



Comparative molecular studies of the human oral microbiota at different altitudes and its relation to systematic disease: A Review

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The oral cavity is a vital organ that connects the human respiratory and digestive systems with the outside world. more than 700 different microbial species inhabit the mouth cavity, making it the second-biggest microbial ecosystem on the planet. Inflammatory bowel disease, pancreatic cancer, diabetes, obesity, and cardiovascular disease have all been linked to an imbalance in the oral microbiota. There are comparatively fewer studies on the impact of different altitudes on the oral cavity, and many of them lack definitive results or call for further research. This review provides an overview of the key research findings on the oral pathology associated with people from high-altitude and coastal residents. our goal from the studies and articles is to look into the effects of various altitudes and ethnicities on the oral micro biome and the diversity of diseases associated with it. Finally, we conclude that there are some differences in the mouth microbes between the populations of high altitude and coastal residents areas which provide some insight for further studies to understand the association between various geographical and ethnic characteristics and oral micro biome dysbiosis-related disorders.

Keywords: oral micro-biome, 16S rRNA gene sequencing, Polymerase chain reaction, altitudes, oral disease

INTRODUCTION

Humans are biological units that include several microbial symbionts and their genomes, similar to all complex multicellular eukaryotes (Bordenstein and Theis, 2015)

We collectively make up a "superorganism," or holobiont, with our symbiotic microbial residents. The human holobiont contains a considerable amount of microorganisms—at least as many as our cells (Sender et al. 2016). In order to better understand how bacteria affect their hosts' physiology, Research on the human micro biome has undergone a revolutionary change. (Gilbert et al. 2018). The mouth cavity, second only to the gastrointestinal system, is a major micro biome habitat (Grice and Segre, 2012). The oral cavity is a vital organ that connects the human respiratory and digestive systems with the outside world (Diamond et al. 2008). More than 700 different microbial species inhabit the mouth cavity, making it the second-biggest microbial ecosystem on the planet (Paster et al. 2006). In the oral cavity, there are about 700 prokaryotic species, 35 percent of which have not been grown, and 482 taxa

have complete genome sequences. (Verma et al. 2018). Only 54% of these species are cultivable and known, with the remaining 32% not even being cultivated and the remaining 14% being both cultivable and unknown (Wade, 2013). The micro biome refers to the microbial population that lives in our bodies. To define the biological population of symbiotic, commensal, and pathogenic microorganisms, Nobel Laureate Joshua Lederberg invented the term "micro biome." These bacteria occupy a physical space within our bodies (Kilian et al. 2016). Our bodies have about the same amount of bacteria as our cells, if not more (Scotti et al. 2017). There has been an increase in the identification and classification of microbial species within microbial communities as a result of the development of culture-independent approaches. To ascertain the content and genetic makeup of the human micro biome, technology, biomarker sequencing, and shotgun Metagenomics have become common tools. Proteomics and metabolomics are examples of "omics" technologies that support mechanistic ideas involving causative microbial pathways linked to states of health and sickness. Oral

microbial communities have been the subject of in-depth research ever since Antonie van Leeuwenhoek looked at dental plaque under a microscope and made the first discovery of microorganisms in the 1700s. The majority of the oral micro biome is made up of bacteria, although there are also minor amounts of fungi, viruses, Achaea, and protozoa. Firmicutes, Fusobacteria, Proteobacteria, Actino-bacteria, Bacteroidetes, and Spirochaetes are the major bacterial phyla found in the oral microbiome, but their abundance varies amongst populations. (Deo and Deshmukh, 2019). Living in a high-altitude environment for an extended period of time is associated with increased levels of oxidative stress, which can persist even when the person returns to sea level. While it's unknown how increased oxidative stress affects the body's systems and overall health (Koivisto et al. 2019). In addition to discussing the oral characteristics of high-altitude life, numerous studies have documented the health issues associated with living at or traveling to high altitudes[(Kumar Singh, 2019), (Terrizzi et al. 2016)]. The microbial community has a direct impact on each individual's physiology, diet, and defense. The maintenance of oral and general health is significantly aided by the commensal microbiota. Its delicate balance is easily disturbed, resulting in oral pathologies such as caries, pulpal and periapical infections, osteitis, and tonsillitis (Abusleme et al. 2013). as well as the emergence of several systemic illnesses such as cardiovascular disease, ictus, preterm birth, diabetes, pneumonia, obesity, colon carcinoma, and psychiatric issues. Therefore, for studies featuring people in good health prior to attempting to demonstrate any association, it is necessary to accurately characterize the human oral microbiota in health. oral microbiota with odd illness circumstances The oral cavity's micro biome is incredibly complicated because each surface provides a distinct ecological niche with nutritional and environmental variables that are only suited for a small number of microbial organisms. As a result, each microhabitat needs to be sampled using the right tools. For instance, it has been reported to employ sterile nylon micro-brushes and sterile brushes for oral mucosa. Gracey curettes that are sterile are typically used to sample plaque from hard tissues like teeth. Whole Genome Sequencing (WGS) has just recently been developed, although it has only been used in a small number of oral cavity samples to concurrently analyze the presence and quantity of all the microorganisms that may be present in the oral cavity. Although the oral microbiota is a complex community, strain-level data can be obtained by whole metagenome shotgun sequencing if sufficient coverage is obtained. However, because of its high expense and computational complexity, large-scale investigations have not been found to be suitable for this method. (Yu et al. 2023 ; Franzosa et al. 2015 ; Avila et al. 2009)

A rising amount of research suggests that the

composition and function of microbiota play critical roles in human development, physiology, immunity, and nutrition (Ley et al. 2006). Gao et al. (2018) found that oral bacteria are critical for maintaining oral homeostasis and preventing oral illnesses (Gao et al. 2018). Moreover, Hypoxia, low temperatures, host sex, age, and oral illnesses all have an impact on the oral microbiota (Grant et al. 2010). Dental caries, periodontitis, peri-implantitis, mucosal disorders, and oral malignancies are all linked to problems with the oral microbiota (Jorth et al. 2014). Over time, a comprehensive method for comprehending oral illnesses from a polymicrobial standpoint has emerged, connecting the pathophysiology of the sickness to networks of coexisting bacteria and serious infections, both of which work together to contribute to the pathogenesis of the disease.. Therefore, among the primary domains of study for oral health is how to comprehend the variations in the bacteria that comprise the oral health and illness, together with their relative abundance and functional activity, genetic influences, and ecological constraints that cause such changes. Diabetes, obesity, cardiac disease, ductal carcinoma, and Crohn's disease have all been linked to an imbalance in the oral microbiota. (Liu et al. 2021). The exogenous foreign substance exposure to the oral microbial environment is ongoing Due to these conditions, founding bacteria and their capacity to survive in this setting are defined, and specific connections between the microbe and host that depend on selective forces are produced. Four species of bacteria: *Streptococcus gordonii*, *Streptococcus sanguinis*, *Streptococcus salivarius* and *Streptococcus mitis* are examples of early oral cavity colonizers that exhibit key traits that make them particularly well suited to this particular niche [(Baker and Edlund, 2019). Prior research endeavored to investigate the impact of diverse elevations and ethnicities on the oral micro biome and associated disorders.

Human oral microbiome database

According to the Human Oral Microbiome Database (HOMD), a database including the genomic sequences of oral bacteria and an extensive resource with descriptions of oral bacteria bacterial taxonomy, a method for 16S rRNA identification. The National Institute of Dental and Craniofacial Research created this one-of-a-kind database in 2010 to keep track of information on isolates produced from oral fluids that can be grown and those that cannot . (Chen et al. 2010). More than 700 prokaryotic species are represented in the eHOMD, with about 500 taxa having complete genome sequences, 70% of them being cultivable, and 30% of them belonging to the uncultivable taxonomic group. Of the seventy-seven percent of culturable species, fifty-seven have names already. By using 16S rDNA profiling of the healthy oral cavity, six main phyla—

Actinobacteria, Firmicutes, Proteo-bacteria, Spirochaetes, Betadeletes, and Fusobacteria—which together account for 96% of all oral bacteria—were found. (Verma et al. 2018)

Function of the oral microbiome

The oral microbiota is typically found as a biofilm. It is necessary to protect the oral cavity and preserve oral health, avoiding disease progression, and it's crucial to understand the identification of the microbiome as well as the neighbors it commonly interacts with. (Jia et al. 2018) The oral microbiome play a role in key physiological, metabolic, and immunological functions such as food digestion and nutrition, energy generation, differentiation, and maturation of the host mucosa and development of immunity, defense against pathogens, processing and detoxification of ambient toxins, host nutrition involves the synthesis of vitamins, fat storage, and short-chain fatty acid generation, all of which are crucial for the metabolism of host energy. (Kilian et al. 2016) For the human microbiome to have beneficial benefits, the host and microbiome must coexist in equilibrium. Among the systemic ailments that dysbiosis, or an imbalance in the microbiome, causes include asthma and atopic diseases, inflammatory bowel diseases, autoimmune diseases, obesity and metabolic syndrome, colon cancer, peripheral vascular disease, depression, and autism. (Kinross et al. 2011)

Method of studying the oral microbiome:

Culture and microscopy

Bacterial taxonomy identification techniques in the past were culture-dependent. These involve sucrose, growing conditions, microscopy, antibacterial sensitivity, metabolic and other morphological testing. (Krishnan et al. 2016). The oral microbiome's true diversity cannot be fully disclosed by culture-based techniques. Several researchers have succeeded in isolating, cultivating, identifying, characterizing, and classifying about half of the seven hundred recognized types of bacteria that are often found in the mouth cavity.. (Aas et al. 2005). Many of the bacterial species found in biological samples cannot be grown, which makes traditional culture and culture-based analytical tools challenging to use for research (Pozhitkov et al. 2010).

Polymerase chain reaction-based methods

is a frequently used method that swiftly makes multiple copies of a specific DNA sample, allowing researchers to amp up a very small amount of DNA (or a piece of it) to the necessary extent to enable in-depth research.

Real-time quantitative PCR (RT-PCR), Conventional polymerase chain reaction, PCR-DGGE, repetitive element-based PCR, PCR-RELP, terminal-RELP and repetitive element-based PCR, PCR-RELP are some of

the PCR-based techniques available for recognizing microbe resistance and avoiding attack and the development of illness. (Aas et al. 2005).

16S rRNA sequencing

16S rRNA sequence analysis and metagenomics are two typical DNA sequencing methodologies used to explore uncultivated oral microbial ecosystems. Metagenomics entails whole-genome shotgun sequencing, whereas 16S rRNA sequencing entails sequencing of the conserved 16S rRNA gene (WGS). A "shotgun" approach is used to shear all of the DNA samples at random. 16S rDNA gene profiling is utilized in the latest research to evaluate the microbes contained within a specimen, or shotgun metagenomics is employed if a thorough profile of gene content in a given habitat is necessary (Xu and Gunsolley, 2014 ; Kuczynski et al. 2011). The existence or abundance of bacterial species is determined by 16S rRNA sequencing. Consequently, scientists are limited in drawing conclusions from their findings. By using shotgun metagenomics sequencing, the metabolic pathways linked to it will also be found. (Le Bars et al. 2017)

DNA microarrays

Phylogenetic DNA micro-arrays have been discovered as useful methods for High productivity, analytical, and methodical investigation of microbiological populations in many microbiological habitats, such as the bacteria in the mouth, by the scientific community. (Parolin et al. 2017)

Oral microbiota in human systematic diseases

Oral infection has effects on systemic illness in addition to local (periodontal) disease. Infection spreading from the mouth due to transitory bacteremia, the movement of microbial toxins, and systemic inflammation brought on by unfavorable immune reactions to oral microorganisms are the three main mechanisms linking oral infection to systemic pathology that have been discovered .(Li et al.2000). Most oral bacteria are not well adapted to survive in a healthy lower GI tract. There is frequent oral-to-gut bacterial transmission. However, patients with inflammatory bowel illness, HIV, liver cirrhosis, and colon cancer have higher amounts of mouth-associated germs in their guts than healthy individuals (Atarashi et al.2017) Oral infection and cardiovascular disease are closely related. (Glurich et al. 2002) Due to the elevated levels of systemic pro-inflammatory cytokines, inflammatory cells and infiltrates, and white blood cell counts caused by periodontal disease, humans are more susceptible to cardiovascular diseases such as atherosclerosis and myocardial infarction. (Page, 1998) A persistent autoimmune condition called rheumatoid arthritis is characterized by synovial inflammation, which can harm

bone and cartilage in the joints. (McInnes and Schett, 2011).

Table 1: shows some types of oral microbes that cause systematic diseases and their characteristics

| Disease | Common Oral micro-biome associated with disease | Characteristic features |
|----------------------------------|--|---|
| Cancers | Porphyromonas gingivalis | Anaerobic, gram-negative, rod-shaped bacteria that grow anaerobically on blood agar and needs heme or hemin and vitamin K for growth. (Yilmaz, 2008) |
| | <i>Fusobacterium nucleatum</i> | <i>The Gram-negative bacterium Fusobacterium nucleatum is not motile and does not produce spores. The G-C composition of this bacteria is between 27 and 28 mol%. (Bolstad et al. 1996)</i> |
| | Treponema denticola | <i>A gram-negative, helically-shaped, motile, and flexible bacterium belonging to the Spirochetes family is called Treponema denticola. (Charon et al. 1992)</i> |
| | Aggregatibacter actinomycetemcomitans | <i>A facultative anaerobe, nonmotile, gram-negative bacterium is called Aggregatibacter actinomycetemcomitans. (Henderson et al. 2010)</i> |
| Cardiovascular diseases | Lactobacillus | <i>The genus Lactobacillus contains rod-shaped, microaerophilic, gram-positive, aerotolerant anaerobes that do not form spores. (Makarova et al. 2006)</i> |
| | Streptococcus Sanguis | <i>Healthy human mouth contain the Gram-positive, nonmotile, nonspore-forming cocci Streptococcus sanguinis. It can live in the heart valves and cause bacterial endocarditis since it is frequently present in the bloodstream. (Pramesti, 2016)</i> |
| | Streptococcus gordonii | Gram-positive, mesophilic, nonmotile cocci in the genus Streptococci grow in pairs or in chains that resemble beads. (Telford et al. 2006) |
| Diabetes | Corynebacterium | <i>Anaerobic, Gram-positive, non-motile, non-spore-forming, non-capsulated, pleomorphic coccobacillus that typically has a club-like structure is Corynebacterium. (Sharma et al. 2019)</i> |
| | Neisseriaceae | <i>Gram-negative bacteria belonging to the Pseudomonadota, a broad class of Gram-negative forms, include Neisseria species. Under a microscope, Neisseria diplococci resemble coffee beans. (Ryan and Ray, 2004)</i> |
| | Actinomyces | group of bacteria. Every one of them is gram-positive. Because they are facultatively anaerobic, Actinomyces species grow in anaerobic environments. (Hsiao et al. 2021) |
| Periodontal diseases(24) | Porphyromonas gingivalis, | Anaerobic, gram-negative, rod-shaped bacteria that grow anaerobically on blood agar and need heme or hemin and vitamin K for growth.(Yilmaz, 2008) |
| | Treponema denticola | <i>A gram-negative, helically-shaped, motile, and flexible bacterium belonging to the Spirochetes family is called Treponema denticola. (Charon et al. 1992)</i> |
| | Tannerella forsythia | Tannerella forsythia is a Gram-negative, anaerobic bacterium species. It belongs to the red complex of periodontal pathogens and has been linked to periodontal disorders.(Tanner and Izard, 2006) |

Oral microbes and digestive system diseases:

One of the various ways that oral microorganisms can affect the digestive system is by invading the intestines, disrupting the intestinal microecology, and harming the digestive system's organs. (Gao et al. 2018). Metabolic products of oral bacteria reach the systemic circulation through the blood, which causes minimal inflammation in an individual's body and encourages the onset and development of chronic inflammatory illnesses in the digestive system. (Hashioka et al. 2018). The use of this approach is gradually becoming more and more accepted in the study of systemic disorders brought on by an imbalance in the intestinal flora, as seen by the results of oral microbiome research. Therefore, this strategy may be the primary function of oral bacteria in the digestive tract as well as a significant means of influencing systemic disorders.

Oral bacteria and cancers:

Through certain cytokines and pathways, oral microbes can directly influence the onset and development of cancers. They can also facilitate the growth, deterioration, and spread of tumors by controlling the immunological response between the body and the tumors. (Han et al. 2014; Zhang et al. 2020).

The throat and mouth cancers are the sixth most common systemic malignant tumors (Hussein et al. 2017). The best ways to stop and manage oral cancer are early identification and treatment.

UV radiation, HPV, alcohol usage, smoking, and other factors are typical risk factors for oral malignancies. (Islami et al. 2017 ; Irani, 2020).

Oral microbes and cardiovascular diseases

Coronary heart disease, endocarditis, and myocardial infarction are just a few examples of the illnesses referred to as cardiovascular problems that affect the heart and vascular circulation system. [58]. Periodontitis is a significant risk factor for cardiovascular disease, according to numerous cross-sectional research, case reports, and epidemiological examinations. (Beck and Offenbacher, 2005 ; Persson and Persson, 2008). Patients with periodontitis are more likely to have broken gingival epithelium in their periodontal pockets, which allows bacteria to enter the bloodstream and cause bacteremia or ectopic colonization in other body organs. When bacteria from periodontal disease enter the bloodstream through damaged periodontal tissue, they cause irritation, damage to vascular endothelial cells, and the development of atherosclerotic plaques. These bacteria can also undermine the body's immunity. (Forner et al. 2006 ; Zheng et al. 2017).

Oral microbes and diabetes

Systemic disorders like diabetes mellitus that affect the mouth have been linked to changes in the composition of the oral bacteria. (Saeb et al. 2019) The degenerative changes associated with diabetes can aggravate and accelerate the onset and progression of periapical inflammation. Therefore, effective treatment of periapical inflammation is essential to controlling blood sugar levels. Periodontal pockets in patients with periapical infection contain oral bacteria that complexly communicate with the immune system to produce persistent, chronic inflammation. Compared to periodontitis patients without diabetes, the gum microbiota of diabetic patients has experienced significant changes, and a variety of microorganisms are differently enriched in the two groups. (Matsha et al. 2020).

Oral Micro biome in Periodontal Diseases

Porphyromonas gingivalis, *Treponema denticola*, and *Tannerella forsythia*, collectively referred to as the "red complex" of oral anaerobic bacteria, have traditionally been considered the main infectious pathogens associated with periodontitis. (Marsh and Zaura, 2017)

Furthermore, *Candida albicans* is one of the major fungi that exist in the oral microbiome. This obligate colonizer may contribute to the development of bacterial gum infections by protecting *P. gingivalis* from immune cell recognition. (Bartnicka, et al. 2020)

Following microflora perturbations, gingivitis and finally periodontitis develops. Systemic diseases and abnormalities in oral homeostasis may be influenced by elements such as the availability of oxygen, nutrients, and pH variations. (Willis and Gabaldón, 2020; Liu et al. 2012)

A change from a symbiotic to a dysbiotic microbial community is also linked to changes in the periodontal microbiome. *Actinomyces* and *Streptococcus* are examples of facultative bacteria found in symbiotic structures. After that, the majority of bacteria are anaerobic, belonging to the groups Firmicutes, Proteobacteria, Spirochaetes, Bacteroidetes, and Synergistetes. (Wilson, 2009)

Dietary behaviors including smoking, drinking alcohol, being overweight, having diabetes, experiencing stress, and growing older all have an impact on Malodour. (Hampelska et al. 2020)

Wu et al.'s study is noteworthy since they discovered that obese patients with malodor had changed oral microbiomes and more numerous species than healthy people. (Wu et al. 2018)

According to Zhang et al. alterations in the tongue coating's microbiota can help in halitosis early diagnosis. The development of more accurate techniques for diagnosis, prognosis, and therapy, however, can benefit from the use of changes in the micro biome of the

tongue covering as indications of halitosis in its early phases. (Zhang et al. 2021)

Effect of the oral microbiome on different altitudes

There are currently few studies showing how altitude affects the bacteria in the human mouth. The variety of oral microbiota is altered by living at different elevations, and the environmental mechanisms linked to it react differently than they do in low-altitude locals, according to a large body of recent information gathered from the Tibetan plateau. Studies conducted recently on animal models exposed to chronic hypoxia revealed an increased risk of periodontitis development because of elevated oxidative stress and inflammatory markers in the submandibular glands. (Terrizzi et al. 2016 ; Terrizzi et al. 2018) Acute mountain sickness and chronic or intermittent hypoxia are two serious health issues that people who live or travel in high-altitude regions may encounter. Additionally, they may be highly susceptible to both high-altitude cerebral and pulmonary edema. (Miller et al. 2013; Stellingwerf et al. 2019) .The amount of oxidative stress, which may continue for some time even after the person descends to sea level, is correlated with the length of time spent living in a high-altitude environment.

However, the effects of increasing oxidative stress on the body's physiological and medicinal systems remain unclear (Koivisto et al. 2019). A series of signaling processes that result in adaptation to high altitudes may be triggered by hypoxia. These signaling events that could result in adaptive responses also include the production of reactive oxygen species. At high altitudes, excessive reactive oxygen species generation may lead to decreased capillary perfusion and poor muscle function, which could lead to more significant health issues. [(Miller et al. 2013 ; Irrázaval et al. 2017). Low atmospheric pressure causes healthy people's oxygen saturation to drop at altitudes above sea level. (Ucrós et al. 2020). Increased red blood cell synthesis (polycythemia) can happen at high elevations to make up for insufficient tissue oxygenation (Fan et al. 2018). Long-term residents of high-altitude regions have been shown to have bodies that have genetically adapted to the hypoxic conditions present there. Adaptation of the body to high altitude and the altitude level, which can be divided into three categories: high altitude, or 4900–11,500 feet above sea level; very high altitude, or 11,500–18,000 feet above sea level; and extreme altitude, or more than 18,000 feet above sea level, are the two main factors that influence the development of polycythemia in these individuals. (Zhao et al. 2017). Numerous studies have documented the health issues associated with high-altitude living and travel, as well as the oral aspects of high-altitude living (Kumar Singh, 2019).

There are comparatively fewer studies on the impact of different altitudes on the oral cavity, and many of them

lack definitive results or call for further research. This review provides an overview of the key research findings on the oral pathology associated with people from high-altitude and coastal residents

CONCLUSIONS

From the previous studies, we conclude that there are some differences in the mouth microbes between the populations of high altitude and coastal residents areas which provide Some information for future research to understand the association between various characteristics related to geography and ethnicity and oral microbiota dysbiosis-related disorders.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: <https://www.isisn.org/article/>

Author contributions

All authors contributed equally to this study. Each author was involved in designing the experiments, conducting research, analyzing data, and writing and reviewing the manuscript. Their collaborative efforts have culminated in this comprehensive work. All authors have read and approved the final version of the manuscript.

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Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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