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Molecular studies of the human oral microbiota from populations of high altitude and coastal residents in west Saudi Arabia

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The human body, similar to other complex multicellular organisms, is an intricate holobiont composed of human cells and a vast array of microbial symbionts. This study focuses on the oral microbiota, the collective genetic makeup of the microorganisms inhabiting the oral cavity, which plays a crucial role in overall health. By comparing the oral microbiota of individuals from Jeddah City at sea level and Taif City at a high altitude (2200 meters), this research aims to understand how altitude affects microbial diversity and composition. The study involved collecting saliva samples from 30 participants aged 20–50, controlling for health status and abstinence from antibiotics prior to the study. High-throughput sequencing techniques were used to analyze the microbial communities. Results showed significant differences between the two groups, with higher microbial diversity observed in the high-altitude samples. Specifically, the Taif samples had greater relative abundances of Firmicutes and Actino-bacteria, while Proteo bacteria were more prevalent in the Jeddah samples.These findings suggest that environmental factors such as altitude significantly influence oral microbiota. The higher diversity in high-altitude samples may reflect adaptations to the unique stresses of lower oxygen levels and colder temperatures. This research contributes to the understanding of how environmental factors shape human microbiota and highlights the importance of considering these factors in public health and clinical practices. Future studies with larger sample sizes are necessary to further explore these interactions and their implications for health.

Keywords: oral microbiota, alpha diversity , altitudes, Microbial Diversity, Sequencing data processing

INTRODUCTION

Like all other complex multicellular organisms, humans are made up of many microbial symbionts and their DNA. (Bordenstein and Theis, 2015) .Together with our symbiotic microbial occupants, we form a "superorganism," or holobiont. There are a lot of microbes in our body—at least as many as our cells, according to Sender et al. (2016).Oral microbiota" refers to the collective genetic makeup of the bacteria that inhabit our mouth cavities. Humans have two distinct types of micro-biomes: a dynamic microbiome and a core microbiota. Every individual has a unique microbiota that changes according to their lifestyle and physiological variables. (Zaura et al. 2014) .The cavity in the mouth is home to one of the most important microbiome ecosystems, after the intestinal tract. (Grice and Segre, 2012) .The mouth cavity, which is constantly exposed to them through intake and inhalation, is home to roughly 700 different species of bacteria, fungus, viruses, archaea, and protozoa (Bäckhed et al. 2012) .The mouth is a perfect biological home for bacterial colonization since it has a wide variety of locations within its complex microbial ecology. These include, among other places, the tongue, larynx, hard and soft palates, teeth, and saliva . (Dewhirst et al. 2010) (Benn et al. 2017) .The genetics, environment, diet, age, and location of the host all influence the oral microbiota's makeup, suggesting that the interaction between the host and the microbial community as a whole controls the development of periodontal health or illness. More

specifically, the variety of oral microorganisms is a good indicator of human health in general and oral health in particular. Many human illnesses, both local and systemic, such as diabetes, dental caries, obesity, and cardiovascular disease, have been related to dysbiosis, an imbalance in the oral microbiome. (Kumari et al. 2023) .According to the "High Altitude Medical Handbook," They seem to have developed the most useful classification approach, which is now widely recognized in the field of mountain medicine. This classification creates five categories: low, defined as anything below 4900 feet; moderate or intermediate, defined as anything between 4900 and 8200 feet; high, defined as anything between 8200 and 11500 feet; very high, defined as anything between 11500 and 19000 feet; extreme high-altitude, defined as anything above 19000 feet; and death zone, defined as anything above 26000 feet. And Taif city is regarded as one of Saudi Arabia's high-altitude cities. (Basnyat, 2004). A vast amount of recent data collected from the Tibetan plateau indicates that living at different elevations alters the variety of oral microbiota and that the environmental systems associated with it react differently than they do in low-altitude localities. Recent research using animal models subjected to prolonged hypoxia has shown that heightened levels of oxidative stress and inflammatory markers in the submandibular glands are associated with a higher risk of developing periodontitis. (Terrizzi et al. 2016 ; Terrizzi et al. 2018) . The study aims to find out how human oral microbiota are impacted by high-altitude situations. The specific goal of the study is to comprehend how the diversity and makeup of the oral microbiota are impacted by the particular physiological difficulties associated with high altitudes, such as lower oxygen supplies and air pressure. Additionally, the study aims to compare these effects with low-altitude environment observations.

MATERIALS AND METHODS

Aim of the study:

This rigorous research project aimed to investigate the possible differences in oral microbiota between people living in two different locations: Jeddah City, which is at sea level, and Taif City, which is located atop Al Hada Mountain, which represents a highaltitude environment. The goal of conducting this study was to offer a detailed understanding of how variations in oral microbiota may be impacted by altitude, a notable environmental factor

Study area/setting

This study was carefully planned and carried out in two strategically selected western regions of the Kingdom of Saudi Arabia, each providing a distinct altitudinal viewpoint.

Jeddah City: The city is situated at sea level. Its distinct coastal climate in addition to its city surroundings provide a rich environment for the research.

Taif City: is the perfect place for this research since it is elevated at a considerable 2200 meters above sea level and is part of the Al Hada Mountain range, which provides a collection of characteristics that are intrinsic to mountainous, high-altitude places.

Data collection methods

The study's participant selection strategy focused mostly on adults between the ages of 20 and 50.

Their condition and state of health were carefully examined. Ethnicity was taken into consideration while choosing study participants in order to ensure comprehensive and trustworthy research results. Ethnicity has been related to several characteristics, including genetics and health, which are shared by the groups being examined. The study's emphasis was narrowed down to an age group of 20 to 50 in order to reduce the variables being compared.

Study plan

Throughout the past two-year period (2022–2023).

A thorough statistical analysis was performed on the data sets, both combined and gender-specific.

Compiling the descriptive mean ± standard deviation and running an unpaired t-test with Welch's correction were the analytical steps concerned.

Sample size

A total of 30 samples were initially screened for possible study subjects. After applying the inclusion and exclusion criteria.All participants provided informed consent and had abstained from using any antibiotics one month before the study. The participants fasted for a minimum of three hours before collecting the samples.

The selected people were screened for their health and medical status through a questionnaire for each person participating in the study.

Ethical considerations

The study procedure was carefully designed with ethical considerations in mind, adhering to the highest standards of academic research.

From the beginning of data collection until the end of analysis, the values of ethical research were upheld.

RESULTS

This investigation involved the collection of thirty saliva samples in total.

The samples were split up into groups according to altitude: low and high. Age, height, weight, and BMI did not differ significantly from one another.

Sequencing data processing

The amplicon was sequenced on Illumina paired-

end platform to generate 250 bp paired-end raw reads (Raw PE), and then merged and pre-treated to obtain Clean Tags. The chimeric sequences in Clean Tags were detected and removed to obtain the Effective Tags which can be used for subsequent analysis. The summarizations obtained in each step of data processing are shown in the table 1 as follow:

Taxa relative abundance in phylum

According to the taxonomic annotation results, the top 10 taxa of each sample or group at each taxonomic rank (Phylum, Class, Order, Family, Genus, Species) were selected to form the distribution histogram of the relative abundance of taxa. This allows the visualization of the taxa with a higher relative abundance and their proportion in different classification levels for each sample or group. The relative abundance of taxa in the phylum is illustrated below in figure 1:

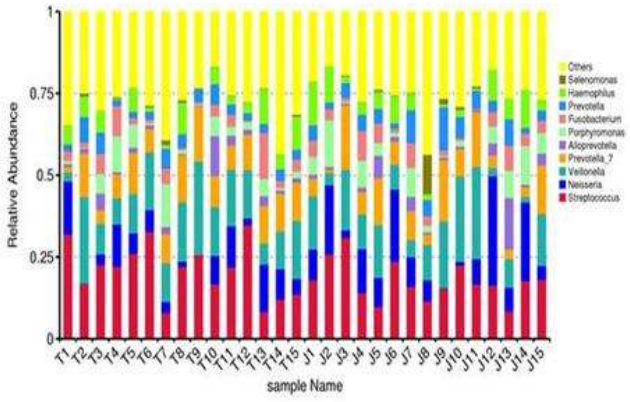

Figure 1: Taxa relative abundance in phylum

Table 1: QC statistics

Sample	RawPE	Combined	Qualified	Nochime	Base(nt)	Avglen (nt)	GC	Q20	Q30
T1	205796	202048	198111	184123	78187835	424.65	52.82%	98.62%	95.44%
T2	201801	199044	195314	176928	74952250	423.63	52.60%	98.62%	95.45%
T3	204541	200580	196734	178782	75640988	423.09	51.60%	98.57%	95.30%
T4	202794	199209	195636	180697	76532252	423.54	52.00%	98.55%	95.26%
T5	202689	199621	195736	170383	72455409	425.25	52.18%	98.55%	95.25%
T6	203106	200069	196246	171945	73052074	424.86	52.85%	98.60%	95.42%
T7	107137	105644	103789	92808	39162287	421.97	52.61%	98.70%	95.68%
T8	205588	201877	197818	179607	76269358	424.65	52.45%	98.55%	95.21%
T9	204596	201284	197192	179855	76463938	425.14	52.70%	98.55%	95.27%
T10	206774	201298	197262	176521	74908684	424.36	51.44%	98.36%	94.77%
T11	204068	200206	196082	168325	71350003	423.88	52.02%	98.58%	95.34%
T12	204285	201597	197652	170233	72462980	425.67	52.86%	98.63%	95.45%
T13	204335	200431	196919	170403	71878596	421.82	51.05%	98.71%	95.68%
T14	205013	201471	197975	177578	75047317	422.62	52.88%	98.68%	95.60%
T15	301001	297990	291333	232541	98398778	423.15	52.65%	98.50%	95.11%
J1	164185	161961	158716	142356	60468971	424.77	52.35%	98.62%	95.41%
J2	205845	203299	199662	179201	76308688	425.83	51.85%	98.75%	95.71%
J3	214698	211104	206978	176319	75036632	425.57	52.52%	98.54%	95.27%
J4	149488	147460	145021	136995	57889362	422.57	51.60%	98.71%	95.67%
J5	206270	201424	197251	179619	76054942	423.42	51.74%	98.52%	95.23%
J6	204456	200571	196697	184153	78335229	425.38	52.96%	98.58%	95.34%
J7	215788	211601	207659	197583	83703251	423.64	51.29%	98.54%	95.18%
J8	207120	201526	197129	165987	70116312	422.42	52.92%	98.33%	94.75%
J9	203529	199989	196107	158044	66937156	423.53	52.12%	98.67%	95.60%
J10	205162	200942	196702	185149	78655393	424.82	52.96%	98.51%	95.12%
J11	206370	203219	199344	175040	74483903	425.53	52.93%	98.60%	95.42%
J12	203748	199133	195287	168024	71378556	424.81	51.90%	98.62%	95.42%
J13	204228	201221	197516	172256	72597471	421.45	50.77%	98.69%	95.56%
J14	202834	199648	196041	170620	72625419	425.66	51.90%	98.72%	95.63%
J15	205833	202861	199087	169577	71804398	423.43	51.76%	98.69%	95.64%

T1=sample number one from taif J1= sample number one from jeddah

Ternary plot

To find the differences in dominant taxa among the three groups of samples at each taxonomic rank (Phylum, Class, Order, Family, Genus, Species), the top 10 taxa with the average abundance of the three groups of samples at each taxonomic rank were selected to generate ternary plot (Bulgarelli et al. 2015) The ternary plot in phylum shows as follows:

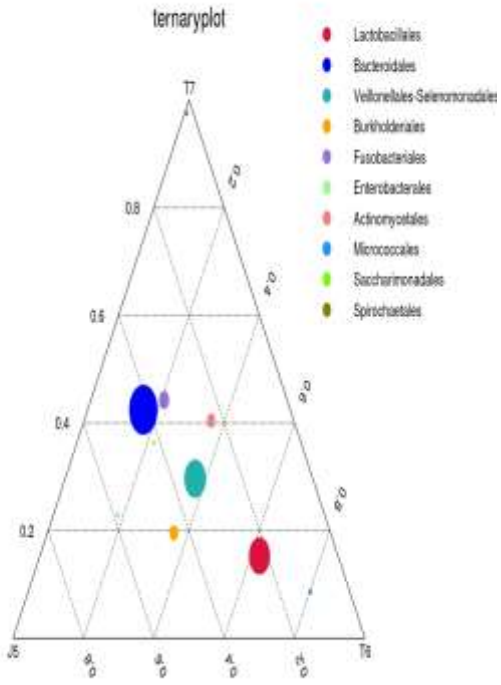

Figure: 2 Ternary plot

Results directory:

Ternary plot: result/03. Taxon Visual/ ternary/ (sample,group)/*/*ternary.(pdf,jpeg), Including Phylum, Class, Order, Family , Genus and Species.

Difference of alpha diversity indices between groups

Boxplots were formed to analyze the differences in Alpha Diversity indices between groups. T-test, Wilcox,Kruskal-Wallis and Tukey tests (Wilcox test are for 2 groups while Kruskal-Wallis and Tukey tests are for more than 2 groups) were performed for the significant difference analysis between groups. Boxplots based on Observed_species and Shannon indices are shown as follow, which can reflect the maximum, minimum, median and abnormal value of index from each groups:

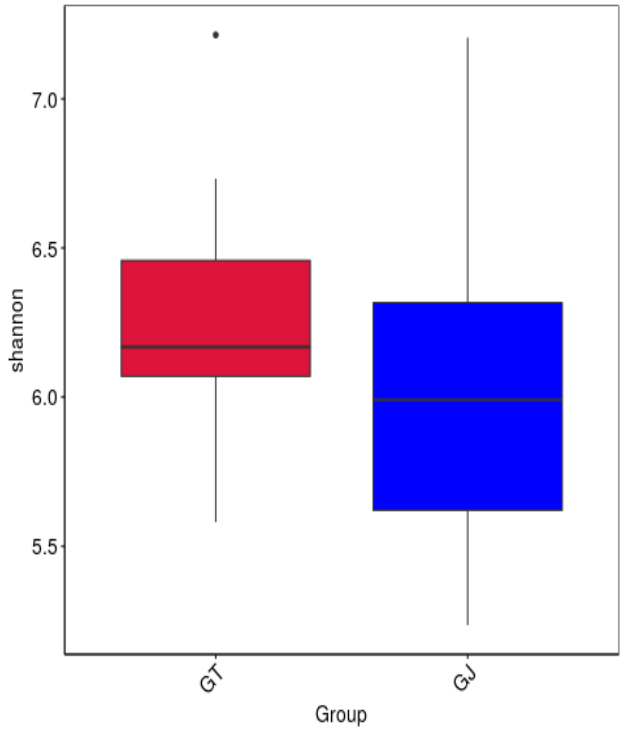

Figure 3: Boxplots for difference of alpha diversity indices

Note: The horizontal axis of the box plot represent the groups, while the vertical axis represents the corresponding alpha diversity index value.

Box plots with Alpha Diversity indices：

Results directory: result/ 04.Alpha Diversity/ visual alpha Diversity/group***/***

DISCUSSION

The objective of this research was to examine the variations in oral microbiota between residents of Jeddah City, which is situated at sea level, and Taif City, which is situated in a high-altitude area. The results showed that the two groups' oral microbiota composition and diversity differed significantly. Certain species were notably more abundant in Jeddah or Taif than in other places, suggesting that environmental conditions and altitude could affect oral micro-biome patterns.

The results indicate that altitude may significantly impact the oral microbiota. The higher diversity observed in the Taif samples could be attributed to the unique environmental conditions associated with high altitudes, such as lower oxygen levels, different dietary habits, and lifestyle factors compared to sea-level environments. These environmental pressures could select for distinct microbial communities better adapted to high-altitude conditions

Taxa Relative Abundance and Diversity

According to our analysis, the Taif and Jeddah samples had different relative abundances of bacterial phyla. To be more precise, samples from Jeddah had larger concentrations of Proteobacteria phylum, while Firmicutes and Actinobacteria phylum had higher prevalence in Taif. A possible explanation for this uneven abundance is that different elevations have distinct living circumstances and environmental stresses. For example:

Firmicutes:

Known for their resilience in diverse environments, their higher abundance in Taif might be a response to the stressors associated with high altitude, such as lower oxygen levels and colder temperatures. (Hird, 2017)

Pro-teobacteria:

This phylum, which includes many pathogens, was more prevalent in Jeddah. This might be influenced by the coastal climate and potentially different lifestyle and dietary habits prevalent in a sea-level urban environment. (Arrieta et al. 2014)

Alpha diversity indices, including observed species and Shannon indices, provided insights into the microbial diversity within each group. The statistical analysis confirmed significant differences in alpha diversity between the low-altitude and high-altitude groups. Higher diversity in oral microbiota is often associated with better oral health, while lower diversity can be linked to diseases. (Zaura et al. 2014) The findings suggest that the high-altitude environment of Taif City may foster a distinct microbial ecosystem compared to the sea-level environment of Jeddah City.

The findings of this study are consistent with previous research that highlights the impact of environmental factors on human microbiota. Studies on gut microbiota, for example, have shown significant variations between populations living at different altitudes (Li et al. 2017). However, research specifically focusing on oral microbiota and altitude is limited. This study contributes to filling this gap and provides a basis for future investigations .

The study was designed with several strengths, including a well-defined participant selection criteria, control of confounding variables (age, health status, and antibiotic use), and the use of high-throughput sequencing technology. These methodological approaches ensured robust and reliable data.

However, the study also had limitations. The relatively small sample size (30 participants) may limit the generalizability of the findings. Future studies with larger cohorts are necessary to validate these results and provide more comprehensive insights. Additionally, while this study controlled for several variables, other environmental and lifestyle factors not accounted for could also influence oral microbiota.

CONCLUSIONS

In conclusion, this study provides valuable insights into how altitude influences oral microbiota, revealing significant differences between individuals living at sea level and at high altitudes. These findings contribute to the growing body of literature on the environmental determinants of human microbiota and highlight the need for tailored public health and clinical interventions based on specific environmental contexts. Further research is essential to elucidate the underlying mechanisms and broader implications of these findings.

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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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the king Abdulaziz University

Informed Consent Statement

Approval was obtained from King Abdulaziz University in cooperation with Khulais General Hospital, and the article was reviewed by the Ethics Committee

Data Availability Statement

All of the data supporting the findings of this study are included within the article and its supplementary materials.

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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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