell membranes and impair host defense mechanisms in cells. Due to its binary form, type III toxin, also known as A/B toxin, compromises the host cell's defenses to enable spread to distant organs¹⁸ According to CDC 2013, Relatively modest amounts of blood-derived proteins can diffuse through the tiny space between epithelial cells and into the saliva. As a result, saliva has a concentration of 0.016 mg/ml and the mean serum IgG content of 11 mg/ml. To identify the presence of a virus, only a small amount of IgG needs to be present in the saliva.¹⁹

In conclusion, the review demonstrated that a forensic odontologist can examine a variety of oral cavity-derived biomarkers to determine whether they are relevant to a case. Because the incidence of bioterrorism is unpredictable, it needs to be handled properly. Experts in the field of odontology forensics must comprehend the nature of the catastrophic effects of bioterrorism in order to carry out their duties in assisting with the identification and analysis of cases brought on by exposure to biological agents.

Conclusion:

The establishment of population- and location-specific oral microbial databanks can be facilitated by the forensic dentist. Additionally, a variety of oral and systemic disorders can be investigated for certain salivary genomes or

biomarkers, which always has a lot of research potential in forensic dentistry.

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