UNIVERSITY OF PADOVA

Department of Comparative Biomedicine and Nutrition

Master's degree course in Food biotechnologies

Final dissertation

Microbiome composition of human dental calculus correlates with dietary intake

Supervisor

Professor Maria Elena Martino

Co-supervisor

Dr. Andrea Quagliariello

Candidate:

Khatereh Kheirkhah

Student ID number: 1227876

Academic Year 2021/2022

Abstract

Within and beyond our bodies, bacteria, archaea, viruses, and eukaryotes make up our microbiota. Our native microbiota can interact with host health and disease, as well with host behaviour and quality of life. Microorganisms can colonize a variety of niches on and inside the human body, adapting to the unique ecological characteristics of each one. The oral microbiota, which is part of the human microbiome, interacts with host health and with the host immune system. Microbe-host imbalances can lead to oral disorders, as well as chronic diseases. The microorganisms that live in human digestive tracts are known as gut microbiota which might be regarded as a virtual organ of the body, with several functions that play a critical part in health maintenance. Dietary factors can have an impact on their composition and health. We collected 40 samples of dental calculus from varying ages and gender, analysing their microbial composition and inspecting the influence of the dietary elements constituting the Mediterranean diet on its composition. We highlighted the presence of four clusters, each characterized by a different microbial biomarker. The systematic review's major finding was an increase in *Streptococcus* in cluster 1 as a result of a fibre and vegetable diet, also the result demonstrated the high abundance of the *Porphyromonas* and *Tanerella* as one of the main periodontal diseases in cluster number 4 that were associated with the animal-based products such as meat and dairy products.

Index

List of figures

List of tables

Acknowledgment:

As we have come to the end of this journey, I would want to thank everyone who has allowed me the opportunity to finish this report.

First and foremost, I am extremely grateful to my supervisor Professor Martino for allowing me to complete the internship inside her research group, for her assistance and ongoing supervision, as well as for supplying the required project information.

I also want to thank Professor Quagliariello for his patience with me throughout the internship, for teaching me the lab procedures, and for his constant support and suggestions as I wrote this report. I also want to thank him for taking the time to proofread and correct all of my mistakes.

My appreciation also goes out to my parents and my sisters especially Roya my lovely sister, for their encouragement, economic and moral support all through my studies. To say that we have had some ups and downs as a family over the past three years would be an understatement. I will always be grateful that you kept me from giving up each time I was about to.

Finally, I'd like to express my gratitude to my sweetheart fiancé Andrea, who never stopped supporting and motivating me. This dissertation is proof of your continuous support and encouragement. More than anything in this world, I love you.

1-Introduction

1-1. Basic introduction of microbiota:

The human microbiome is a complex and dynamic microbial community made up primarily of bacteria, but also include protozoa and archaea, virus, and fungi, that lives in and on the oral cavity, throat, stomach, and respiratory tract, and skin. Even though our interior tissues are generally sterile, these microorganisms become residents of our bodies shortly after birth. Under normal circumstances, they are commensal in our bodies and provide certain benefits, such as preventing bacteria from entering our organs and tissues, while other bacteria, known as opportunistic pathogens, can have an impact on human health, these pathogens have evolved to colonize and invade human organs and they counterpart dominates over commensals.[1,2]

There are an estimated 100 trillion microbial communities that live on and within the human body, and there is a relationship between the host and the microbiota, with one benefiting from the other. Microbiotas are important for maintaining homeostasis because they provide many benefits to the host, including pathogen displacement, immune system development, and nutrient absorption. While the host provides stability and a nutrient-rich environment for the microbiota, the microbiota improves digestion, immunity, and neuronal development.[3] The gastrointestinal tract is the primary site of bacterial colonization in the human body, which aids in the digestion of nutrients, starches, carbs, protein catalysis, and the production of important vitamins and amino acids. They also contribute to fat capacity and the production of anti-inflammatory substances. [4]

Viruses such as plant viruses and eukaryote-derived viruses, as well as bacteriophages, are found in the human microbiome. Bacteriophages, or phases, in short, carry genes from one bacterium to another and modify the genetic content of bacteria. As a result, viruses play an important role in the maintenance of the microbial community in terms of resistance to unfavourable conditions, and they also prevent infectious microorganisms from colonizing the body. [5]

Exposure to numerous environmental factors such as nutrition, xenobiotics, medications, and infections can alter the composition of the gut microbiota which eventually plays a role in the pathophysiology of a variety of metabolic, neurological, immunological, and cancer-promoting illnesses Inflammatory bowel disease (IBD) and Crohn's disease (CD).[6]

The human microbiome is unique to each individual, much like a fingerprint.[7] They aid in the development and adaptation of the body's immune system throughout human life to be compatible with the body's environment, defend the host from invading diseases, and produce antimicrobial chemicals. As a result of environmental factors and lifestyle, various changes in microbial communities occur after birth. where the microbiota composition exhibits the greatest intra- and interindividual variation before becoming more stable at about three years of age. [8]

1-2 Discovery of the human microbiome:

In 1970, Antoine van Leeuwenhoek, the pioneer of microbiology, became the first, to identify the diversity of the human microbiome, from water, mud, and dental plaque samples. He obtained bacteria of various shapes, fungus, and protozoa. Among all samples, he discovered the interaction inside complex biofilm ecosystems. After discovering microbial infections in humans and animals that led to the formation of disease, Robert Koch developed the idea of pathogenicity in microbiology as well as the role of microbes in human health and disease. He classified microbial communities into two groups: those that are useful to the ecosystem and interact with their hosts or other bacteria, and the opportunistic microorganisms that can cause disease.[9]

In 1909, American bacteriologist Arthur I Kendall demonstrated the effect of nutrition on the composition of intestinal bacteria in monkeys, as well as the consequences of these microorganisms on their health. Some researchers investigated the interactions between non-pathogenic microbes and the host. Microorganism detection was time-consuming at the time, and the available technologies couldn't distinguish between a wide variety of bacteria species. Furthermore, the number of colonies counted under a microscope was never the same as the number of colonies produced on an agar plate. [10]

Rene Dubos of the Rockefeller Institute for Medical Research (later renamed Rockefeller University) discovered in the mid-1950s that some bacteria are beneficial to the body because they participate in a well-defined ecosystem and convert into virulent pathogens under certain conditions. [11]. Following the finding of the above-mentioned fact, researchers recognized the mechanisms of tuberculosis immunological resistance. Because bacteria can inhibit, promote, and change the behavior of their neighbors. Dubos stressed the importance of evaluating microbes while taking into account the entire ecosystem and their interactions with one another and the

environment, which appears to be unachievable. [12]. In the late 1950s, Dubos and colleagues began a series of experiments in which they introduced different types of microbes into previously germ-free mice to see how they colonized the stomach. They discovered some gut microflora in the middle of the 1960s, it is discovered that the microenvironment of the gastrointestinal tract plays a major role in the colonization of definitive microorganisms. The colonized microorganism might be able to figure out the link between stress, nutrition, and antimicrobial agent in bacterial colonization and human health. For example, germ-free female mice fed with a low-protein diet during pregnancy produced offspring with decreased dopamine and norepinephrine levels in their brains. [13,14].

For the first time, scientists can more properly characterize and quantify the types of microorganisms present in every community, as well as determine their biological activities, thanks to the advent of novel tools such as PCR and sequencing. These improvements increased DNA sequencing capacity while also speeding up and lowering the cost of the process. By 2005, DNA technology had progressed to the point that most scientists could now sequence the DNA of an entire microbial community in a sample affordably and efficiently. These enabled researchers to move away from studying the features of particular types of organisms in isolation and toward studying the full network of entire communities. [9]

The Human Microbiome Project (HMP) was founded in 2007 and marked a turning point in the study of the human microbiome to improve our knowledge of the microbiota's role in human health and disease and understand the development of the diseases and how can scientists prevent or even treat diseases. This study comprises two phases: the first phase (HMP1) identifies human microbiota, and the second phase, also known as the integrative human microbiota project (iHMP), identifies bacteria and their roles in health and disease.[15] The Human Microbiome Project was a group of roughly 80 universities and academic institutions from all over the world that came together to launch a series of coordinated projects. The goal of the NIH-funded initiative was to learn more about the microbial components of human genetic and metabolic landscapes, as well as their involvement in normal physiology and disease vulnerability. HMP researchers had established the usual bacteria composition in a healthy Western population by 2012. 5,000 samples of total human and microbial DNA were purified and analyzed from the mouth, nose, skin, and vaginal areas of 242 healthy American volunteers. This was a massive task that could only be

accomplished by drastically lowering the cost of DNA sequencing. Researchers were able to identify between 81 and 99 percent of the estimated 10,000 microbial species that live in human environments. Given that just a few hundred bacteria species had previously been isolated, their accomplishments were astonishing. [15]

Researchers at HMP are also looking at the link between microbiota and disease. They discovered that around the moment of birth, bacterial species richness in a woman's vagina was drastically reduced. Researchers at Baylor College of Medicine in Houston, for example, compared changes in the vaginal microbiome of 24 pregnant women to 60 non-pregnant women and discovered that the vaginal microbiome undergoes a dramatic shift in bacterial species in preparation for birth, characterized primarily by decreased species diversity.[16]. Another study found a fivefold increase in viral DNA in nasal samples from children with fevers compared to children without fevers.[17]

Individual bacterial species and strains were isolated and cultured in the early stages of research into human-associated microorganisms. Using the 16S rRNA gene, which is the most common culture-independent technique to analyze the microbiome is based on the 16S ribosomal RNA (16S rRNA) gene indicates the bacteria inhabiting the human body cannot be cultured in a laboratory, while quantitative PCR (qPCR) that shows these unculturable members, by using PCR amplification of 16S rRNA genes with the use of the universal primer can sequence the human microbiome. Next-generation sequencing (NGS) allows for the sequencing of a large number of nucleotides in a short amount of time at a low cost, resulting in massive amounts of data. [18,19]. The field of microbiome research has been developed by recent advancements in metagenomics technologies, as well as the availability of quick and cost-effective sequencing platforms for the understanding of the human microbiome that is still critical. Bioinformatics and high-throughput sequencing techniques have made it easier to identify the amount and variety of human microbiota in various bodily niches and find the probable link between the microbiome and certain diseases.[20]

1-3. Members of human microbiota:

Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcus, PeptoStreptococcus, and *Bifidobacterium* are the most prevalent genera among the bacterial species found in human guts. *Escherichia* and *Lactobacillus* are also present in the gut but to a lesser extent. Several of these bacteria can affect host homeostasis, and some of them are

pathogens. [21] . Microbiotas are typically thought of as living microorganisms that have colonized a certain part of the human body, but bacteria, archaea, fungi, algae, and small protists are considered members of the microbiome that habitat in various environments. Hence, phages, viruses, plasmids, prions, viroid, and free DNA are commonly thought to survive in living microorganisms. They do not belong to the microbiota community since they're not considered living microorganisms.[9]. The term microbiota refers to their activities, which include community microorganisms. As a result, the microbiome should contain all mobile genetic components, including phages, viruses, "relic" and extracellular DNA. The microbiome and the metagenome are both terms that are frequently misinterpreted. The term "metagenome" refers to a collection of genomes and genes from the microbiome. [22]

The microbiota is made up of a large number of microorganisms such as bacteria, viruses, and yeast that live in different regions of the human body, often known as the "hidden organ." As a result, the microbiome refers to the entire genome of a microorganism found in the environment, such as plants, nature, or animals. The term microbiome refers to the majority of microorganisms, as well as structural elements, metabolites, and environmental factors. [23]. As members of the microbiome, bacteria, archaea, fungus, algae, and tiny protists should indeed be considered. [24]

1-4 - Microbial diversity in healthy humans:

The diversity of microbial community within the body is characterized by the frequency of different types of organisms that have been linked to various human disorders, such as inflammatory bowel disease and vaginal bacterial vaginosis. [25]. The Human microbial communities contain roughly 81-99 percent of all microorganismal genera, according to studies on microbiome diversity conducted on healthy people in two different geographic areas in the United States. In terms of community membership, oral and fecal communities are complex, whereas the bacterial community of vaginal areas is very simple. [26]

The classification of human body microbiota into the oral, cutaneous, intestinal, and vaginal groups is based on studies on microbiome diversity conducted on healthy persons. As a result, the individual uniqueness of the microbial community appears to stay consistent throughout the time (in comparison to the population as a whole). [25] .*Streptococcus* is the most common species in the oral cavity [27]; however, *Haemophilus* species colonize the buccal mucosa, *Actinomyces* species

colonize superregional plaque, and *Prevotella* species colonize the lower oxygen regions of subvaginal plaque. [26] .Previously, data from 16s RNA profiling of members' microorganisms in healthy humans revealed that some species, such as vaginal *Prevotella amnii* and intestinal *Prevotella copri*, and vaginal *Lactobacillus spp*., are environment specific. [27].

As a result, no opportunistic "pathogens" as defined by Pathosystems Resource Integration Center (PATRIC) Canonical pathogens such as *Vibrio cholera*, *Mycobacterium avium, Campylobacter jejune*, and *Salmonella entries* were found. *E. coli* was detected in an abundance of 0.1 percent in 15 percent of the stool microbiome (in an abundance of 0.0 percent in 61 percent) and *Helicobacter pylori* was found in 0.01 percent of the stool microbiome.[27]

1-4-1 Human microbiota in maintenance of health:

Commensal microbiota can affect human health, such as gut microbiota, which contributes to the healthy development of the human system by maintaining pathogen displacement, immune system development, vitamin generation, and nutrient absorption. [28]. For example, on the surface of the mouth, there are few harmful microorganisms because it is colonized by the beneficial microbiota; this could be the effect of the commensal microbiota on human health. [29].

Because the microbiota composition is relatively stable within healthy adults over time for bacteria and viruses due to the presence of antimicrobial compounds that can be produced by intestinal bacteria to compete against pathogens, understanding the stability of an individual's microbiota is critical for predicting disease onset and developing therapies to correct dysbiosis (imbalances in the microbial community). The microbiota and the host have a beneficial connection in the normal gut. The microbiota is given stable growing circumstances and a consistent supply of nutrients from its host. In exchange, the microbiota supports the host's angiogenesis, digestion, immune system growth, and fat storage. This complex network of interactions is expected to keep the microbiota's population structure stable and prevent disease invasion. [30,31]

After birth, commensal bacteria quickly invade the host. With the growth of the body, this simple community rises steadily during the development of bacteria, compromising a very diversified ecology. [32]. Host-bacterial interactions have developed into valuable connections over time. Symbiotic bacteria metabolize indigestible chemicals, deliver essential nutrients, defend against opportunistic pathogen colonization, and aid in the creation of gut architecture. [33] . The intestinal

microbiota, for example, plays a critical role in maintaining power homeostasis by breaking down meals that cannot be digested through the stomach and small intestine action. These meals are high in general nutritious fibres, such as xyloglucans, which are commonly found in vegetables and must be digested by *Bacteroides* species. [34] . Fructo-oligosaccharides (FOS) and oligosaccharides are examples of non-digestible fibres that can be used in conjunction with *Lactobacillus* and *Bifidobacterium* activities. [35].

Short-chain fatty acids (SCFAs), which include acetic, propionic, and butyric acids, are produced by the typical gut microbiota at a rate of 50–100 mmolL-1 per day and serve as a power source for the host intestinal epithelium. [36] . These SCFAs are quickly absorbed in the colon and play a variety of roles in regulating intestinal motility, inflammation, glucose homeostasis, and energy supply. [37] Furthermore, it has been demonstrated that the gut microbiota provides nutrients to the host, including folates, vitamin K, biotin, riboflavin (B2), cobalamin (B12), and maybe other B vitamins. B12 could be made using delta-amino levulinate (ALA) as a precursor, according to a previous study. In addition, bacteria that colonize the intestine stimulate the development of the humoral and cellular immune systems, as well as the balance of immunity components in the gut mucosa. [38] .

Microorganisms' signals and metabolites can be detected and translated into physiological responses using hematopoietic and non-hematopoietic cells of the innate immune system. [39]. Germ-free (GF) mice show noticeable deficiencies in the improvement of intestine-associated lymphoid tissue and antibody production, according to studies comparing normal mice to GF mice. The intestinal microbiota also produces a tolerogenic reaction that operates on intestine dendritic cells and inhibits the type 17 T-helper cell (Th17) anti-inflammatory pathway, according to a study. However, not all bacteria play a role in being beneficial. Some cause inflammation when certain conditions are met. [39]

1-4-2 Human microbiota in maintenance of disease:

The gut microbiota is made up of six phyla: *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria,* and *Verrucomicrobia. Firmicutes* and *Bacteroidetes* are the most important because they can reflect gut microbiome disorders, which can alter the structure and function of intestinal flora and lead to diseases. [24,40]. Alterations in the commensal microbiota

make-up, (both structure and function) can cause metabolic and nutritional changes in the intestine, which can allow infections to flourish and inhibit beneficial bacteria and it can affect human health. [41]. Modern western lifestyle and diet, which include red meat, animal fat, high sugar, and low fibre foods in the diet, can have an impact on microbial dysbiosis and on how the microbial community of the human gut is shaped. [42]. Dysbiosis microbiota is defined as an imbalance in the microflora, modifications to their functional composition and metabolic processes, or a change in their local distribution that disturbs the homeostasis of the microbiota, that one might fail to provide the host with the full complement of beneficial properties, and they become a consequence of the disease, including liver diseases, the human microbiota, and infectious diseases, the human microbiota associated with gastrointestinal malignancy, the human microbiota and metabolic disorders such as obesity. [3,42]

1-4-2-1 Inflammatory Bowel Disease (IBD):

Over time, a balanced and organized relationship between the human microbiome and the immune system takes place. A change in the host's microbiota modifies this interaction, impairing the immune response and raising the possibility of an inflammatory illness. Inflammatory bowel diseases (IBD) are a chronic disorder of the gastrointestinal (GI) tract, as a result of the direct contact between the intestine and the microbiota, the microbiota's composition can change and the pathophysiology of numerous intestinal diseases including ulcerative colitis (UC) and Crohn's disease (CRD). So, IBD development is strongly correlated with the gut microbiome. In recent years, this condition has become a major health concern according to geographical location, and its frequency rises with age. Some environmental factors, such as commensal microflora, pathogenic infections, metabolic factors, diet, smoking, and social stress, have a role in the development of IBD. For example, a pro-rich western diet can increase the risk for the development of CRD and UC. [43,44]

Metagenomic analysis of CD revealed a decrease in *Firmicutes*, specifically *F. prausnitzii,* (which resulted in increased levels of proinflammatory cytokines (IL12, IFN-c), and a reduction in anti-inflammatory cytokines (IL-10)), and an increase in *Enterobacteriaceae*, particularly the virulent invasive *E. coli*. Patients with CD and UC have much lower levels of the beneficial microorganisms *Bacteroides, Eubacterium,* and *Lactobacillus*. [24,45,46]

CD affects the GI system from the mouth to the stomach with symptoms including abnormal pain, fever, and weight loss. The pathology of UC is restricted to the mucosal surface of the colon. CD is characterized by the T helper 1 Th1 response and is mediated by high levels of tumour factors TNF, while the pathology of UC is distinguished by the atypical Th2 response and is mediated by high levels of Th17 cells, it affects the large intestinal and gradually extends through the entire colon. [47,48]. With the development of sequencing technology, it was discovered that *Helicobacter pylori* were the dominant phylotype in the stomachs of chronic gastritis patients and that *Helicobacter pylori's* interactions with other microorganisms in the gut microbiota can increase the risk of gastric cancer. The healthy human stomach is dominated by *Prevotella, Streptococcus, Veillonella, Rothia*, and *Haemophilus*. [3,49]. Before chronic gastritis develops, *Helicobacter pylori* removal can reduce the chance of developing the gastric disease. The genetic diversity of the *H. pylori* strains, variability in host responses, and host-microbe interactions are some of the causes of the carcinogenic issues. [3]

1-4-2-3 Metabolic disorders:

Over the past few decades, obesity and related conditions including type 2 diabetes T2D have become more prevalent. The use of antibiotics and the lifestyle of the human host, including exercise, diet, and cleanliness habits, have an impact on the composition of the gut microbiota and its involvement in controlling host metabolism. In addition to interactions between the gut microbiota and the host genotype or dietary changes, changes to the gut microbiota have a significant role in the ethology of obesity and diabetes. [3]. Obesity and type 2 diabetes (T2D) are co-occurring health issues, and environmental factors including nutrition and gut microbiota might increase the risk of T2D. [3]

Obesity is linked to altered metabolic pathways and dysbiosis of the gut microbiota (Dysbiosis in gut microbiota has been found to play a role in obesity), it has been demonstrated that the composition of the gut microbiota varies between obese and lean individuals in humans and change in the gut microbiota is indicated by a decline in the *Bacteroidetes* and an up with rapid in the *Firmicutes*.[50]. Recent metagenomics investigations revealed that type 2 diabetes (T2DM) patients have fewer butyrate-producing bacteria such as *Roseburia spp*. and *Faecalibacterium spp*., suggesting that butyrate-producing bacteria may protect against T2DM. [50]

1-5 Human dental calculus:

Dental calculus which forms both above (supragingival) and below (subgingival) in gumlines a hard deposit created by the mineralization of dental plaque on the surfaces of natural teeth and dental prosthesis, which are generally covered by a layer of unmineralized plaque. [51]. Bacterial plaque retention, biochemical factors (defined by saliva or crevicular fluid), microorganisms and nutritional factors, the presence of *S. mutans* bacteria, and genetic polymorphisms in the salivary glands are all linked to calculus development. [51,52]. Microorganisms colonize the tooth surface or below at the gingival margins, causing periodontal disorders, as a result, there is always a correlation between the presence of calculus and the prevalence of gingivitis. Dental calculus was considered to be a primary etiologic factor in the initiation and progression of periodontal diseases.[52]. Gingivitis is caused by dental plaque, which is linked to poor oral hygiene. It is a common condition in people of all ages, with variable degrees of severity, and it is practically common in children and teenagers. Periodontal diseases include the gingival illnesses caused by dental plaque, aggressive periodontitis, chronic periodontitis, eruption gingivitis, and puberty gingivitis. [53]

1-6 Oral microbiota:

The infant's mouth and oral cavity are sterile before birth, but after birth, it encounters the internal and external environments and acquires nourishment, resulting in microbial colonization. The microbe began to establish itself in a specific area of the oral cavity over time, posing a threat to the oral microbiota. [54] . *Streptococci* species were first discovered in the environment. The diversity of colonized microorganisms grows over time, until they become a permanent resident of the colonized area, relying on compensating mechanisms to maintain suitable conditions in the oral cavity. The mucosa and teeth in the oral cavity are constantly in contact with external microbiota, and dentition health is also influenced by the proportions of different microorganisms. The provision of inappropriate food can disrupt the oral ecosystem's balance, resulting in the spread of pathological conditions throughout the mouth. [55]

Bacteria, fungi, viruses, and archaea make up the oral microbiota, with bacterial populations dominating. [55]. The number of fungal species in the oral microbiome has been shown to range from 9 to 23. However, according to the 16srRNA detection approach, more than 700 bacterial

species are found in the oral cavity of humans; additionally, roughly 50% of bacterial species are not grown, and their significance in microbial ecology and oral cavity is unknown. [56]

Approximately 1000 bacterial species can colonize the oral cavity, with 50-200 different species able to coexist. [57]. While circulating through saliva, most types of bacterial microbiota produce biofilm on teeth or Musa. The phenotypic of colonized bacteria is overestimated, according to biodiversity studies such as pyrosequencing 454 and Illumina sequencing of mouse cavities. Plaques are colonized by around 600 bacterial strains. Other sequencing methods identified 3621 phylotypes in saliva and 6888 in a subgingival plaque. [55]. Oral cavity bacteria are divided into several strains based on the phylogenetic study, including *Firmicutes, Fusobacteria, Bacteroidetes, Actinobacteria, Proteobacteria, Spirochaetes,* and *Synergistetes*. The majority of strains are dominant. [55]. The mouth cavity defends the gastrointestinal system against germs, in addition to being the source of food and water intake, taste discrimination, temperature, and pressure. [58]. Due to anatomical and physiological conditions, the microbiota of the oral cavity varies; there is also diversity in different sections of the oral cavity, such as at the orifices of salivary glands, on the surface of teeth, in the gingival sulcus, on the tongue, at tonsils, or in the buccal mucosa. Temperature, pH, oxidation-reduction potential, availability of nutrients and water, oral architecture, saliva flow, and the presence of antimicrobial chemicals that maintain the microbial balance, are some of the elements that contribute to the proliferation of oral microorganisms. [59].

The following are the most common bacterial communities found in the gingival sulcus:

Staphylococcus epidermidis, Streptococcus sanguis, Streptococcus mitis, Micrococcus spp., Mycoplasma spp., Trichomonas tenax, Entamoeba gingivalis, Streptococcus ntermedius, Micrococcus spp., Micrococcus spp., Micrococcus spp., Micrococcus spp., Micrococcus spp., Micrococcus spp., Micro, Streptococcus billorum, Streptococcus c stellatus, PeptoStreptococcus mi os, Veillonella parvula, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus rhamnosus, Lacto, Eubacterium\slentum, Propionibacterium acnes is a bacterium that causes acne. *Catonella spp., Johnsonella spp., Rothia dentocariosa, Catonella spp., Catonella spp., Catonella spp., Catonella spp. Actinomyces viscosus, Actinomyces odontolyticus, Actinomyces naeslundii, Actinomyces naeslundii, Actinomyces naeslundii, Actinomyces naeslundii Capnocytophaga gingivalis, Capnocytophaga ochracea, Capnocytophaga ochracea,*

Capnocytophaga ochracea, Capnocytophaga Prevotella denticola, Prevotella oralis Bacteroides melaninogenicus, Bacteroides melaninogenicus, Bacteroides melanin Fusobacterium nucleatum, Fusobacterium nucleatum, Fusobacterium nuclenucleienella corro-dens, Eikenella corro-dens, Eikenella corro-den, Wolinella spp., Campylobacter sputorum, Selenomonas sputigena, Treponema spp., and Leptotrichia spp., Granulicatella spp., Wolinella spp., Campylobacter sputorum, Selenomonas sputigena, Treponema spp.*, Treponema spp*. The parasitic protozoa *Entamoeba gingivalis* and *Trichomonas tenax* are two of the more notable representatives. [59]

1-6-1 Oral *Streptococci*:

In healthy people, commensals or non-pathogenic bacteria like *Streptococci, Actinomyces,* and *Veilonella* make up the majority of the oral microbiota.[60]. A higher proportion of commensal with beneficial qualities, such as *S.gordonii, S.sanguinis*, and, is generally linked to dental health. Dental plaque caries can be triggered by diminished salivary flow and a diet high in fermentable carbohydrates. [61]. Other alternate strains such as *S.vestibularis, S.salivarius,* and *S.sobrinus* are also acid producers in caries beginning. Carbohydrates have the potential to disturb the ecology of this microbial community by favouring acidogenic and acid-tolerant species that cause tooth caries. [62]

Streptococci, one of the most common bacteria in the human mouth, are facultative. Oral *Streptococci* are categorized into four groups based on the 16srRNA gene, which is used to establish phylogenetic relationships within the oral cavity. The anginous, mitis, mutants, and salivary groups are the biggest ones found in the oral cavity and detected in the mouths of new-born infants. [61]

1-6-2 Pathogenic bacterial species:

Periodontal disease is dependent on a succession of intricate host/bacterial interactions, a complex microbial biofilm that colonizes the tooth surface and gingival margins. They are bacteria that are found in the gums and tooth support structure, and they play a vital part in the development of periodontitis. Both *P. gingivalis* and *T. forsythia* have been consistently found in high numbers in periodontitis patients, and while the pathological mechanisms of these bacteria are still being

investigated, it is their unique virulence qualities that allow them to evade the host immune system and produce the disease's destructive characteristics. [63]

Oral microbiota also has the main effect on the progression of systemic diseases especially in gastrointestinal disease. The periodontitis pathogens, for example, can disseminate through the whole body in the case of periodontal inflammation which enter to the bloodstream and migrates to other regions [64], also, the metabolite of oral microbiota could enter to the blood circulation promoting the development of chronic inflammation especially in gastric regions [65], and could cause the inflammatory bowel disease especially in developed country. *Porphyromonas gingivalis* and *F. nucleatum* as the main risk factor of periodontal disease that while passed through intestinal barrier render the systemic inflammatory response. The bacteraemia following tooth extraction and endocarditis mostly affected by *S.mutans. Streptococcus*, *Prevotella*, *Neisseria*, *Haemophilus*, *Veillonella*, *Campylobacter* and *F. nucleatum* are the main cause of IBD, whereas *Veillonella, Streptococcus, Prevotella, Haemophilus, Lactobacillus,* and *Clostridium* render the occurrence of liver cirrhosis. *P. gingivalis*, *F. nucleatum*, *Treponema denticola, P. gingivalis*, and *Candida* have been shown to mediate in occurrence of oral squamous cell carcinoma (OSCC). Furthermore, *F. nucleatum* has been observed to effect on occurrence of colorectal cancer and *P. gingivalis* promotes the development of diabetes, Alzheimer's disease, atherosclerotic plague. [66]

1-6-2-1 *Porphyromonas gingivalis*:

Porphyromonas gingivalis is a gram-negative, non-motile, saccharolytic, anaerobic bacteria, in the shape of short sticks.[67]. Because they are indole positive, they cannot ferment sucrose. [68]. *P. gingivalis* is found in low abundance in healthy oral flora, but this quantity rises during the transition from a symbiotic to a dysbiosis microbiota, due to the accumulation and multiplication of the bacteria within the tooth plaque. Even though the cause of periodontitis is unknown, the prevalence of *P. gingivalis* inside the disease, as well as the pathogen's ability to manipulate the oral bacterial community, has led to *P. gingivalis* being classified as a 'keystone' pathogen within the periodontal disease. [69,70]

1-6-2-2: *Tannerella forsythia*:

Tannerella forsythia, a gram-negative anaerobic bacterium belonging to the *Cytophaga Bacteroides* family, was first isolated from the oral cavity and named *Bacteroides forsythus*. [68] While the prevalence of *T. forsythia* in periodontitis has been thoroughly proven through clinical investigations, the bacterium's virulence factors remain unknown. *T. forsythia* has been found to produce enzymes that shield the bacterium from the innate immune system while also digesting host proteins for molecular resources. It has also been proven to upregulate the expression of a variety of host genes, which has a direct effect on host cell transcription. *Forsythia* in the oral cavity can induce gingivitis and periodontal illnesses due to virulence factors such as cutaneous abscesses in mice due to the synergistic effect of *T. forsythia* and *P.gingivalis*, and another serpin protein that can shield bacteria from neutrophil proteolytic actions. [68,71] .

1-6-3 Oral microbiota on the surface of teeth:

Bacteria that form a complex matrix, as well as extracellular products of microorganisms and saliva components, make up dental plaque. Gram-positive, facultative anaerobic bacteria, particularly *Streptococci*, and members of the genus *Actinomycetes*, are the most common bacteria recovered from supragingival plaques. *Veillonella, Haemophilus*, and *Bacteroides* are typically found in the deeper layers. The stages of plaque formation are the creation of pellicle, early bacterial adhesion, bacterial colonization, plaque maturation, and lastly plaque mineralization and calcification. For instance, dental calculus formation. [72]

Because of virulence characteristics such as acidogenesis, acid survival, and proton ATPase activity in *S.mutans*, some bacteria can attach to the tooth surface. Tooth decay can be caused by metabolites and other bacterial disorders in other regions of the oral cavity. [73]. The incidence of bacterial disease in the oral cavity increases if the balance between potentially harmful microorganisms and the indigenous microbiota is disrupted. As a result, these microbes may be pathogenic under certain conditions, such as pH imbalance, and are thus classified as opportunistic pathogens. *Streptococcus Sanguis, Streptococcus mutans, Neisseria, Lactobacillus, Propionibacterium, Actinomycetes, Lepttrikia, Fusobacterium, Veillonella, Bacteroides*, and *Bacterionema* have also been isolated from the surface of teeth. [59]

1-6-4 Oral microbiota of the tongue:

Streptococcus Salivarius was isolated from the tongue shortly after birth, and later in the teething stage, *Streptococcus mutans* and *Streptococcus Sanguis* emerged in the oral cavity. [74]. The tongue can act as a reservoir for bacteria that cause periodontal disease. Saliva contains bacteria from many areas of the oral cavity, and the microbial composition of saliva is similar to that of the tongue. [75]. The microbial community of tongue is highly stable and play a major role exogenous NO production and biosynthesises, the bacterial community such as *Veillonella, Actinomyces, Prevotella, Neisseria*, and *Haemophilus* have an outmost importance in no production pathways. According to Chinese traditional medicine each of five tastes have a main effect on tongue surface microbiota. The five tastes in tongue have carbohydrate, amino acids, fat, and bitter receptors indicating the type of food closely related with metabolic system and the tongue microbiota directly associated with taste function; people consumed beverages mostly are insensitive to salty flavours than others, whereas people who are insensitive to sweet flavour intended to eat more sweety food. The frequency of *Prevotella* is higher in vegetarian people and the frequency of *Clostridia* is higher in people using hight protein/ fat diet. Furthermore, tongue microbiota interacts with chemosensory on tongue and effect on metabolic systems. [76]

1-6-5 Oral microbiota of saliva:

Saliva fluid, which contains free fluoride ions in concentrations ranging from 0.01 to 0.05 ppm, is a key role in dental enamel remineralization. Some calcium, fluoride, and phosphate in saliva indicate a possible remineralization action on tooth tissue. Saliva has a beneficial effect on caries formation, which is due to the unsaturated content of phosphate, fluorine, and calcium in saliva, as well as the continual replacement of ions between the tooth and saliva. At Neutral PH, there is a balance between enamel minerals and saliva, but this balance is disrupted when bacteria in the oral cavity produce acid, which contributes to the demineralization of the tooth surface. As a result, saliva is produced to prevent dental decay by reducing the acidic environment and decay rate. Saliva has a high buffering capacity due to phosphates, bicarbonate, and proteinaceous buffers. [77].

Glycoprotein mucin promotes chewing, swallowing, and talking by acting as a lubricant for the mouth surface and a protective barrier from the external environment. Bacterial aggregation is caused by one of the agglutination factors saliva. *Streptococcus sanguis, Streptococcus mitis,*

Streptococcus gordonii, Aggregatibacter actinomycetem comitans, Pseudomonas aeruginosa, and *Escherichia coli* can all interact with the glycoprotein. Also, saliva contains hormones, glucose, cholesterol, fatty acids, and urea, among other biologically active chemicals. Because the PH of the oral cavity is somewhat neutral, between 6.75 and 7.25, microorganisms are unstable when the PH fluctuates. Saliva demonstrates remineralization abilities, although the remineralization process takes time. [59]

1-6-6 Factors shaping the gut microbiota:

Host and environmental selective factors can influence the composition of the microbiota. The gastrointestinal tract limits the host immune system's exposure to the microbiota by employing a multifactorial and dynamic intestinal barrier. Because the mammalian intestine microbiota and their host have a symbiotic relationship up until the opportunistic microorganisms break down their symbiotic host-microorganism connection and cause disease, this limits the host immune system's exposure to the microbiota. The human oral microbiome is unaffected by geographic variation. [78]

Many aspects, like contacts with the outside environment, network institutions, internal interactions among specific organs, diet, feeding methods, geographical location, smoking, depression, living arrangement, and so on, are used to motivate microbial network homeostasis and to shape the gut microbiome. [79]

1-6-6-1 Mode of delivery:

Using vertical transmission, the microbiota can be transmitted from mother to infant at the time of birth. The type of microorganisms encountered first is determined by the mode of distribution. Microorganisms can be obtained from the vaginal canal in the case of normal shipping or from the pores and skin in the case of caesarean delivery . The taxonomic variation of oral microbiota was shown to be higher in 3-month-old babies delivered vaginally, according to studies [80]. When new-borns are exposed to an environment, adaptive immunity develops, and the microbiome is influenced by the environment, even though the impact of the environment is minor in comparison to delivery modalities. The oral microbiota is mostly affected by immunization with attenuated

microorganisms, fitness, age, and people's regular habits, as well as smoking (active and passive smokers). [81]

1-6-6-2 Feeding habits:

Feeding patterns can also alter an infant's oral microbiome. Oral lactobacilli with antibacterial properties are present in the oral cavity of breast-fed babies, but are absent in spontaneous formula-fed babies, according to studies. [82]. Oral microbiome diversity can also be acquired through horizontal transmission among humans who share similar environments and habitats. [83], and while infants' adaptive immunity to the environment is formed, the microbiome is influenced by the environment, though the impact of the environment is negligible in comparison to delivery methods. The oral microbiota is mostly affected by immunization with attenuated microorganisms, fitness, age, and people's regular habits, as well as smoking (active and passive smokers). [81]

Dietary components can shape the bacterial composition, for example, the *Bacteroides* genus is highly associated with the animal protein, and amino acids that are found in western diets, while *the Prevotella* genus is associated with high carbohydrate and sugar consumption. Some studies describe the relationship between exercise and diet microbiota. [84]. In athletes who need to consume more calories, protein, fat, and carbohydrate, exercise plays a crucial role in the interaction between the microbiota, host immunity, and host metabolism, as well as diet. [85], for example, dietary carbohydrate sources such as diet-rich sugar are served as an energy source for oral bacteria. Those who consume a lot of carbohydrates in their oral cavities have an abundance of acidogenic (acid-producing) and aciduric (acid tolerating), bacteria such as *Streptococcus mutans* can lead to tooth decay and deterioration of saliva buffering caries and has cariogenic properties . *S. mutans* in their mouths and the acid produced by these bacteria can cause dental caries. *Streptococcus mutans* and *Streptococcus Sobrinus* can metabolize simple sugars and stimulate growth. [86]

1-6-6-3 Smoking:

Smoking causes nasal mucociliary passage disruption and attachment to the epithelial surface; also, bacteria such as *Acinetobacter, Bacillus, Burkholderia, Clostridium, Klebsiella, Pseudomonas aeruginosa*, and *Serratia lineages* have been detected in cigarettes, contributing to infections . The presence of pathogens such as *Haemophilus influenzae, Streptococcus*

pneumoniae, and *Moraxella catarrhalis* in the nasopharynx of smokers has been documented, as has the absence of indigenous microbiome members such as *Prevotella* and *PeptoStreptococcus* , which prevents the spread of invading pathogens . Periodontitis-causing bacteria such as *Fusobacterium, Parvimonas, Campylobacter*, and *Bacteroides* have been discovered in the subgingival environs of cigarette smokers' oral hollow spaces. It has been discovered that once smoking is stopped, the nasopharynx and oral cavity are once again governed by the pleasant indigenous microflora. [87]

In people who smoke, the number of *Megasphaera spp., Firmicutes, Streptococcus, Vellionella, and Atopobium spp., Eggerthella, Erysipelotrichaceae I.S., Dorea, Anaerovorax,* and *Eubacterium spp*. has increased in comparison to nonsmokers, according to some studies based entirely on the most recent techniques such as univariate evaluation and system. The majority of the species mentioned above have a strong link to oral infections. *Shigella spp*. is the most basic species whose abundance has been reported to decrease in the nasopharynx of smokers. [87,88] .

1-7 Gut microbiota:

The human microbiota of the gastrointestinal tract is considered a large part of the host genome and even that of host cells, containing various species of known bacteria, the majority of which are obligate anaerobes from the genera *Bacteroides, Eubacterium, Clostridium*, and less prevalent facultative anaerobes such as *Lactobacillus, Escherichia, Enterobacter*, and *Enterococcus*. [89] Bacteria, yeast, and viruses make up the gut flora. *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacterium,* and *Verrucomicrobia* are the primary bacterial strains in the intestine. 90% of the gut flora is made up of two *Firmicutes* and *Bacteroidota* strains. [89,90]. Over 200 genera make up the *Firmicutes phylum*, which includes *B. Lactobacillus, Bacillus, Clostridium, Enterococcus,* and *Ruminicoccus*. Approximately 95% of *Firmicutes* species are *Clostridium* genera. The most common genera are *Bacteroidetes*, and *Prevotella. Actinomycetes* are proportionally less prevalent, with *Bifidobacterium* being the most common species. [90]

The gut microbiota influences the immune system, intestinal integrity, brain development, vitamin biosynthesis, pathogen defence, nutrition processing, xenobiotic metabolization, antifungal, antiviral, and antibacterial substances production, and infection risk reduction [89] . The bacteria in the gut perform a variety of functions, including nervous system modification, food compound breakdown, immune system development, and epithelial injury protection. These bacteria can have

an impact on the gastrointestinal tract by colonizing a large number of microbiota communities in contact with the host. [20]

The intestine is sterile at birth; however, intestinal colonization begins shortly after birth, and microbes from the mother swiftly colonize the new-born's digestive tract (vaginal, faecal, skin, breast, etc), microbiota becomes more stable and similar to adults as they grow older. *Lactobacillus* spp and *Bifidobacterium* spp, which are comparable to their mother's vaginal canal, are present in vaginally delivered infants, whereas skin microorganisms such as *Staphylococcus* are present in caesarean-delivery infants. The gut microbiome of a breastfed new born is dominated by *Bifidobacterium*, but the gut microbiome of a baby-fed infant formula is dominated by gram-negative bacteria. [91]

Dietary components can influence gut bacteria and affect babies' composition. For example, a diet high in fibre is vital for microbe growth, and prebiotics that is not digested by humans can be sustained by beneficial colonic microorganisms, leading to the proliferation of gut microbes. *Bifidobacterium adolescentis* and *Ruminococcus bromi* consume starch and can develop as a result of their ingestion. [92].

Probiotics, defined as live microorganisms by the FAO/WHO, can provide health advantages when given in sufficient quantity to the host, such as *Bifidobacterium* and *Lactobacillus*, to preserve health. These products have several key impacts, including immunological regulation, bioactive chemical generation, and prevention of diarrhoea, acute upper respiratory tract infection, and eczema in children. [92]

The gut microbiome can also influence obesity since *Staphylococcus aureus* can cause obesity in children. As a result of increased caloric storage in the intestinal microbiota, gene expression changes coding for enzymes involved in nutrition and digesting mechanisms, and early alterations in faecal microbiota composition in children may predict overweight. On the other hand, some microbial populations, such as *Faecalibacterium Prausnitzii*, have been negatively linked to obesity. [89]. The anatomical location of the intestine, physiology, pH, O2 tension, digestive flow, substrate availability, and host secretion all influence the intestinal flora. [93]. The small intestine provides a challenging environment for microbial colonization due to bile secretion, which contributes to the short and transient colonization time, whereas the large intestine provides a suitable environment for microbes, primarily obligate anaerobic species, due to slow flow rates and a neutral to slightly acidic pH. [94]

1-7-1 Development of gut microbiota:

The intestines are sterile and bacteria-free at birth. After delivery, the microbial flora of the mother's skin, vagina, and feces meet the microbial flora of the surroundings, creating a rich and dynamic ecosystem. The sort of labor determines how the microbial flora colonizes. [95]. Newborns acquire a microbiome makeup like their own maternal vaginal microbiota during vaginal delivery. Indeed, a strong association was found between newborn gastrointestinal microbiota and the microbial community of the mother's vagina, which comprised *Lactobacillus, Prevotella, Sneathia, Bifidobacterium longum*, and *Bifidobacterium catenulatum,* according to the examination of infant meconium. Other facultative anaerobic species that colonize the infant's intestine include *Escherichia coli, staphylococci, Bacteroides fragilis, and streptococci* [96].

Babies born via cesarean section, on the other hand, receive germs from the cesarean section, the hospital environment, and the mother's skin: *Staphylococcus, Corynebacterium, and Propionibacterium . E. coli, shigella, and Bacteroides* species are overestimated in infants born via Caesarean section. While the diversity of intestinal microbiota was lower in Caesarean sections than in vaginal deliveries, the diversity of intestinal microflora was higher in vaginal deliveries. [97]

Solid food and milk supplies are being introduced. With the prevalence of *Bifidobacterium* and *Clostridium coccoides*, weaning contributes to profound alterations in gut microbiota. Eating habits and weaning are major determinants in the establishment of gut flora. *Firmicutes and Prevotella* increase with the introduction of high fiber and high carbohydrate diets (traditional foods), but with the introduction of high fiber and animals, *Firmicutes* and *Prevotella* decrease. The phylum *Bacteroides* grows in response to protein-rich foods. [98]

1-7-2: The impact of the diet on the gut microbiome:

People's eating habits have gradually changed over time, from traditional farming to the industrial era, and food in the industrial era has not strengthened our gut microbiota due to society's development and the increasing occurrence of numerous non-communicable diseases. Diet and nutritional status are among the most important modifiable determinants of human health. The nutritional value of food is influenced in part by a person’s gut microbial community (microbiota)

and its component genes (microbiome). The microbiota is dependent on food residues for survival and metabolism. [99]

The composition of the gut microbiome can be influenced by dietary habits in healthy individuals; it can remain stable for years, but the relative abundance of each member is very varied. Long-term eating habits and foodborne bacteria that colonize the stomach rapidly can influence the formation of the gut microbiome. As a result, changes in the food type can dramatically alter the organization of the gut microbiome. [100]. Administration of various type of food have a direct effect on microbial community of gut. The administration of food rich in plants and vegetables promote the colonization of alpha microbial taxa, whereas consumption of polyphenols, foods containing antioxidants such as fruits, vegetables, cereals, coffee, tea and wine can inhibit the growth of some specific taxa which decrease the risk of occurrence of chronic diseases. [101]

1-7-2-1: Carbohydrates:

Dietary carbohydrates are categorized as digestible and nondigestible carbohydrate sources, digestive carbohydrates can utilize a source of energy by digestive enzyme degradation, Nondigestible carbohydrates, on the other hand, play a vital function in the gut microbiome because they cannot be broken down in the small intestine and reach the large colon, where they are fermented into short-chain fatty acids by the gut microbiota (SCFA). Because the gut microbiome may produce short-chain fatty acids, which are crucial for gut health, dietary fibre can impact the structure and function of the microbiome. [99,102]

Peter J. and colleagues, for example, studied how a high-fat, high-sugar western diet affected the microbiota of mice and change the microbial community who had previously consumed a high-sugar, low-fat diet, it shows that changes or adjustments of food types can quickly change the structure of the gut microbiome. [103]. Gary D. Wu and *etc* investigated the impact of a long-term diet shift from a high-fat/low-fibre to a low-fat/high-fibre diet on the human gut microbiome, observing changes in bacterial communities in the control group in the gut microbiome within 24 hours of starting their experiment. [84]. In contrast to those who consume low-fibre diets, increased dietary fibre composition can modify the nutritional niches in the intestine and enlarge the bacterial population. Exposing mouse strains to two separate diets, such as low fat/high fibre and high fat/high sugar, revealed that the high fat/high sugar diet had a greater impact on microbial

composition and that diet-induced alterations can happen within days, [104] while the lower consumption of fibre has the risk of colon cancer, extra dietary fibre supplements seem to be beneficial and can reduce the risk of type two diabetes. [105]

1-7-2-2: Fats:

Dietary fat also affects the makeup and metabolic activity of the gut microbiota. Fat is digested in the upper region of the small intestine, where it serves as a source of calories and energy. Dietary fat and caloric consumption can impact the relative number of *Fimicutes* and *Bacteroides* phyla in the gut microbiota by the interaction of the gut microbiome with the diet composition. [106]. Gut microbiota can influence the distribution of fat in the body and the formation of various body shapes in men and women, suggesting that they may have a sex-specific microbiome and gut bacteria with varied fat distribution potential according to Yan Min e et al. [107]. A high-fat diet can lower intestinal bacterial diversity, unbalance the gut microbiota composition (due to long-term dietary intake), increase permeability and lipopolysaccharide translocation, change the immune system, cause low-intensity systemic inflammation, as well as induce liver cancer. [108]

1-7-2-3: Proteins:

According to various research dietary patterns directly associated with bacterial community in gut microenvironment. Protein, particularly amino acids (as a result they are building blocks for microbial protein, making them important for microbial growth) are essential for human body function and are the primary supply of nitrogen for gut bacteria. Protein digestion begins in the stomach and is an important aspect of a balanced diet because humans are unable to manufacture certain amino acids and must get them from proteins in foods to stay healthy. Protein-rich foods, such as meat, eggs, and nuts, can help overweight people lose weight. [109] Consumption of higher protein diet than carbohydrate decrease the rate of obesity induced by fatty acid consumption. Gut microbiotas are able convert the protein content in to some essential amnio acid which in some cases contributes to development of diseases. [110]

Protein can be hydrolysed into amino acids and peptides by protease and peptidase produced by intestinal microbiota. Also, the undigested amino acids could ferment into fatty acid, hydrogen sulphate and ammonia which are the bacteria metabolites and involved in physiological functions on host based on their concentration. [111]. The composition of dietary amino acid and metabolic depend on the bacterial composition, the *Lactobacillus johnsonii* a small intestine microbiota can metabolize the exogenous peptides to synthesis the protein. the genera of *Bacteroides*, *Propionibacterium*, *Streptococcus*, *Fusobacterium*, *Clostridium*, and *Lactobacillus, Clostridium spp.*, *Fusobacterium spp.*, *Peptostreptococcus spp*., *Veillonella spp.*, *Megashark Elsden*, *Acidaminococcus fermentans*, and *Selenomonas ruminantium* are accounts as a large intestinal microbiota have the proteolytic activity which digested the absorbed proteins in to amnio acid and administered for production of protein mediated by gut microbiota. But some metabolites can transmit to the colon and can either have a beneficial effect or toxic effect. The microbial diversity, amnio acid balanced and type of protein directly effect on intestinal microbiota and maintenance of health in human. [111]

1-7-2-4: Micronutrients:

Micronutrients account for an essential element in human health and are categorized into four major groups microminerals, microminerals, water-soluble, and fat-soluble vitamins. They have a pivotal role in metabolism through absorbance of essential elements of food intake eighter direct effect or with the help of gut microbiota. Micronutrients mediate energy metabolism, cell growth, differentiation, and proper function of the organ and immune system. The large intestine microbiota can produce water-soluble vitamins such as Thiamine, Riboflavin, Niacin, Biotin, pantothenic acid, and folate besides dietary intake. These vitamins produced by large intestine microbiota are associated with energy metabolism and combability of colon environment with the various condition. *Streptococcus thermophilus* and *Lactobacillus helveticus* are responsible for the production of Thiamin (Vitamin B1), while *Helicobacter Pylori* is responsible for de novo synthesis of B6 vitamin which is the cofactor of more than 100 enzymes in humans. Other bacteria which are responsible for the production of micronutrients including vitamins are*: Bifidobacterium bifidum* and *Bifidobacterium longum* to produce folate, *Propionibacterium freudenreichii*, *Salmonella enterica*, *Listeria innocua*, and *Lactobacillus reuteri* produce the cobalamin (VB12). It has been discovered that the production of Vitamin B12 by B. *thetaiotaomicron* neutralize the

major virulence factor of Shigella toxin 2 and enterohemorrhagic *E.Coli* (EHEC) in the colon. The fat-soluble vitamins including vitamins A, D, E, and K have mediated immune response regulation, especially T cell-mediated response. Vitamin K is mainly synthesized by *Bacteroides* sp *Enterobacter* sp. *Veillonella* sp, and *Eubacterium lentum,* whereas *Bifidobacterium infantis* is mediated in the synthesis of vitamin A. [112]

2-Aim of the study:

The current study aimed to determine the oral composition microbiota starting from the dental calculus of patients, collected during routine dental health check-up, and evaluate the effect of diet pattern on the bacterial community, to date, we lack information regarding the microbial biodiversity harboured in the dental calculus, and regarding whether it presents a certain level of inter-individual variability or, conversely, a flattened microbial biodiversity. Moreover, we currently have no evidence to disclose whether lifestyle and health variables are able to shape its composition, as observed in microbiomes belonging to other body niches.

In this study, we analysed the microbiome composition of modern dental calculus in order to define (i) the level of inter-individual variability and (ii) the role of the diet in driving its structure. To this end, taxonomic diversity and microbial community functional profiles were investigated by applying 16S amplicon sequencing methodology to 40 omnivorous female and male subjects, ranging from 20 to 77 years of age, following a Western lifestyle. This work can be considered a pilot study testing how the dental calculus microbiome could be exploited to obtain relevant clinical information on diet effects and patient health status.

2-Material and methods

2-1 Sample collection:

The samples were taken from the Clinica Odontoiatrica at the Azienda Ospedaliera of Padova under the supervision of Professor Edoardo Stellini and Dr. Adolfo Di Fiore during a routine dental examination for hygiene. Patients were asked to complete a questionnaire prior to their dental examination. Samples were collected in 1.5 mL Eppendorf tubes and sent to the Department of Comparative Biomedicine and Food Science's (BCA) Legnaro (PD) laboratory for DNA extraction. A total of 40 samples were collected.

The questionnaire asked for a variety of information, including:

1-Personal data such as age, gender, weight, height, where they reside, whether they live alone or not, if they live with animals and the study degree.

2-Information on the patient's health status, such as the presence of specific cardiovascular, metabolic, or chronic disorders, pharmaceutical use, and antibiotic therapy.

3 - Data on the Mediterranean diet derived from Francesco Sofi et al article The process can be broken down into a few steps, which include: [113]

2-2 DNA Extraction:

Samples must be crushed using a standard micro pestle, which is an accessory for tissue culture and homogenization of cells and tissues in reaction tubes by hand or with laboratory stirrers, before DNA extraction can begin.

There are five steps to DNA purification:

(1) cell lysis; **(2)** separation of soluble DNA from cell debris and other insoluble material; **(3)** binding of the desired DNA to a purification matrix; **(4)** washing of proteins and other contaminants from the matrix; and **(5)** elution of the DNA

For DNA extraction, we utilized the DNeasy Power Soil kit with a few modest modifications to the usual methodology.

All DNeasy products are designed for use in molecular biology. Using patented Inhibitor Removal Technology®, the DNeasy Power Soil Kit also contains an innovative and proprietary approach for isolating genomic DNA from environmental materials (IRT). This kit is for environmental samples with high levels of wet acidity, such as compost, sediment, and manure, as well as challenging soil types like compost, sediment, and manure.

- Microcentrifuge (10,000 x g) and performing the centrifuge steps at room temperature at 15-25 ℃
- Pipettors (50 µl–500 µl)
- Vortex-Genie 2 Vortex
- Shake to mix Solution C4 before use

Procedures:

1. Add 0.25 g of soil sample to the Power Bead Tube provided. Gently vortex to mix.

2. Add 60 µl of Solution C1 and invert several times or vortex briefly.

Note: Solution C1 may be added to the Power Bead tube before adding soil sample

3. Secure Power Bead Tubes horizontally using a Vortex Adapter for 24 (1.5–2.0 ml) tubes (cat. no. 13000-V1-24).

4. Vortex at maximum speed for 10 min. Note: If using the 24-place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.

5. Centrifuge tubes at 10,000 x g for 30 s.

6. Transfer the supernatant to a clean 2 ml Collection Tube. Note: Expect between 400–500 µl of supernatant. Supernatant may still contain some soil particles.

7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min. Note: You can skip the 5 min incubation. However, if you have already validated the DNeasy Power Soil extractions with this incubation we recommend you retain the step.

8. Centrifuge the tubes for 1 min at 10,000 x g.

9. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml Collection Tube.

10. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

Note: You can skip the 5 min incubation. However, if you have already validated the Power Soil extractions with this incubation, we recommend you retain the step

11. Centrifuge the tubes for 1 min at 10,000 x g.

12. Avoiding the pellet, transfer up to 750 µl of supernatant to a clean 2 ml Collection Tube.

13. Shake to mix Solution C4 and add 1200 µl to the supernatant. Vortex for 5 s.

14. Load 675 µl onto an MB Spin Column and centrifuge at 10,000 x g for 1 min. Discard flow-through.

15. Repeat centrifugation step 14 twice or even three times, until all of the sample has been processed.

16. Add 500 µl of Solution C5. Centrifuge for 30 s at 10,000 x g.

17. Discard the flow-through. Centrifuge again for 1 min at 10,000 x g.

18. Carefully place the MB Spin Column into a clean 2 ml Collection Tube. Avoid splashing any Solution C5 onto the column.

19. Add 100 µl of Solution C6 to the centre of the white filter membrane. Alternatively, you can use sterile DNA-free PCR-grade water for this step (cat. no. 17000-10).

20. Centrifuge at room temperature for 30 s at 10,000 x g. Discard the MB Spin Column. The DNA is now ready for downstream applications.

Note: We recommend storing DNA frozen (–20°C to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA, see the Troubleshooting Guide.

2-3Quantification:

Prior to proceeding with downstream studies, DNA quantification, also known as nucleic acid quantification, is widely used to measure the average concentration of DNA or RNA in a sample.

When calculating the amount of DNA or RNA in a sample, its purity is also a factor to consider.

How it works:

- Qubit assay dyes bind selectively to DNA, RNA, or protein, making it more specific than UV absorbance
- More sensitive than UV absorbance, detecting as little as 10 pg/µl of DNA
- Uses as little as 1 µL of sample, even with very dilute samples
- New integrated reagent calculator to quickly generate working solution calculations
- Flexible options for exporting results: Wi-Fi, USB drive, or direct connection with a USB cable
- Highly accurate Qubit Flex Fluorometer increases throughput capacity, measuring 8-samples at once

2-4 Amplification and Sequencing:

We performed a nucleic acid amplification by using the polymerase chain reaction (PCR), that can amplify many amounts of target DNA within a few hours. To perform amplification process, we used Taq platinum HiFi (Termofisher).

primer with tail for 16s rRNA regions v3-v4: [114]

Pro341F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG -3′

Pro805R: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC -3′

Table1: PCR program for detection microbiota

MIX	Volume (µl)
Buffer	2.5
MgSO4 (50 mM)	1
dNTPs mix (10mM)	0.5
primer forward (10 µl)	1
primer reverse (10 µl)	1
Taq	0.2
H20 DNA-free	13.8
Genomic DNA	5

1 cycle	25 cycles	1 cycle
94°C for 1 min	94°C for 30 sec, 55°C for 30 sec, 68°C for 45 sec	68°C for 7 min

Amplified amplicon has been sent to the BMR Genomics (Padova) for the Next-Generation Sequencings (NGS) using Illumina MiSeq platform with 300 Paired Ends (PE) strategy.

2-5 Bioinformatic analysis:

The produced sequences were analysed according to Callahan et al. 2016. [115]. Paired-end sequenced reads were processed by quality filtering using the package dada2 (v. 1.14.1) on R version 3.6.3. Reads were trimmed according to their base quality at the 5' ends (where the average quality decreases), using a size of 300bp for the forward and 270bp for the reverse. Then, the fragments were de-replicated, resulting in the collapse of every identical sequence into a unique one as a representative, and finally, they were merged together. The resulting sequences were then clustered using the Amplicon Sequence Variants approach (ASV), removing chimera sequences at the end of the process. ASVs were assigned to their respective taxonomical classification until genus level using a training set provided by the SILVA 16S Database (NR98 version 138.1). Another filtering strategy was applied by removing all taxa for which the prevalence was under the threshold of 0.05% (out of 1) for the entire dataset, resulting in the exclusion of all the unique sequences (singletons). Taxon raw counts (ASVs) were transformed into relative abundance levels, reporting the percentage of each one with respect to the total number of sequences accounted for in every sample. Taxonomic information, ASV counts, and sample metadata were merged together using the phyloseq package (v. 1.30.0), and a set of explorative analyses was performed to investigate the taxonomical abundances for each individual and sample's inter- and intra-variability regarding microbiome composition.

The core microbiota was determined using the microbiome package (v 1.8.0), using a 0.1% compositional abundance threshold and a prevalence of 0.5, focusing on the genus level. A principal coordinate analysis (PCoA) using the Bray–Curtis distance was used to observe the main differences in our dataset. Then, we performed a cluster analysis based on the taxonomic differences among samples. To do so, a hierarchical clustering algorithm was applied to the whole

dataset, and the optimal number of clusters was computed using the gap statistic, applying the PAM clustering algorithm, with a maximum number of 20 clusters and 500 bootstraps. Moreover, we evaluated the goodness of the gap statistic using the clusGap function from the cluster (v. 2.1.3) package in R. An ANOVA was additionally executed on the resulting clusters using the Shannon and observed indexes. In order to understand which taxa significantly changed in terms of abundance and diversity along the resulting clusters, a Kruskal–Wallis test was also applied to the whole genus-level community. Finally, computing the LDA effect size with LEfSe3 allowed us to assign each of the significant taxa to the cluster for which they were representative.

Food intake associations with the host microbiome were checked using all available metadata on daily consumption of meat, vegetables, fruit, alcohol, olive oil, milk and cheese, cereals, legumes, and fish. In addition, the Mediterranean score of each sample was considered as part of the microbiome correlation analysis, as done previously4. Daily consumption values, represented by nominal and qualitative variables in our dataset, were then transformed into ordinal values, allowing a quantitative evaluation: we assigned '<1' to 0, '1–2' to 1, and '>2' to 2. The association between diet type and microbiome composition was checked through a combined statistics approach. First, based on the detrended correspondence analysis (DCA) result, redundancy analysis (RDA) was found to be the most appropriate method with which to investigate the relationship between dietary and taxonomic variables. A permutation analysis to assess the significance of dietary variables that fitted with the ordination was applied through the envifit function from the vegan (v. 2.4-2) package in R. Then, the Mediterranean scores of each cluster were analysed using an all-versus-all t-test to find possible associations between different diet styles and microbial communities. Finally, a Spearman test was performed to determine which and to what extent each genus-level taxon correlated with Mediterranean scores.

3-Results

3-1 Data collection results:

In order to determine the influence of diet on the human dental calculus, we collected 40 dental calculus samples from a cohort of patients from the UOC Clinical Odontoiatrica of Padova, with an emphasis on some key factors such as gender, diets, particularly the Mediterranean diet, and smokers and non-smokers.

The questionnaire was divided into sections, with each component requiring the patients to respond. Individual questions like age, sex, weight, height, where they were born, and where they live, for example, are covered in the first section. The second section contains information about their habits, such as their educational qualifications, whether or not they live alone and with how many people, whether or not they keep any domestic animals, whether or not they participate in sports and how frequently they do so, and whether or not they smoke and how often they do so. The third part is on their health conditions, which includes pathology reports and whether or not they've used antibiotics or probiotics, as well as how much they've used in the last month. Their diets are discussed in the last part, which includes whether they eat fruits, vegetables, legumes, grains, fish, meat, milk, olive oil, or alcohol, as well as how much of each they consume.

ID	Dieta Mediterranea	Cluster2	Sesso	Fumatore	Farmaco	Frutta	Verdura	Legumi	Cereali	Pesce	Carne_e_Salumi	Latte_e__latticini	Alcol	Olio_oliva	Speck	Prosciutto_Crudo	Salame	Soppressa	Parmigiano	Formaggi_Freschi	Gorgonzola	Birra	Yogurt	Kefir	Salsa_di_soia	Verdure_Fermentate	Crauti	Cren	Aceto	Olive_in_salamoia
ID1	8	cl1	f	no	si	>2	>2	<1	<1	>2	<1	<1	<1	regolarmente	<1	<1	<1	<1	>2	<1	<1	<1	<1	<1	<1	1_2	<1	<1	1_2	<1
ID15	11	cl4	m	no	no	<1	1_2	1_2	>2	1_2	1_2	1_2	>2	regolarmente	<1	<1	1_2	<1	>2	<1	<1	>2	<1	<1	<1	<1	<1	<1	>2	<1
ID16	9	cl1	f	no	no	1_2	1_2	1_2	1_2	1_2	1_2	1_2	<1	regolarmente	<1	<1	<1	<1	1_2	<1	<1	<1	1_2	<1	<1	<1	<1	<1	<1	<1
ID17	11	cl3	f	no	no	1_2	1_2	1_2	>2	1_2	1_2	>2	<1	regolarmente	1_2	>2	<1	<1	>2	>2	<1	<1	>2	<1	<1	<1	<1	<1	<1	<1
ID18	10	cl1	f	no	si	1_2	>2	1_2	1_2	1_2	>2	<1	<1	regolarmente	<1	<1	<1	<1	>2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID19	9	cl2	m	no	si	1_2	1_2	1_2	1_2	1_2	1_2	1_2	1_2	frequentemente	<1	<1	<1	<1	<1	<1	<1	>2	>2	<1	<1	<1	<1	<1	<1	<1
ID2	6	cl1	m	no	si	<1	1_2	>2	>2	1_2	<1	<1	1_2	regolarmente	1_2	1_2	1_2	<1	>2	1_2	1_2	1_2	<1	<1	<1	<1	<1	<1	>2	1_2
ID20	7	cl4	f	si	no	<1	<1	1_2	1_2	1_2	1_2	1_2	<1	regolarmente	<1	1_2	<1	<1	1_2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID21	11	cl4	f	no	no	1_2	>2	1_2	1_2	1_2	1_2	1_2	1_2	regolarmente	<1	<1	<1	<1	1_2	<1	<1	>2	>2	<1	<1	<1	<1	<1	<1	<1
ID22	8	cl3	f	no	no	1_2	1_2	<1	<1	>2	1_2	1_2	<1	regolarmente	<1	<1	<1	<1	1_2	1_2	<1	<1	>2	<1	<1	<1	<1	<1	<1	<1
ID23	8	cl1	f	no	si	1_2	1_2	<1	>2	>2	1_2	<1	<1	frequentemente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID24	9	cl4	f	no	no	1_2	1_2	1_2	1_2	1_2	1_2	<1	1_2	regolarmente	<1	<1	<1	<1	>2	<1	<1	1_2	<1	<1	1_2	>2	<1	<1	>2	>2
ID25	12	cl1	f	no	si	1_2	>2	1_2	1_2	>2	>2	<1	1_2	regolarmente	<1	1_2	<1	<1	1_2	<1	<1	<1	1_2	<1	<1	<1	<1	<1	<1	1_2
ID26	7	cl3	m	no	no	<1	1_2	<1	>2	1_2	1_2	<1	<1	regolarmente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID27	9	cl2	f	si	si	1_2	1_2	1_2	1_2	1_2	>2	<1	<1	regolarmente	<1	>2	>2	<1	1_2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	>2
ID28	9	cl1	f	si	si	1_2	>2	>2	1_2	1_2	<1	<1	<1	regolarmente	<1	<1	<1	<1	>2	1_2	1_2	1_2	<1	<1	1_2	>2	1_2	<1	>2	<1
ID29	9	cl1	f	no	no	1_2	1_2	1_2	1_2	1_2	1_2	1_2	<1	regolarmente	1_2	1_2	<1	<1	<1	1_2	<1	<1	>2	<1	<1	<1	<1	<1	<1	<1
ID30	7	cl1	f	no	no	<1	1_2	1_2	1_2	<1	1_2	>2	<1	frequentemente	<1	<1	<1	<1	1_2	1_2	<1	<1	1_2	<1	<1	1_2	<1	<1	<1	<1
ID31	10	cl4	m	no	no	1_2	1_2	<1	1_2	1_2	>2	1_2	1_2	regolarmente	1_2	1_2	1_2	1_2	1_2	1_2	<1	>2	>2	<1	<1	<1	<1	<1	<1	<1
ID32	11	cl3	f	no	no	1_2	>2	1_2	1_2	>2	1_2	1_2	<1	regolarmente	<1	<1	<1	<1	>2	<1	<1	<1	>2	<1	<1	>2	<1	<1	1_2	<1
ID33	8	cl1	m	si	no	1_2	1_2	1_2	1_2	1_2	1_2	<1	1_2	frequentemente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID34	10	cl1	m	no	no	1_2	1_2	1_2	1_2	1_2	1_2	1_2	1_2	regolarmente	<1	<1	<1	<1	1_2	1_2	<1	1_2	>2	<1	<1	<1	<1	<1	<1	<1
ID35	7	cl3	f	no	no	1_2	1_2	1_2	1_2	1_2	1_2	1_2	<1	occasionalmente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID36	8	cl4	m	no	no	<1	1_2	1_2	1_2	1_2	1_2	1_2	1_2	frequentemente	<1	<1	<1	<1	>2	<1	<1	>2	<1	<1	<1	>2	<1	<1	<1	<1
ID37	10	cl1	f	no	no	1_2	1_2	1_2	>2	1_2	1_2	1_2	<1	regolarmente	<1	<1	<1	<1	1_2	1_2	<1	<1	>2	<1	<1	<1	1_2	<1	>2	<1
ID38	10	cl1	f	no	si	1_2	1_2	>2	1_2	1_2	1_2	1_2	<1	regolarmente	<1	1_2	<1	<1	>2	1_2	<1	<1	>2	<1	<1	<1	<1	<1	>2	<1
ID39	10	cl3	m	no	no	1_2	1_2	>2	1_2	1_2	1_2	1_2	1_2	frequentemente	<1	1_2	<1	<1	1_2	>2	<1	<1	>2	<1	<1	>2	<1	<1	1_2	<1
ID40	10	cl4	f	si	no	1_2	>2	>2	>2	<1	<1	<1	1_2	regolarmente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1_2	>2	<1	<1	>2	<1
ID41	11	cl2	f	no	si	1_2	1_2	1_2	1_2	>2	1_2	1_2	1_2	regolarmente	<1	<1	<1	<1	1_2	1_2	<1	1_2	>2	<1	<1	<1	<1	<1	1_2	>2
ID42	6	cl1	f	no	no	<1	1_2	<1	1_2	1_2	>2	<1	1_2	occasionalmente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1_2	<1	<1	<1	<1
ID43	5	cl2	f	si	si	<1	1_2	1_2	1_2	<1	1_2	1_2	<1	occasionalmente	<1	1_2	<1	<1	1_2	1_2	<1	<1	<1	<1	<1	<1	<1	<1	1_2	<1
ID44	8	cl4	m	no	si	1_2	1_2	1_2	1_2	<1	1_2	1_2	1_2	frequentemente	<1	<1	<1	<1	>2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID45	11	cl1	f	si	no	1_2	1_2	1_2	>2	<1	>2	>2	<1	regolarmente	<1	<1	<1	<1	<1	>2	<1	<1	<1	<1	<1	>2	<1	<1	<1	<1
ID46	8	cl1	m	no	no	<1	1_2	1_2	1_2	>2	1_2	<1	1_2	frequentemente	<1	<1	<1	<1	1_2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

ID47	6	cl4	f	no	no	1_2	1_2	<1	<1	1_2	1_2	1_2	<1	frequentemente	<1	1_2	<1	<1	1_2	<1	<1	1_2	1_2	<1	<1	1_2	<1	<1	<1	<1	<1
ID48	7	cl2	m	si	no	<1	1_2	<1	1_2	1_2	1_2	1_2	1_2	frequentemente	<1	<1	<1	<1	1_2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1_2	<1
ID49	11	cl3	f	no	no	1_2	>2	1_2	>2	1_2	1_2	1_2	<1	regolarmente	<1	<1	<1	<1	>2	1_2	<1	<1	1_2	<1	<1	<1	<1	<1	<1	<1	<1
ID50	6	cl4	f	no	no	<1	1_2	<1	1_2	1_2	1_2	1_2	<1	frequentemente	<1	<1	<1	<1	1_2	1_2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID51	9	cl2	m	no	si	1_2	1_2	1_2	1_2	1_2	1_2	1_2	1_2	frequentemente	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
ID52	10	cl2	f	no	si	1_2	1_2	1_2	1_2	1_2	1_2	1_2	1_2	regolarmente	<1	<1	<1	<1	1_2	1_2	<1	1_2	>2	>2	<1	>2	<1	<1	<1	<1	<1

Table 2 - 1: Transposition of the data collected through the questionnaire for each patient involved in the study. (dietary information)

ID	Diabetes	Gastritis	Chron	Obesity	Cardiopathies	Asma	Depression	Anisa	Artrite_reumatoide	Pathologies	patologie_vascolari	Disturbi_psicologici	Dieta_fermentata
ID1	no	no	no	no	no	no	no	no	no	no	no	no	no
ID15	no	no	no	no	no	no	no	no	no	no	no	no	si
ID16	no	no	no	no	no	no	no	no	no	no	no	no	no
ID17	no	no	no	no	no	no	no	no	no	no	no	no	si
ID18	no	no	no	no	no	no	no	no	no	si	si	no	no
ID19	no	no	no	no	no	no	no	no	no	no	no	no	si
ID2	si	no	no	si	si	no	no	no	no	si	si	no	si
ID20	no	no	no	no	no	no	no	si	no	si	no	si	no
ID21	no	no	no	no	no	no	no	no	no	no	no	no	si
ID22	no	no	no	no	no	no	no	no	no	no	no	no	no
ID23	no	no	no	no	no	no	no	no	no	no	no	no	no
ID24	no	no	no	no	no	no	no	no	no	no	no	no	si
ID25	no	si	no	no	no	no	no	no	no	si	no	si	si
ID26	no	no	no	no	no	no	no	no	no	no	no	no	no
ID27	no	si	no	no	no	no	no	no	no	si	no	no	si
ID28	no	no	no	no	no	no	no	no	no	no	si	no	si
ID29	no	no	no	no	no	no	no	no	no	no	no	no	si
ID30	no	no	no	no	no	si	no	no	no	si	no	no	no
ID31	no	no	no	no	no	no	no	no	no	no	no	no	si
ID32	no	no	no	no	no	no	no	no	no	no	no	no	si
ID33	no	no	no	no	no	no	no	no	si	si	no	no	no

ID34	no	no	no	no	no	no	no	no	no	no	no	no	si
ID35	no	no	no	no	no	no	no	si	no	si	si	si	no
ID36	no	no	no	no	no	no	no	no	no	no	no	no	si
ID37	no	no	no	no	no	no	no	no	no	no	no	no	si
ID38	no	no	no	no	no	no	no	no	no	si	si	no	si
ID39	no	no	no	no	no	no	no	no	no	no	no	no	si
ID40	no	no	no	no	no	no	no	no	no	no	no	no	si
ID41	no	no	no	no	no	si	no	no	no	si	no	no	si
ID42	no	no	no	no	no	no	no	no	no	no	no	no	no
ID43	no	si	no	no	si	no	si	si	no	si	no	si	no
ID44	no	no	no	no	si	no	no	no	no	si	si	no	no
ID45	no	no	no	no	no	no	no	no	no	no	no	no	no
ID46	no	no	no	no	si	no	no	no	no	si	si	no	no
ID47	no	no	no	no	no	no	no	no	no	no	no	no	si
ID48	no	no	no	no	no	no	no	no	no	no	no	no	no
ID49	no	no	no	no	no	no	no	no	no	no	no	no	no
ID50	no	no	no	no	no	no	no	no	no	no	no	no	no
ID51	no	no	si	no	no	no	no	no	no	si	no	no	no
ID52	no	no	no	no	si	no	no	no	no	si	si	no	si

Table 2-2: Transposition of the data collected through the questionnaire for each patient involved in the study. (health information)

3-2 DNA extraction and PCR results:

The polymerase chain reaction (PCR) is a technique for amplifying DNA that is also used in DNA sequencing to obtain DNA copies, reduce contamination, and find DNA mutations and clones. As a result, for two reasons, amplification is crucial. First, some contaminants from the extraction kit, such as chemical compounds that can inhibit amplification, may remain in the DNA samples if we did not purify DNA well enough during extraction, making the sequencing process difficult. The second objective is to test that the quality of the DNA extraction is satisfactory for sequencing.

The Qubit is used to do quantification to determine the amount of Ng/L of DNA in each sample. Only sample 40 in the amplification test result could not be easily amplified because of a small amount of DNA, prohibiting Qubit from detecting the amount of DNA there in, despite the fact that all other samples could be amplified for the V3 and V4 sections of the 16s. (Figure 1 and Table 3).

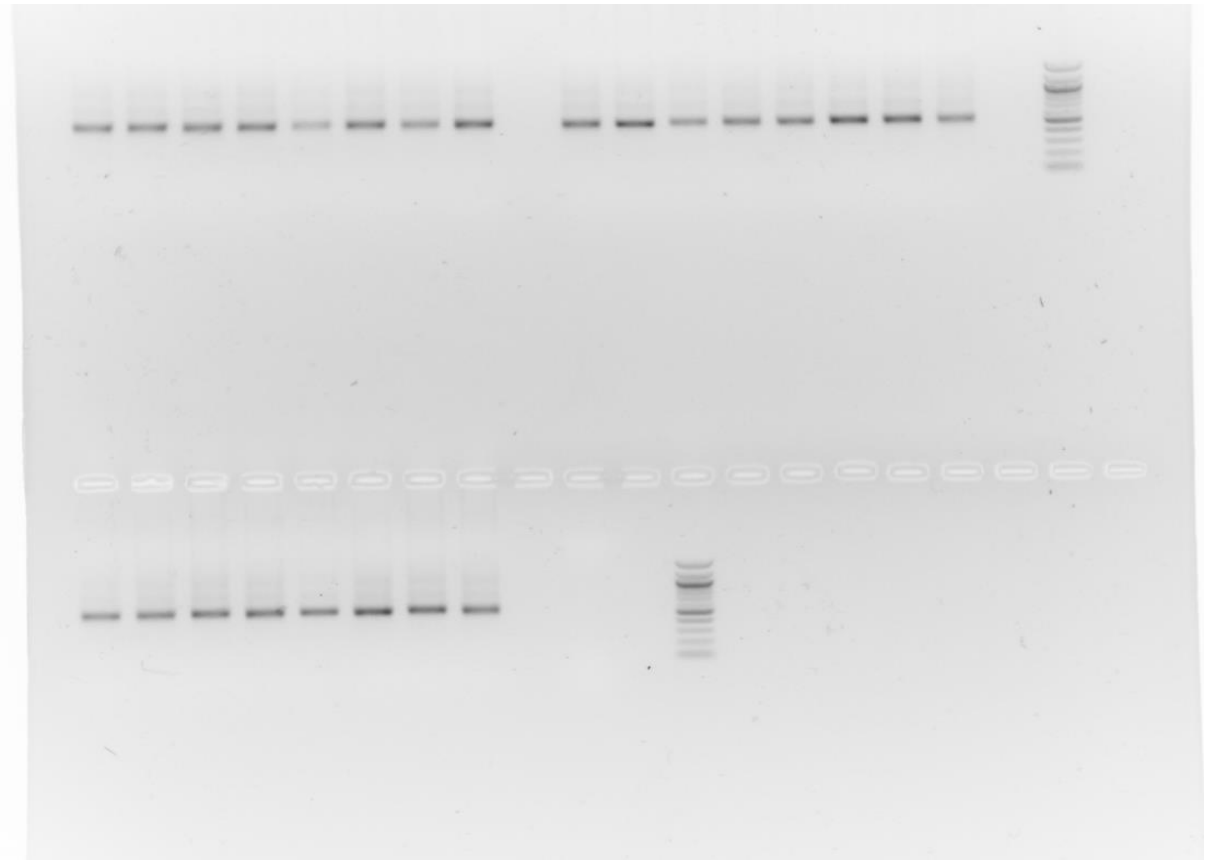

Figure 1: The PCR amplification result visualized in the agarose gel.

Table 3: DNA quantification results obtained by Qubit.

samples	DNA Quantification (ng/uL)
1	4.8
2	3.14
3	8.89
15	3
16	11.6
17	11.9
18	8.73
19	29.6
20	3.63
21	9.45
22	24.7
23	10.7
24	3.89
25	12
26	8.42
27	5.69
28	9.57
29	2.89
30	5.29
31	22
32	4.76
33	2.37
34	3.69
35	4.51
36	18.9
37	4.48
38	0.511
39	16.09
40	no result
41	4.5
42	11.7
43	5.24
44	6
45	18.6
46	42.2
47	6.97
48	0.38
49	13
50	4.69
51	8.59
52	5.35

3-3 The distribution of sex on the age ranges:

Two pie charts and two bar charts represent the gender distribution and age range of each sex, respectively. In both, sex has been depicted in two distinct colours, feminine red and man blue. In the pie chart (Figure 2A), women account for 67.5%of the participants, while men account for the remaining 32.5%. The age categories are depicted in the boxplot (Figure 2B), which span from 20 to 75 years old. The mean distribution of women age is between the ages of 40 and 55, while men are between the ages of 35 and 65 years old. Overall, the plots reveal that there are no age differences in terms of age distribution between males and girls.

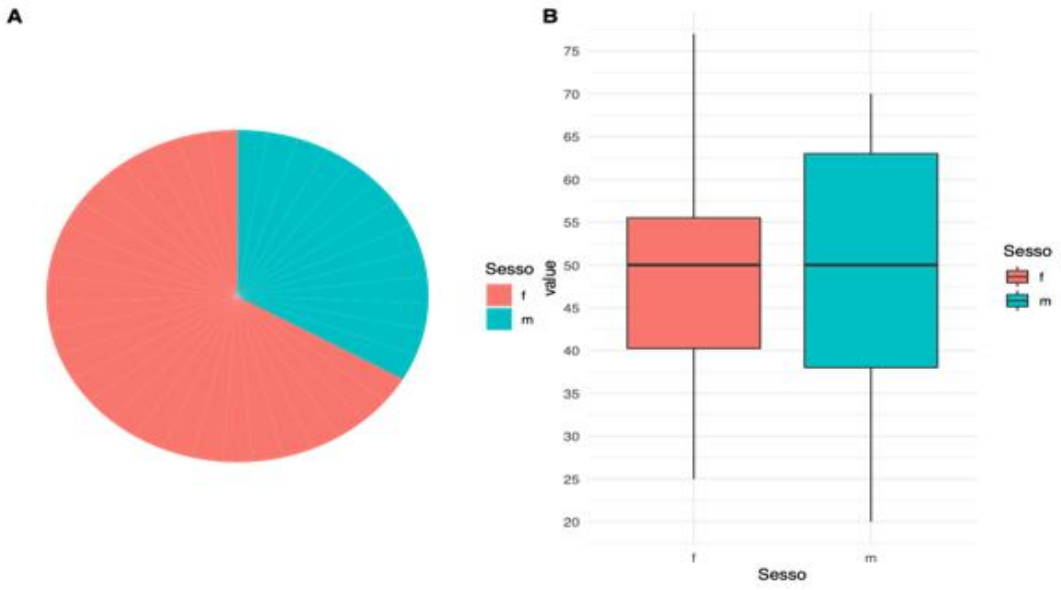

Figure 2: (A) Pie chart on division male and female. (B) Boxplot shows the distribution of genders among the age ranges.

3-4: Quantity of smokers and non-smokers:

The number of smokers and non-smokers in each sample is depicted in the bar graph (Figure 3). The X-axis represents the “yes” or “no” responses reported by patients on the questionnaire, while the Y-axis represents the percentage. Overall, non-smokers appear to outnumber smokers by a significant margin, with 60% and 20%, respectively.

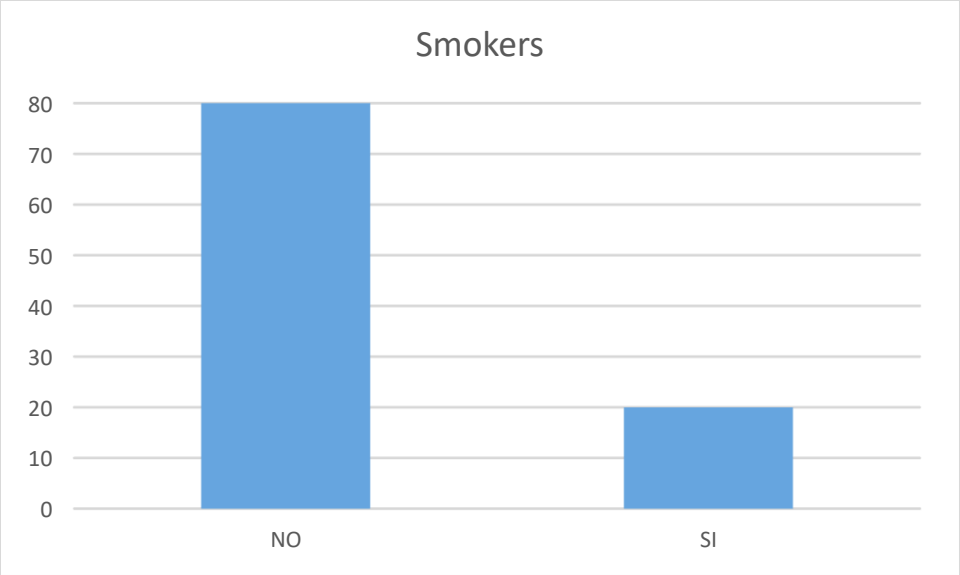

Figure 3: Bar plot represents distribution the smokers and non-smokers within our cohort.

3-4-1 Body mass index distribution among samples:

It's represented as a bar chart, with each gender's Body Mass index (BMI) scenario (overweight, pre-overweight, and normal weight) represented by lines. The X-axis displays the number of samples, while the Y-axis displays the BMI value. The BMI is a simple calculation that takes a person's height and weight into account. BMI is a metric for measuring a person's nutritional health as well as a disease risk indicator, because as it rises, so does the chance of certain disorders like obesity. [116]

However, in the ID1,16,25,30,35,43 (female samples) and ID 26,44,46 (male samples), BMI is greater than 25, indicating pre-obesity. When their BMI is less than 25 and larger than 18, ID17,21,22,23,24,28,29,37,38,45,47,49,50,52 (females) and ID15,19,33,34,36,46,51 (males) falls into the normal weight category. The rest of the people are underweight, with a BMI of less than 18.

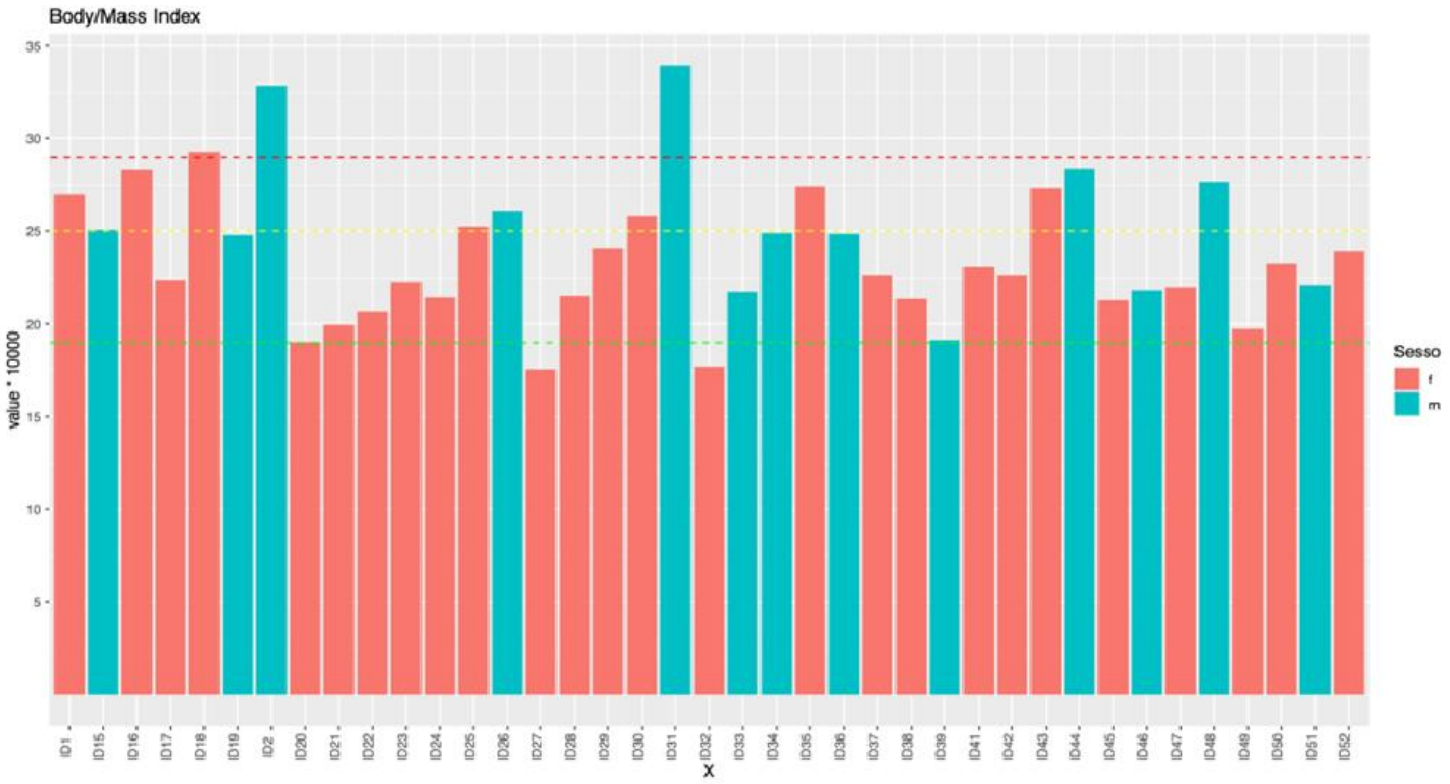

Figure 4: Body mass index distribution among samples. Three different threshold are indicated by lines: green for under-weight; yellow for pre-overweight; red for overweight.

3-5 Mediterranean diet:

The bar chart (Figure 5) was used to investigate the value of the Mediterranean diet in both men and women. The X-axis depicts the number of samples of various colours (red for females and blue for men), while the Y-axis represents the value of adherence to the Mediterranean diet as estimate through the method of Francesco Sofi et al article [113] , which ranges from 0 to 18. Within our cohort, the patients ID25 (female) has the highest value, with a number of 12, while the ID43 has the lowest, with a value of around 5.

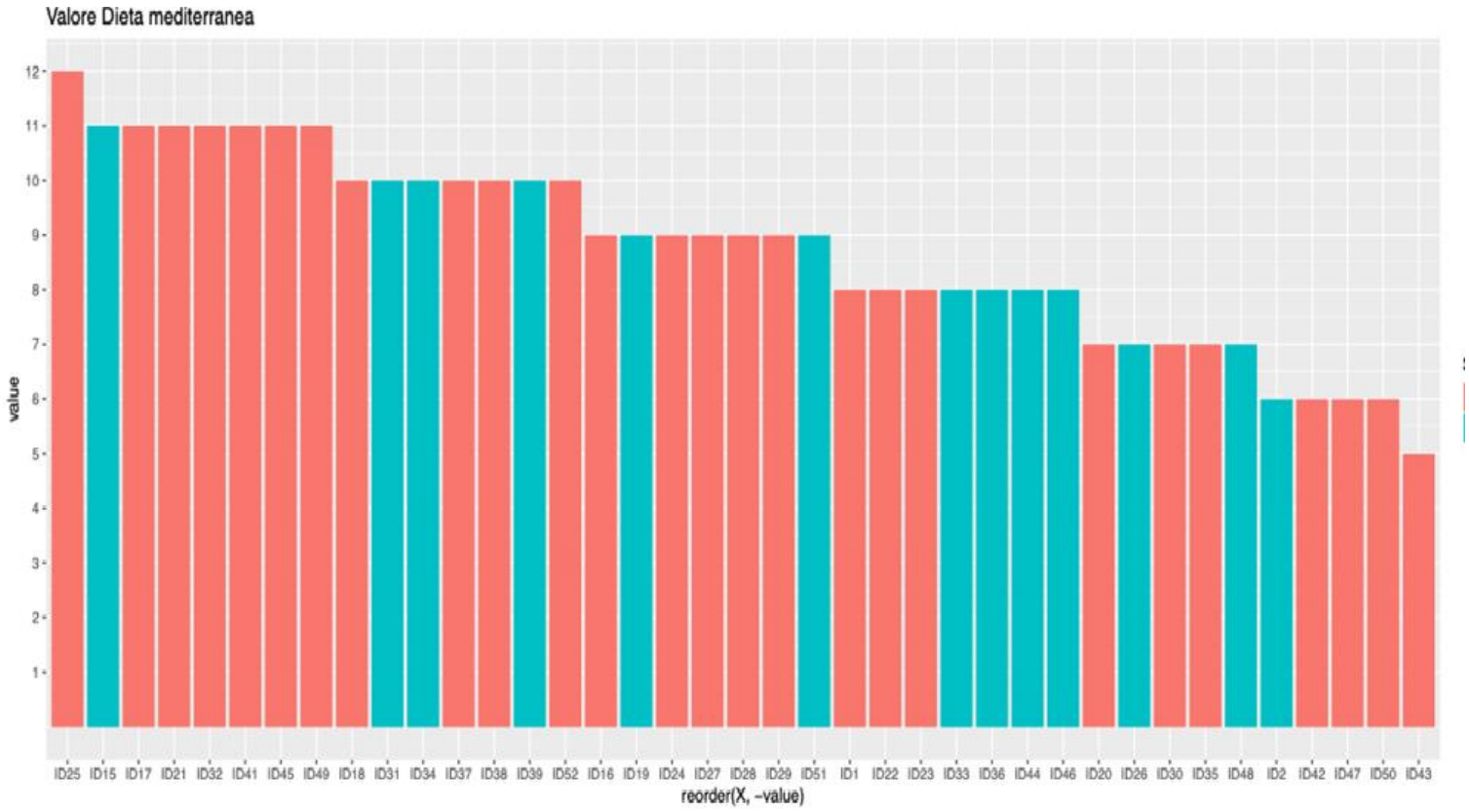

Figure 5: Bar plot reporting the score of adherences to the Mediterranean diet divided per gender.

3-6 Taxonomic analysis and microbial diversity:

The bar chart depicts the detection of phyla that are detected in each sample (Figure 6). The total number of samples is shown on the X-axis, while relative abundance is shown on the Y-axis. Overall, the phyla found within samples' dental calculus include *Actinobacteria*, *Bacteroidetes*, *Campylobacteria*, *Chloroflexi*, *Firmicutes*, *Fusobacteria*, *Palescibacteria*, *Proteobacteria*, *Spirochaetes*, and *Synergistota*. Samples contain some diverse phyla, though in differing degrees of abundance. For example, the *Firmicutes* phylum is more abundant in ID 28 and ID 33, but the phylum *Actinobacteria* is more prevalent in ID48 and ID 43.

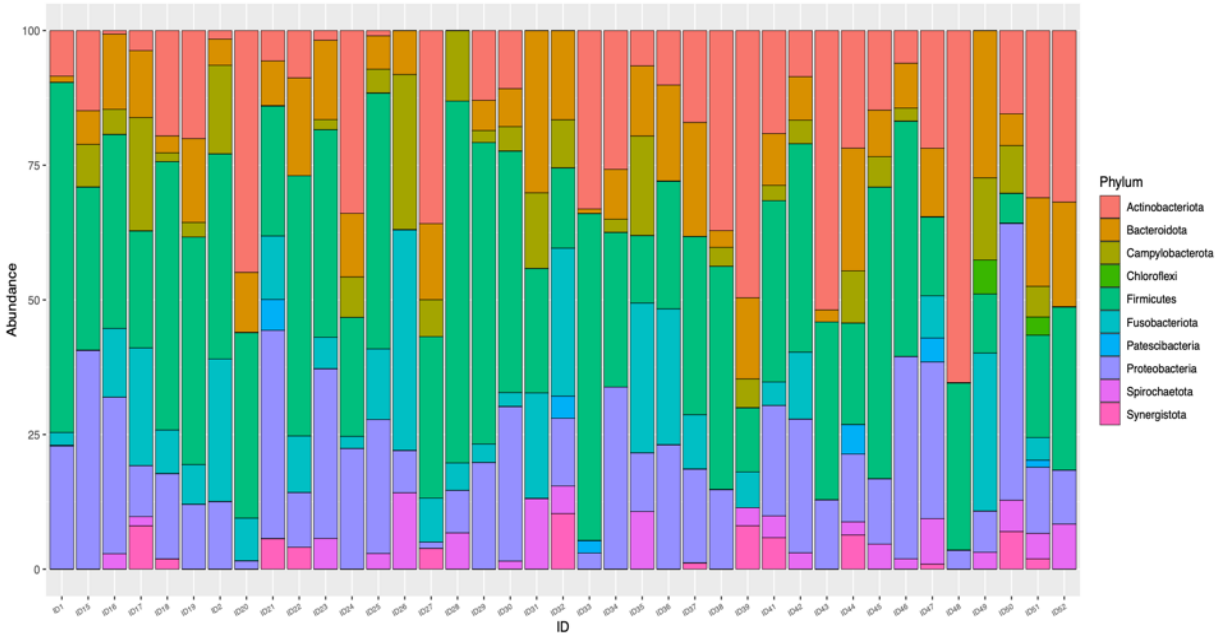

Figure 6: Phylum distribution of among all samples.

Once the phyla in each sample have been established, the next step is to look at the distribution of genera in the bar chart (Figure 7). Like the phylum graph, the ID is displayed by the X-axis, while relative abundance is represented by the Y-axis. Despite the fact that different genera coexist in each sample, there are certain similarities. *Rothia* and *Streptococcus*, for example, have the highest relative abundance. W5053, on the other hand, is a rare genus.

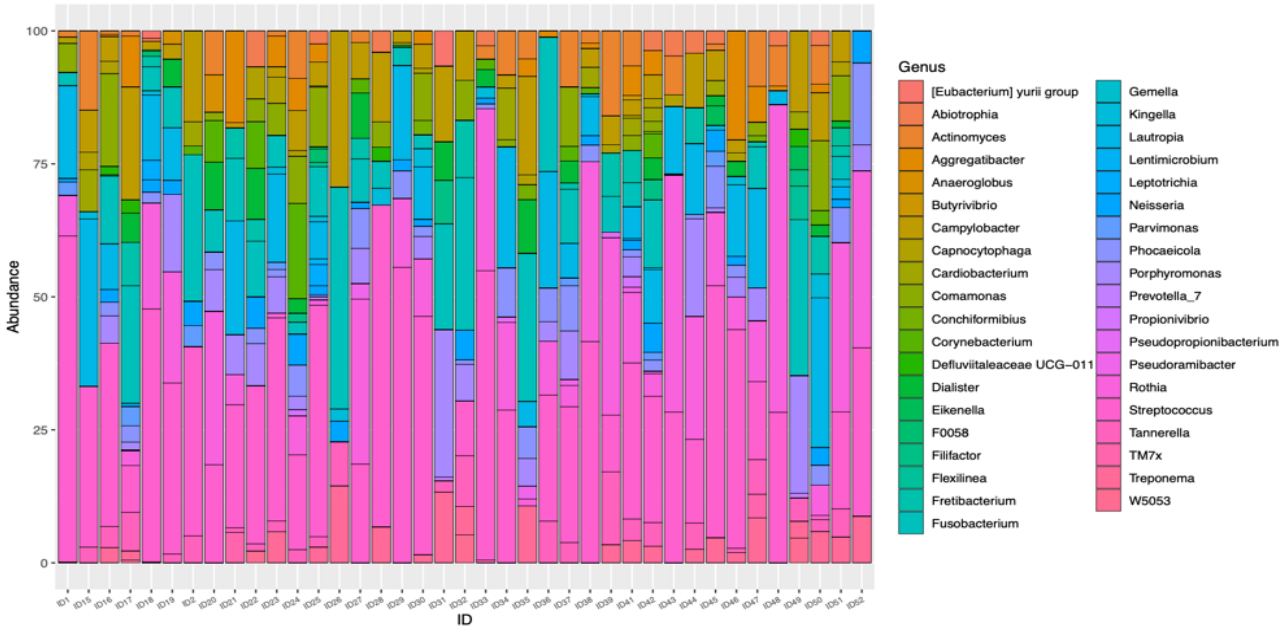

Figure 7: The genus level distribution among all samples

3-7 Cluster analysis:

Following the primary analyses that described the phylum and genera distribution, we proceeded with the identification of clusters within our cohort using GAP statistics and Hierarchical clustering analysis. First, GAP statistical analysis is used to determinate the presence of clusters in the data set, in other words, how many clusters are present.

In this study, there are four clusters based on the taxonomy. Then, using Hierarchical Clustering Analysis, we can figure out which cluster each sample belongs to. The first cluster had 16 samples, the second had 5 samples, and the third and fourth clusters, respectively, had 7 and 9 samples.

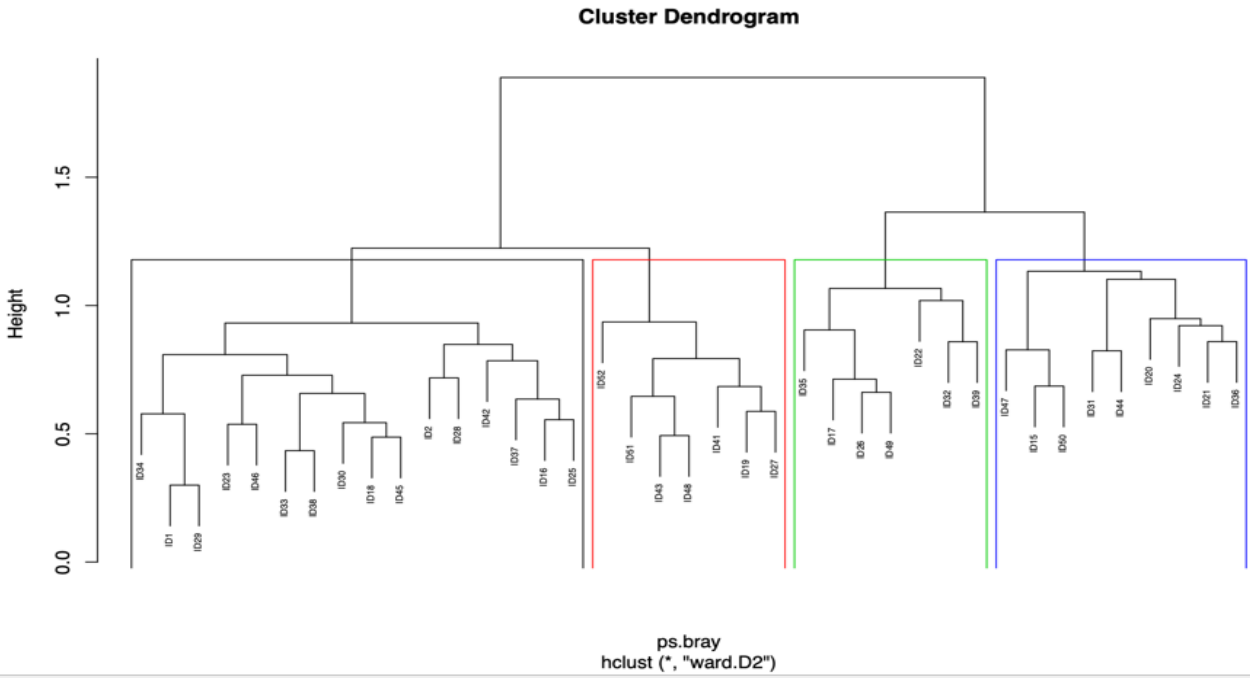

Figure 8: Result from Hierarchical Cluster Analysis represented here through a dendrogram.

Following the detection of the 4-clusters, it can be observed in figure 9 that each cluster is distinguished by a different composition at the genus level, which is represented by different colours. We performed the Kruskal Wallis test and linear discrimination analysis (LDA) to identify which genera are mainly associated to each cluster. Figure 10 show the genera that were

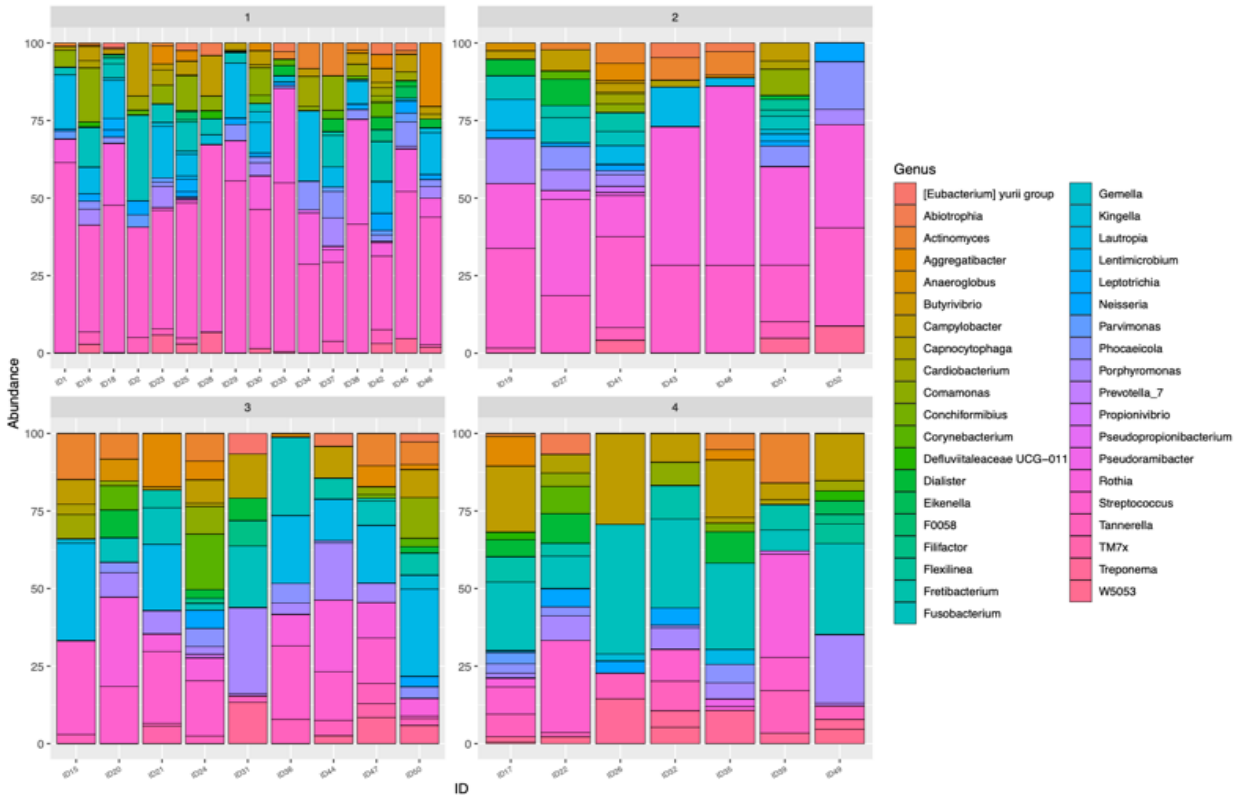

Figure 9: Genera distribution among clusters.

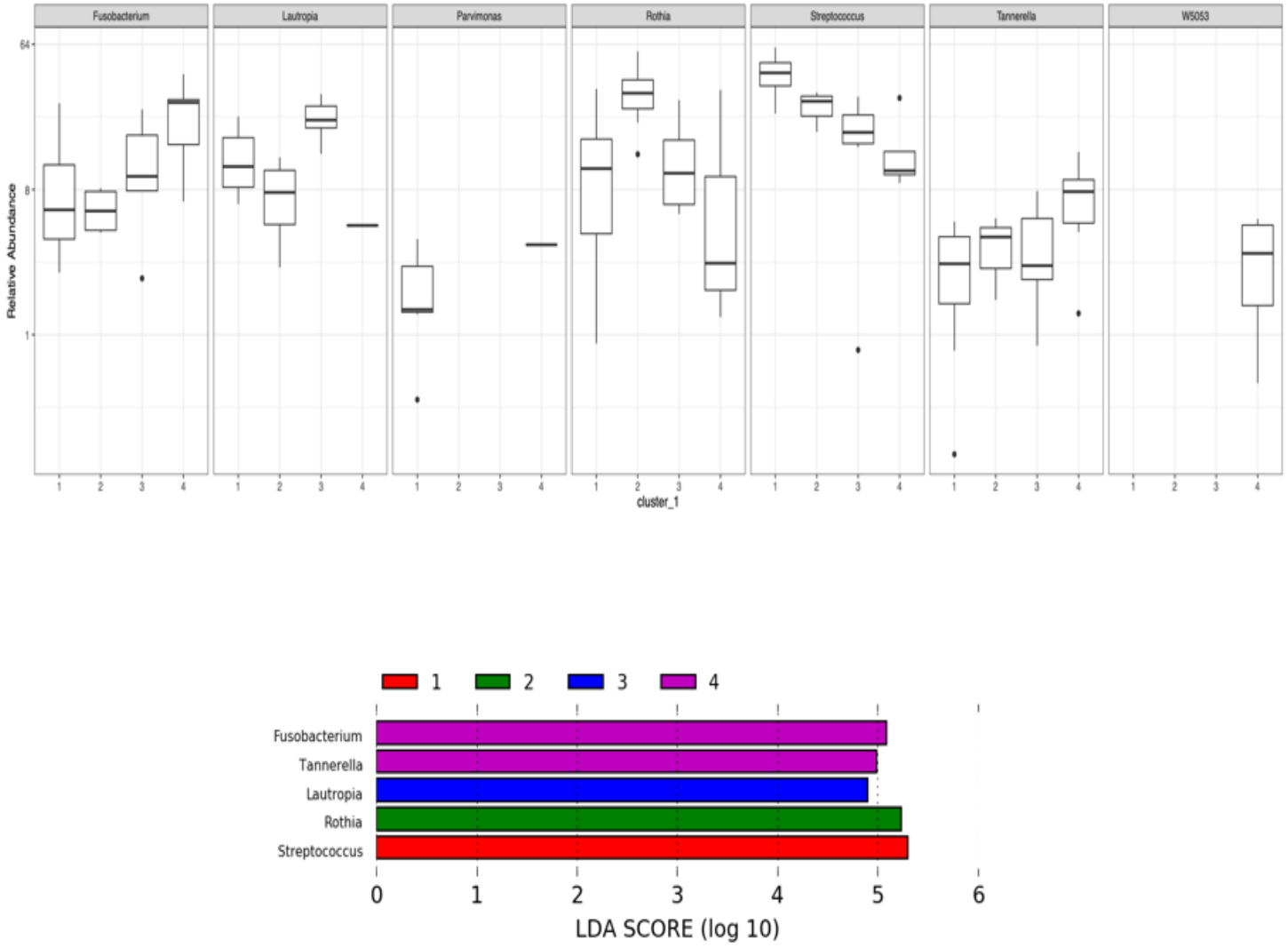

Figure 10: Kruskal Wallis test and LDA analysis results to detect the association of each genus in each cluster.

In addition, using the Richness and biodiversity analysis index in figure 11, identify each set of clusters based on biodiversity. Different colours are used to identify each cluster group. Because they include a larger number of taxa, clusters 1 and 2 have a higher biodiversity index than clusters 3 and 4.

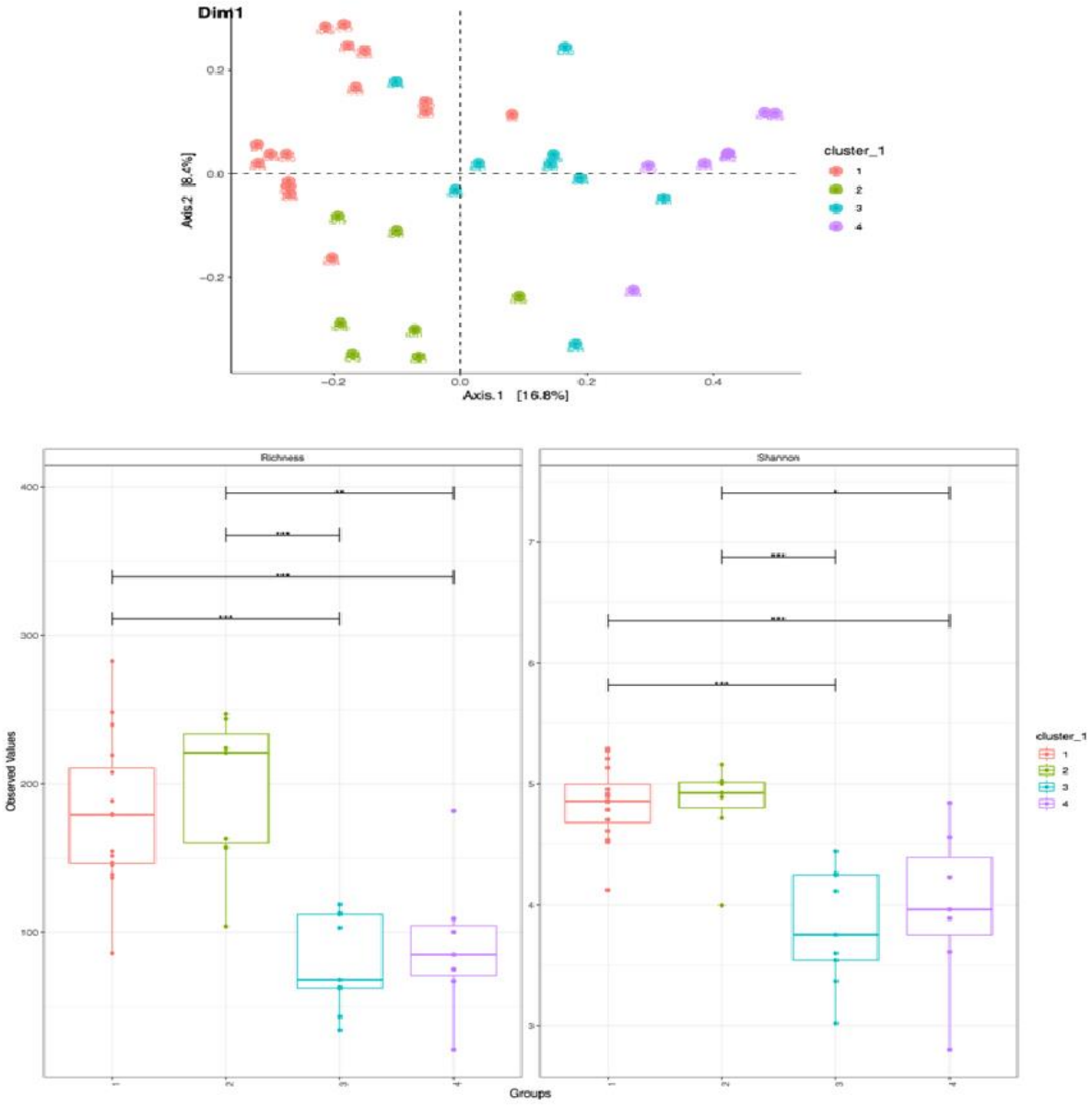

Figure 11: Richness and biodiversity analysis among our groups using alpha diversity indexes

3-8 The association between diet and taxonomic biodiversity:

We used a Canonical Analysis of Principal Coordinates (CAP) to link dietary factors to the microbiome composition of our cohort, as shown in figure 12. We were able to discover some of the dietary variables that have the most impact on the microbial makeup in our sample using this method. Beans, fruits, vegetables, and fish, for example, were linked to cluster 1. Cereals were strongly linked to cluster 3, while milk and its derivatives, as well as meat, were strongly linked to cluster 4.

Furthermore, this research provided insight into the relationship between nutrition and microorganisms. For example, a diet high in vegetables, fruits and legumes, is associated with the genus *Streptococcus* , a key component of a healthy oral microbiota; on the other hand, a diet heavy in animal protein, such as milk and meat, is associated with *Tannerella* and Porphyromonas. Different colours denote different clusters. Clusters 1 and 2 are shown in red, clusters 3 and 4 in green, and clusters 3 and 4 in blue and purple, respectively. In addition, the purple arrows denote bacterial genera, and the grey arrows denote dietary vegetables.

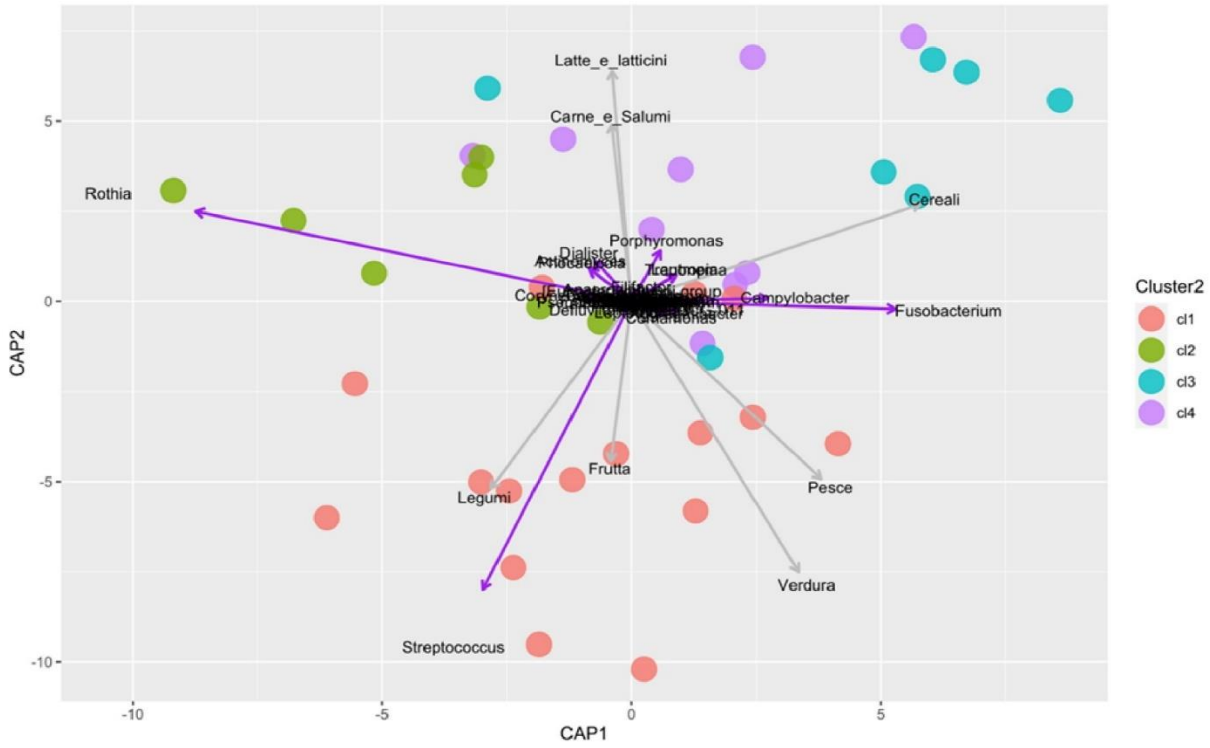

Figure 12: The relationship between the diet and microbiome

4- Discussion

4-1 The effect of the diet on microbiome:

Various types of food intakes can have different effects on gut microbiota composition, according to this research. The Neolithic diet and the recent introduction of industrially processed flour and sugar are two key dietary backgrounds that have had no effect on the composition of the oral microbiome between the ancient era and modern world. The human diet has been subject to vast changes over the last few hundred years, there is a significant difference in nutritional intake between our ancestors, who ate food in different ways. [117]. For example, they consumed more wild plant foods (fibre) per day than non-industrial and industrialized countries, which tend to consume less dietary fibre. [118]. The increased consumption of cereals and fermented carbohydrates in the human diet resulted in dental calculus, which is currently recognized as one of the most common diseases among school-aged children and adults. Diet is one of the key factors on individual microbial fingerprints in humans. Beside the dietary consumption, antibiotic use, and age are all factors that can influence the composition of microorganisms. [86]

Diet can make differences both in terms of community structure and taxonomic composition and also genomic potential of the community. So, diet can lead to tooth decay due to the influence on the oral microbiome. There are different types of dietary intake that are enrich with specific ingredients such as “Mediterranean diet (MD)”, which is omnivore type include diets rich in vegetables, fruits, and olive oil [86,119], and “Western diet”, diets rich in red/ processed meats and low in fibre. [86]

Applying clusters in each group individuals based on the abundance of dominant genera that were identified according to the diet consumption properties imply that certain dietary component influences the oral microbiome community. For example, the intake of total dietary fibres and vegetables is associated with increased abundance of oral community such as *Streptococcus* and *Prevotella* that can be observed in cluster one. the composition of gut microbiota is related to macronutrient intake. Dietary fibre can promote the growth of beneficial bacteria including *Bifidobacterium*, *Lactobacillus*, and *Veillonella.* Various types of fibre (soluble and insoluble) have different effects on microbiota and changing the fibre component can vary the microbial

composition. Fibre-rich diets can help to improve the quality of the gut microbiota (increased diversity, stability and reduced mucus degrading bacteria). [120]

4-2 Results of current study:

In the current investigation, a total of 40 samples were obtained from patients who had been referred to the Clinica Odontoiatrica at the Azienda Ospedaliera, after information about their eating and smoking habits was gathered via study participants' responses to a questionnaire. The dental calculus samples were crushed, and the DNA was then extracted from them after DNA quantification and 16s rRNA primer amplification. BMR Genomics (Padova) sequenced the PCR products for the Next-Generation Sequencings (NGS) using Illumina MiSeq platform with 300 Paired Ends (PE) strategy. With a range of 40-55 years for women and an average age of 35-65 years for men, men were more prevalent than women (32.5 percent versus 67.5 percent, respectively). Twenty percent of the individuals smoked, which suggests that smoking had little impact on the study's findings. Most participants had BMIs that fell within the normal range. The results of NGS indicated the presence of *Actinobacteria, Bacteroidetes, Campylobacteria, Chloroflexi, Firmicutes, Fusobacteria, Palescibacteria, Proteobacteria, Spirochaetes,* and *Synergistota.* Four taxonomically based groupings were validated by the GAP statistical analysis. There were 16 samples in the first cluster, 5 in the second, 7 and 9 samples in the third and fourth clusters, respectively. Kruskal Wallis test and LDA analysis results indicated that the *Fusobacteria and Tannerella spp* have belonged to the cluster 4, *Lautropia* spp is in cluster 3, *Rotial spp* is in cluster 2, and *Streptococcus spp* is categorized in cluster 1. By the applying the canonical Analysis of Principal Coordinates (CAP), we discovered a relationship between food and the frequency of microbiota composition in each cluster. For instance, a diet high in fish, beans, fruits, and vegetables was connected to cluster 1. While milk and its derivatives, as well as meat, were substantially associated to cluster 4, cereals were strongly linked to cluster 3. In the end, we found that administration of plant-based foods—vegetables, fruits, and legumes—is related with the genus *Streptococcus*, a vital part of a healthy oral microbiota, while administration of animal protein—meat and milk—is connected with *Tannerella* and *Porphyromonas*.

4-3 *Streptococcus* / *Prevotella* and oral microbiome:

Streptococcus.mutans is one of the most common bacteria in human oral flora, and it can cause dental caries in people who eat a high-sugar diet, [121]. They are able to consume glucose, starch, and sucrose because carbohydrates are likely accessible to a diverse bacterial composition, resulting in an increase in caries prevalence. [122]. This is prevalent in the oral cavities of those who ingest carbohydrates, which causes dental caries as result of acid production. [123]. It is discovered that *S.mutans* growth and development is linked to carbohydrate consumption in the oral cavity of people who include fibre and carbohydrate in their diet. Vegan diets are linked to increased fibre intake and decreased saturated fat and protein intake.[86]

Several studies have identified a relationship between increased microbial diversity and fibre, fruit, and vegetable consumption, either at the taxonomic or gene level. In many situations, it has been documented that a high-vegetable diet is linked to *Prevotella,* whereas a high-protein/fat diet is linked to *Bacteroides*. [119]. *Prevotella* is a *Bacteroidetes* phylum genus that is abundant in vegan diets compared to omnivore diets that are high in plant-based foods and low in animal items, and vegans have a lower *Bacteroides* and *Firmicutes/Bacteriodes* ratio than omnivores. [29],because there is an agreement that a higher percentage of *Bacteroidetes* is associated with lower consumption of animal-based products, the ratio of *Bacteroidetes* in participants who ate whole grains and plant-based vegetarian foods was higher when compared to those whose diet was higher in animal fat and protein. [29]. Another study found that *Firmicutes* were higher in the faeces microbiota of Italian children who ate a western diet than those who ate a carbohydrate, animal, or plant-based diet, but the *Bacteroidetes* ratio was lower in their faecal sample. [29]. *Prevotella* is detected in children who eat a diet low in fat and animal protein but high in starch, fibre, and plant protein, as opposed to those who eat a diet high in animal protein, carbohydrate, and fat but low in fibre. [86]

Our findings according to figure 12 revealed that *Streptococcus* and *Prevotella* in Cluster 1 were linked to fibre and vegetable consumption, implying that include these foods in our diet can lead to an increase in *Streptococcus* and *Prevotella* in the gut microbiome. The *streptococci* spp accounts as primordial microorganisms in the oral cavity and are generally acquired initially after birth that the growth and development of the *Streptococcus* spp as a microbiota depends on environmental factors such as diet and oral health status which protect the host from colonization

of other pathogenic microorganisms. The dryness of the oral cavity, as well as the administration of sugar-free foods, could negatively disrupt the colonization of bacteria in the oral cavity. Administration of foods containing starch such as potatoes, rice, and wheat, have a major effect on the maintenance of oral microbiota especially *Streptococcus* genus because the polysaccharides are degraded into mono and/ or disaccharides through heating and amylase activity of saliva that accounts for a main source of metabolite for *Streptococcus.* The Saliva contains secretory IgA and other antimicrobial agents hence the commensal *Streptococcus* mitis, *S. oralis*, and *S.Sanguinis* are adopted with these antimicrobial secretions; but if the oral cavity becomes dry as a result of decrement in saliva secretion, it gives an opportunely for colonization of pathogenic microorganism such as fungi. Some other studies had been demonstrated that the presence of *streptococci* would enhance the colonization of oral fungi with moderate virulent efficiency. This current project confirmed that the frequency of *Streptococcus* is higher in people who mostly administered vegetables, fruits, and legume that is accordance with previous finding regarding the effect of vegetarian diet of frequency of *Streptococcus species.*

In oral cavity, most phyla belongs to the *Bacteroidetes*, in which the *Prevotella* species consist of the largest genus. *Prevotella* is a gram negative, obligate anaerobic, and rode shaped bacteria which predominantly colonized from saliva and dental plaque, according to several cohort studies the frequency of *Prevotella* species in Turk, Chinese, Indian, Indonesian, Filipinos, African, Venezuelan, and Moroccans was more prevalent whereas the *Bacteroides* species mostly found in western and American countries. These finding indicated that the *Prevotella* genus have a potential to digest the cellulose and xylene in which the *P.Copri* has been introduced as a main indicator of effect of diet on bacterial community. It should be considered that the western diet is consist of administration of meat, dairy products, processed food contained refined carbohydrates and low level of fibre, whereas in eastern regions such as mentioned to have a high frequency of *Prevotella* spp, the diet is consisting of rice, noodle, vegetables, and natural products. It can be concluded that vegetarian diet shifts the higher colonization of *Prevotella*, hence protein and fat rich diet contributes to the higher colonization of *Bacteroides* spp. [126]

PetiaKovatcheva-Datchary et al evaluated the effect of fibre consumption in abundance of *Prevotella* in gut microbiota using shotgun metagenome analysis. They dived the participant in to two group, group consuming Barley- Kernel- based breads (BKB) and those received white wheat

flour bread (WWB) for 3 days. The results indicated that the ratio of *Prevotella/Bacteroides* was higher in group received BKB diet along with better fermentative metabolism of polysaccharides indicating that the *Prevotella* had a main role in glucose metabolism by enhanced the glycogen storage in BKB group. The fining obtained from European Metagenomics of the Human Intestinal Tract (MetaHIT) project, the American Human Microbiome Project (HMP) or the Asian Microbiome Project, indicated the diversity of gut microbiota in rural and industrialized and confirmed that the *Prevotella* genus had a higher abundance in preagricultural population and the higher level of *Bacteroide*s. [127]

4-4 The influence of diet on *Tanerella forsythia*/ *Tanerella forsythia* and their role in the disease:

Tanerella forsythia and *Porphyromonas gingivalis* are gram-negative, anaerobic oral bacteria that can induce gingivitis and periodontitis in their hosts. *P.gingivalis* is a member of the *Bacteroidetes* phylum, which needs iron to develop and produces virulence factors like enzymes. It has the ability to replicate in the reservoir of its host and to grow in the mouth cavity. Because of their ability to break down sugar for energy, *T.forsythia* belongs to the *Tanerella* genus, which can cause gingivitis in the mouth. [68]

Nutrition and diet have a local effect on the oral cavity. Dietary factors play a significant influence in gingivitis prevention and treatment. As a result of increased sugar consumption, bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Prevotella* intermedia can cause periodontal diseases. Because dietary carbohydrate is one of the most important elements in the development of dental caries and tooth loss, it is possible to describe what happens when carbohydrate is broken down by microorganisms and acid is created. Sugar-fermenting bacteria and biofilms have flourished in places where refined sugar is limited. [128,129]. One key advice for reducing the severity of gingivitis is to limit sugar consumption. For example, some studies have strongly suggested using xylitol as an alternate source strongly suggested using xylitol as an alternate source of sugar to suppress bacterial growth and metabolism, such as that of *P.gingivalis*. [128,130]. Another option is to use honey as a consequence of its phenolic compounds that exert antibacterial properties against *Streptococci*. [128]

Calcium and vitamin D, which can be found in dairy products, leafy vegetables, nuts, and seeds, can aid to reduce dental loss and improve periodontal disease outcomes. Increased consumption of dairy products may assist to minimize the severity of periodontitis later in life. Because the effect of calcium on periodontal disease is related to alveolar bone change, lower calcium consumption can increase hormone production and calcium loss from the skeleton, patients with periodontal disease who were given vitamin D and calcium had better periodontal health indicators than those who didn't consume Vit D and Ca supplement [131].

Many factors, including nutritional composition, which includes carbohydrate and protein, can influence the oral environment, plaque bacteria metabolism, and interaction with tooth and oral surfaces, as well as how food shape affects plaque and gingival inflammation control. Dry pet food is said to be preferable to soft pet food in terms of controlling plaque and calculus and preserving gingival health in pets, and fibre can help to improve gingival health. [132]. Participants who consume a higher percentage of plant-based diets not only have a healthier lifestyle, but also have better dental hygiene, which leads to plaque reduction and the prevention of periodontal disease, because both macronutrients and micronutrients are present in their diets and healthier diet can reduce the risk of many diseases. [133]

According to our analysis, it pointed out that *Porphyromonas gingivalis* and were associated to periodontal diseases and indicating that the consumption of the dairy and meat products can change *Porphyromonas gingivalis* microbial community in oral cavity.

Tannerella spp was initially isolated from patients with progressive periodontitis by Tanner et al as a fusiform *Bacteroides*. It has been indicated that the glycan on the cell surface of *Tannerella s* could suppress the infiltration of neutrophile mediated by Th17 in gingival tissue and induce the persistence of pathogen in dental plaque. [134]. Periodontal disease is characterized by the colonization of microbial community including *Porphyromonas gingivalis, Tannerella forsythiaar*, as well as *, Fusobacterium nucleatum, Prevotella intermedia, Actinobacillus*,a*ctinomycetemcomitans,* and *Eubacterium nodatum*. [135]. Ayaka koga et al for a first time reported the association of *T.forsythia* with onset of fever. The *T.forsythia* produce the trypsin- like protease, SiaH, NanH, as a sialidases, *Bacteroides* surface protein A (Bsp), a-D-glucosidase, Hemagglutinin, N-acetyl- β- glycosaminidase, forsythia detaching factor, methylglyoxal, and bacterial S-layer which induce the necrotic and apoptotic death following

interruption of connective tissues and destructions of epithelial junction. [136]. Also, other study revealed that the oral environment provide a suitable environment for colonization of anaerobic bacteria such as *P.gingivalis, A._actinomycetemcomitans, and T.forsythia promoting the colonization of these bacteria in patients without* teeth *which underwent the orotracheal intubation.* [136].The results of current study illustrated that administration of protein such as meat, dairy products are associated with frequency of *Tannerella* and *Porphyromonas* spp.

4-5 The influence of the diet on *Eikenella:*

Eikenella is a protobacteria bacterium that is commonly found in the mouth and gastrointestinal tract and has been linked to periodontal disease. is gram negative bacillus, microaerophilic, requires 5-10% co2 for its optimal growth . E. corrodes is a normal habitant of human oral cavity that can be detected cultivable bacteria from sub and supra gingival plaque and increase in number and proportion at diseased sites, they may induce the expression of various inflammatory mediators. [137,138]. The presence of *E. corrodens* in the mouth and gingival surfaces appears to be associated with the adherence of the organism to human epithelial cell. [139]. Patients with periodontitis have more sites infected with higher level of *E. corrodens* than healthy individuals that is suggested that this organism maybe associated with pathogenesis of periodontitis. [140]. Our findings reporting *Eikenella* as one of the oral microbiomes is associated with the coronary artery disease that is a chronic inflammatory disease and periodontal disease.

Eikenella corrodens accounts as a normal flora of oral cavity, intestinal tract, and genitals tract. *E.corrodens* is a gram- negative, facultative, and rod shaped bacteria that is mostly detected from sub and supra- gingival plaque and periodontal diseases namely known and putative and non-competitive periodontopathogens. The growth of bacteria directly associated with nitrate supply, so diets rich in nitrates such as green leaves, vegetables would considerably promote the growth of *E. corrodens*. [137]. Yael R nobel et al investigated the effect of dietary fibre on esophageal microbiome and the results indicated that administration of low fibre foods render the increment of gram-negative bacteria such as *Eikenella, Neisseria, and Prevotella* spp. [141]

4-6 The influence of the diet on *Rothia:*

Rothia and *Neisseria* spp have a major role in periodontal health by reduction of nitrate, as they are an obligate aerobic bacteria hence, many species could reduce the nitrate under anaerobic situation through denitrification to NO. [142] .Velmurugan et al reported that consumption of beetroot juice for six month could increase the frequency of *Rothia* and *Neisseria* genera in oral cavity. [143]. Also, vanhatalo et al observed that administration of dietary supplement rich in nitrate for 10 days would increase the frequency of *Rothia* and Neisseria while decrease the frequency of *Prevottella* and *Veillonella*. [144]

Zamakhchari et al evaluated the *Rothia* bacteria in upper gastero-intestinal tract as a gluten-digesting microbial enzyme. They declared that *Rothia mucilaginosa* and *Rothia aeria* as a major bacterial strain in digestion of gluten in dental plaque. Also, *Rothia* could cleavage the immunogenic epitopes of gliadin in celiac disease. *Rothia* spp produce glutamine endo proteases in which cleavage the wheat gluten and other gluten containing foods accounts as a good candidate for treatment of celiac diseases. *Rothia* genus is a family of Actinobacteria phyla which is a gram-positive in cocci form. [145]. Another study observed that vegetarians diet promote the colonization of upper respiratory tract microbiome such as (*Neisseria subflava,* *Haemophilus parainfluenzae* and *Rothia mucilaginosa* as well as colonization *Campylobacter rectus* and *Porphyromonas endodontalis* which are responsible for periodontal disease. [146]

4-7 The influence of the diet on *fusobacterium:*

Tennert Christian et al investigated the effect of healthy diet on composition of supragingival oral plague bacteria including *Streptococcus mitis, Granulicatella adiacens, fusobacterium,* and*Actinomyces* spp. During the experiment participant divided in two group: one group received carbohydrates and processed food namely western diet as a standard group, while other group received lower carbohydrate and rich in omega-3 fatty acid, Vitamin C, D, antioxidant, and fibre for 4 weeks, then the microbial composition of saliva and supragingival plaque samples were compared in both groups. They observed a significant reduction in frequency of *Streptococcus mitis* group, *Granulicatella adiacens*, *Actinomyces* spp., and *fusobacterium* in healthy group. [147]

Fusobacterium nucleatum (FN) accounts as a main cause of cancer among other species. *Fusobacterium nucleatum* is a gram-negative, non-spore forming, and anaerobic bacteria which is classified as a normal flora in the oral cavity, gastrointestinal tract, and vagina. [148]. *Fusobacterium nucleatum* passed through the blood stream and render the occurrence of colorectal disease by strong adhesion to the epithelial cells, fibroblasts, polymorphonuclear leukocytes, and salivary macromolecules following the activation of host immune response.[149]. According to the finding of Dana-Farber Cancer Institute and Massachusetts General Hospital, a diet rich in grains and fibre could significantly reduce of occurrence of colorectal carcinoma (CRC), mediated by *fusobacter*. [150]. Christian Tennert et al investigated the healthy diet on composition of supragingival oral plaque. They samples were taken from saliva and supragingival plaque following 8 weeks follow up. They discovered that administration of diet rich in omega-3 fatty acid, Vitamin C, D, and fibre along with reduction in administration of carbohydrates could significantly reduce the number of *fusobacterium spp, Streptococcus mitis, Actinomyces spp*, and, *Granulicatella adiacens*.[147]. Administration of proinflammatory diet, red meat, and processed foods are associated with higher risk of FN relative colorectal tumours. The components and metabolite of food can directly effect on gut microbiota function and composition. It has been demonstrated that hem components, N-nitroso compounds (NOCs), heterocyclic amino acids, and undigested proteins which are the main components of red meat and processed foods have a major effect of gut microbiota composition and progression of colorectal diseases.

The oral *Fusobacterium nucleatum* (*Fn*) and *Porphyromonas gingivalis* (*Pg*) account as a main pathogenic bacterium which promotes the periodontitis. The FN bacteria attached to the vascular endothelial cadherin (VE- cadherin) and endothelial cells cadherin (E-cadherin) through FadA protein following the secretion of serin protease which promotes the decrement of immune cells and induction of apoptosis. Smoking and alcohol accelerate the biofilm formation of *FN* on teeth, as the alcohol has a dehydration property which reduce the secretion of saliva. Furthermore, administration of red meat, high carbohydrate, processed foods, refined grain, and beverage rich in sugar have a direct association with *Fn*- related colorectal cancers, whereas, diets rich in fibres, render the changes in PH levels of oral cavity, have an impact on bacterial fermentation of foods which create a situation with is not suitable for *FN* bacteria. [151]

6-Conclusion:

A bacterial population known as the microbiota lives in particular parts of the human body. The human digestive tract is home to more than a trillion microorganisms, and each individual has a different microbiota composition. Therefore, nutrition is a crucial contributor to the diversity of the microbiota community, which can be essential for maintaining a diverse microbial population and a healthy environment in the gastrointestinal tracts. Altering one's eating habits may alter the microbiota in the gut and present a potential therapeutic strategy for various diseases connected to the microbiota population. The current study was aimed to determine the frequency of oral microbiota on dental calculus from patients who referred for ordinal check-up of dental health and according to NGS method we found presence of *Actinobacteria, Bacteroidetes, Campylobacteria, Chloroflexi, Firmicutes, Fusobacteria, Palescibacteria, Proteobacteria, Spirochaetes,* and *Synergistota.* Finally according to Kruskal Wallis test and LDA analysis we observed that *Fusobacteria, Tannerella spp*, *Lautropia* spp, *Rotial spp*, and *Streptococcus spp* are associated with type of diet. A diet rich in beans, fruits, vegetables, and fish, for example, was associated with *Streptococcus spp* . Cereals were strongly linked *Lautropia* spp , while milk and its derivatives, as well as meat, were strongly linked to *Fusobacteria and Tannerella spp.* Ultimately, Comparing the diet habits with obtained results of NGS sequencing, we observed that administration of vegetables, fruits, and legumes, is associated with the genus *Streptococcus*, a key component of a healthy oral microbiota; while administration of animal protein, such as milk and meat, is associated with *Tannerella* and *Porphyromonas.* These finding indicated that administration of specific diet pattern could have preventive effect on occurrence of oral cavity diseases such periodontitis, gingivitis, tooth decay, and other gum and teeth related complications. This study was performed on random patients referred for routine teeth examinations and it is suggested that if the diet pattern of people were specified and people divided into different diet group the exact effect of diet on microbiota composition as well as duration of application of specific diet on permanent changes of oral microbiota would be clearly approved. Aslo, as the prevalence of *Porphyromonas gingivalis, Tannerella* and *Porphyromonas, Fusobacteria and Tannerella* are closely related to the administration high sugar content food as well as western diet it is suggested that by modification of diet habits and administration sugar free food could prevent the occurrence of oral cavity diseases.

7-Bibliography:

1. Hoffmann, A. R., Proctor, L. M., Surette, M. G. & Suchodolski, J. S. The Microbiome: The Trillions of Microorganisms That Maintain Health and Cause Disease in Humans and Companion Animals. *Vet Pathol* 53, 10–21 (2016).

2. Cénit, M. C., Matzaraki, V., Tigchelaar, E. F. & Zhernakova, A. Rapidly expanding knowledge on the role of the gut microbiome in health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1842, 1981–1992 (2014).

3. Wang, B., Yao, M., Lv, L., Ling, Z. & Li, L. The Human Microbiota in Health and Disease. *Engineering* 3, 71–82 (2017).

4. Amon, P. & Sanderson, I. What is the microbiome? *Arch Dis Child Educ Pract Ed* 102, 257–260 (2017).

5. Diard, M. *et al.* Inflammation boosts bacteriophage transfer between *Salmonella* spp. *Science* 355, 1211–1215 (2017).

6. Mohajeri, M. H. *et al.* The role of the microbiome for human health: from basic science to clinical applications. *Eur J Nutr* 57, 1–14 (2018).

7. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochemical Journal* 474, 1823–1836 (2017).

8. Belkaid, Y. & Hand, T. W. Role of the Microbiota in Immunity and Inflammation. *Cell* 157, 121–141 (2014).

9. Berg, G. *et al.* Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8, 103 (2020).

10. Weber, K. S. *et al.* Personal microbiome analysis improves student engagement and interest in Immunology, Molecular Biology, and Genomics undergraduate courses. *PLoS ONE* 13, e0193696 (2018).

11. Honigsbaum, M. René Dubos, tuberculosis, and the “ecological facets of virulence”. *HPLS* 39, 15 (2017).

12. Sarma-Rupavtarm, R. B., Ge, Z., Schauer, D. B., Fox, J. G. & Polz, M. F. Spatial Distribution and Stability of the Eight Microbial Species of the Altered Schaedler Flora in the Mouse Gastrointestinal Tract. *Appl Environ Microbiol* 70, 2791–2800 (2004).

13. Dubos, R. & Schaedler, R. W. SOME BIOLOGICAL EFFECTS OF THE DIGESTIVE FLORA: *The American Journal of the Medical Sciences* 244, 265–271 (1962).

14. Morgan, X. C. & Huttenhower, C. Chapter 12: Human Microbiome Analysis. *PLoS Comput Biol* 8, e1002808 (2012).

15. The Integrative HMP (iHMP) Research Network Consortium. The Integrative Human Microbiome Project. *Nature* 569, 641–648 (2019).

16. The VOGUE Research Group *et al.* The vaginal microbiome of pregnant women is less rich and diverse, with lower prevalence of Mollicutes, compared to non-pregnant women. *Sci Rep* 7, 9212 (2017).

17. Wylie, K. M., Mihindukulasuriya, K. A., Sodergren, E., Weinstock, G. M. & Storch, G. A. Sequence Analysis of the Human Virome in Febrile and Afebrile Children. *PLoS ONE* 7, e27735 (2012).

18. Kilian, M. *et al.* The oral microbiome – an update for oral healthcare professionals. *Br Dent J* 221, 657–666 (2016).

19. Warinner, C., Speller, C., Collins, M. J. & Lewis, C. M. Ancient human microbiomes. *J Hum Evol* 0, 125–136 (2015).

20. Laukens, D., Brinkman, B. M., Raes, J., De Vos, M. & Vandenabeele, P. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. *FEMS Microbiology Reviews* 40, 117–132 (2016).

21. Guarner, F. & Malagelada, J.-R. Gut flora in health and disease. *The Lancet* 361, 512–519 (2003).

22. Carini, P. *et al.* Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nat Microbiol* 2, 16242 (2017).

23. Marchesi, J. R. & Ravel, J. The vocabulary of microbiome research: a proposal. *Microbiome* 3, 31, s40168-015-0094–5 (2015).

24. Hou, K. *et al.* Microbiota in health and diseases. *Sig Transduct Target Ther* 7, 135 (2022).

25. Costello, E. K. *et al.* Bacterial Community Variation in Human Body Habitats Across Space and Time. *Science* 326, 1694–1697 (2009).

26. Segata, N. *et al.* Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods* 9, 811–814 (2012).

27. Gillespie, J. J. *et al.* PATRIC: the Comprehensive Bacterial Bioinformatics Resource with a Focus on Human Pathogenic Species. *Infect Immun* 79, 4286–4298 (2011).

28. Moens, E. & Veldhoen, M. Epithelial barrier biology: good fences make good neighbours: Epithelial barrier biology. *Immunology* 135, 1–8 (2012).

29. Wade, W. G. The oral microbiome in health and disease. *Pharmacological Research* 69, 137–143 (2013).

30. Bull, M. J. & Plummer, N. T. Part 1: The Human Gut Microbiome in Health and Disease. *Integr Med (Encinitas)* 13, 17–22 (2014).

31. Caporaso, J. G. *et al.* Moving pictures of the human microbiome. *Genome Biol* 12, R50 (2011).

32. Rogier, E. W. *et al.* Lessons from mother: Long-term impact of antibodies in breast milk on the gut microbiota and intestinal immune system of breastfed offspring. *Gut Microbes* 5, 663–668 (2014).

33. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 9, 313–323 (2009).

34. Larsbrink, J. *et al.* A discrete genetic locus confers xyloglucan metabolism in select human gut Bacteroidetes. *Nature* 506, 498–502 (2014).

35. Goh, Y. J. & Klaenhammer, T. R. Genetic Mechanisms of Prebiotic Oligosaccharide Metabolism in Probiotic Microbes. *Annu. Rev. Food Sci. Technol.* 6, 137–156 (2015).

36. Duncan, S. H., Louis, P., Thomson, J. M. & Flint, H. J. The role of pH in determining the species composition of the human colonic microbiota. *Environmental Microbiology* 11, 2112–2122 (2009).

37. Cani, P. D., Everard, A. & Duparc, T. Gut microbiota, enteroendocrine functions and metabolism. *Current Opinion in Pharmacology* 13, 935–940 (2013).

38. Kang, Z. *et al.* Recent advances in microbial production of δ-aminolevulinic acid and vitamin B12. *Biotechnology Advances* 30, 1533–1542 (2012).

39. Magrone, T. & Jirillo, E. The Interplay between the Gut Immune System and Microbiota in Health and Disease: Nutraceutical Intervention for Restoring Intestinal Homeostasis. *CPD* 19, 1329–1342 (2012).

40. Chen, Y., Zhou, J. & Wang, L. Role and Mechanism of Gut Microbiota in Human Disease. *Front. Cell. Infect. Microbiol.* 11, 625913 (2021).

41. Khan, I. *et al.* Mechanism of the Gut Microbiota Colonization Resistance and Enteric Pathogen Infection. *Front. Cell. Infect. Microbiol.* 11, 716299 (2021).

42. Schippa, S. & Conte, M. Dysbiotic Events in Gut Microbiota: Impact on Human Health. *Nutrients* 6, 5786–5805 (2014).

43. Amre, D. K. *et al.* Imbalances in Dietary Consumption of Fatty Acids, Vegetables, and Fruits Are Associated With Risk for Crohn's Disease in Children. *Am J Gastroenterology* 102, 2016–2025 (2007).

44. Corridoni, D., Arseneau, K. O. & Cominelli, F. Inflammatory bowel disease. *Immunology Letters* 161, 231–235 (2014).

45. Baumgart, M. *et al.* Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 1, 403–418 (2007).

46. Thomas, S. *et al.* The Host Microbiome Regulates and Maintains Human Health: A Primer and Perspective for Non-Microbiologists. *Cancer Research* 77, 1783–1812 (2017).

47. Baumgart, D. C. & Sandborn, W. J. Crohn's disease. *The Lancet* 380, 1590–1605 (2012).

48. Ordás, I., Eckmann, L., Talamini, M., Baumgart, D. C. & Sandborn, W. J. Ulcerative colitis. *The Lancet* 380, 1606–1619 (2012).

49. Li, X.-X. *et al.* Bacterial Microbiota Profiling in Gastritis without Helicobacter pylori Infection or Non-Steroidal Anti-Inflammatory Drug Use. *PLoS ONE* 4, e7985 (2009).

50. Karlsson, F., Tremaroli, V., Nielsen, J. & Bäckhed, F. Assessing the Human Gut Microbiota in Metabolic Diseases. *Diabetes* 62, 3341–3349 (2013).

51. Akcalı, A. & Lang, N. P. Dental calculus: the calcified biofilm and its role in disease development. *Periodontol 2000* 76, 109–115 (2018).

52. Fons-Badal, C. *et al.* Analysis of Predisposing Factors for Rapid Dental Calculus Formation. *JCM* 9, 858 (2020).

53. Siddharth, M., Singla, A. & Kaur, S. Periodontal Diseases In Children And Adolescents -A Review. *Journal of Oral Health & Research* 4, 18–23 (2013).

54. Wilcox, C. R. *et al.* Effectiveness of the probiotic Streptococcus salivarius K12 for the treatment and/or prevention of sore throat: a systematic review. *Clinical Microbiology and Infection* 25, 673–680 (2019).

55. Rosenblatt, R., Steinberg, D., Mankuta, D. & Zini, A. Acquired Oral Microflora of Newborns During the First 48 Hours of Life. *Journal of Clinical Pediatric Dentistry* 39, 442–446 (2015).

56. Peterson, S. N. *et al.* The Dental Plaque Microbiome in Health and Disease. *PLoS ONE* 8, e58487 (2013).

57. Jakubovics, N. S. Saliva as the Sole Nutritional Source in the Development of Multispecies Communities in Dental Plaque. *Microbiol Spectr* 3, 3.3.26 (2015).

58. Siqueira, J. F. & Rôças, I. N. Diversity of Endodontic Microbiota Revisited. *J Dent Res* 88, 969–981 (2009).

59. Stašková, A., Nemcová, R., Lauko, S. & Jenča, A. Oral Microbiota from the Stomatology Perspective. in *Bacterial Biofilms* (eds. Dincer, S., Sümengen Özdenefe, M. & Arkut, A.) (IntechOpen, 2020). doi:10.5772/intechopen.89362.

60. Jenkinson, H. F. & Lamont, R. J. Oral microbial communities in sickness and in health. *Trends in Microbiology* 13, 589–595 (2005).

61. Abranches, J. *et al.* Biology of Oral Streptococci. *Microbiol Spectr* 6, 6.5.11 (2018).

62. Gross, E. L. *et al.* Beyond Streptococcus mutans: Dental Caries Onset Linked to Multiple Species by 16S rRNA Community Analysis. *PLoS ONE* 7, e47722 (2012).

63. Holt, S. C. & Ebersole, J. L. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol 2000* 38, 72–122 (2005).

64. Bourgeois, D., Inquimbert, C., Ottolenghi, L. & Carrouel, F. Periodontal Pathogens as Risk Factors of Cardiovascular Diseases, Diabetes, Rheumatoid Arthritis, Cancer, and Chronic Obstructive Pulmonary Disease—Is There Cause for Consideration? *Microorganisms* 7, 424 (2019).

65. Hashioka, S. *et al.* Implications of Systemic Inflammation and Periodontitis for Major Depression. *Front. Neurosci.* 12, 483 (2018).

66. Peng, X. *et al.* Oral microbiota in human systematic diseases. *Int J Oral Sci* 14, 14 (2022).

67. Koyata, Y., Watanabe, K., Toyama, T., Sasaki, H. & Hamada, N. Purification and characterization of a fimbrial protein from *Porphyromonas salivosa* ATCC 49407. *J. Vet. Med. Sci.* 81, 916–923 (2019).

68. Malinowski, B. *et al.* The role of Tannerella forsythia and Porphyromonas gingivalis in pathogenesis of esophageal cancer. *Infect Agents Cancer* 14, 3 (2019).

69. Hajishengallis, G., Darveau, R. P. & Curtis, M. A. The keystone-pathogen hypothesis. *Nat Rev Microbiol* 10, 717–725 (2012).

70. Haffajee, A. D. *et al.* Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. *J Clin Periodontol* 25, 346–353 (1998).

71. Sharma, A. Virulence mechanisms of Tannerella forsythia: Virulence mechanisms of Tannerella forsythia. *Periodontology 2000* 54, 106–116 (2010).

72. Ghabanchi, J., Zibaei, M., Afkar, Md. & Sarbazie, A. Prevalence of oral *Entamoeba gingivalis* and *Trichomonas tenax* in patients with periodontal disease and healthy population in Shiraz, southern Iran. *Indian J Dent Res* 21, 89 (2010).

73. Zaura, E., Nicu, E. A., Krom, B. P. & Keijser, B. J. F. Acquiring and maintaining a normal oral microbiome: current perspective. *Front. Cell. Infect. Microbiol.* 4, (2014).

74. Edlund, A. *et al.* An in vitrobiofilm model system maintaining a highly reproducible species and metabolic diversity approaching that of the human oral microbiome. *Microbiome* 1, 25 (2013).

75. Marcotte, H. & Lavoie, M. C. Oral Microbial Ecology and the Role of Salivary Immunoglobulin A. *Microbiol Mol Biol Rev* 62, 71–109 (1998).

76. Li, Y. *et al.* Oral, Tongue-Coating Microbiota, and Metabolic Disorders: A Novel Area of Interactive Research. *Front. Cardiovasc. Med.* 8, 730203 (2021).

77. Ellies, M. & Laskawi, R. Diseases of the salivary glands in infants and adolescents. *Head Face Med* 6, 1 (2010).

78. Zheng, D., Liwinski, T. & Elinav, E. Interaction between microbiota and immunity in health and disease. *Cell Res* 30, 492–506 (2020).

79. Brushett, S., Sinha, T., Reijneveld, S. A., de Kroon, M. L. A. & Zhernakova, A. The Effects of Urbanization on the Infant Gut Microbiota and Health Outcomes. *Front. Pediatr.* 8, 408 (2020).

80. Dominguez-Bello, M. G. *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11971–11975 (2010).

81. Stahringer, S. S. *et al.* Nurture trumps nature in a longitudinal survey of salivary bacterial communities in twins from early adolescence to early adulthood. *Genome Res.* 22, 2146–2152 (2012).

82. Holgerson, P. L. *et al.* Oral Microbial Profile Discriminates Breast-fed From Formula-fed Infants. *Journal of Pediatric Gastroenterology & Nutrition* 56, 127–136 (2013).

83. Baca, B. *et al.* Horizontal transmission of streptococcus mutans in schoolchildren. *Med Oral* e495–e500 (2012) doi:10.4317/medoral.17592.

84. Wu, G. D. *et al.* Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science* 334, 105–108 (2011).

85. Clarke, S. F. *et al.* Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63, 1913–1920 (2014).

86. Kato, I. *et al.* Nutritional Correlates of Human Oral Microbiome. *Journal of the American College of Nutrition* 36, 88–98 (2017).

87. Sapkota, A. R., Berger, S. & Vogel, T. M. Human Pathogens Abundant in the Bacterial Metagenome of Cigarettes. *Environmental Health Perspectives* 118, 351–356 (2010).

88. Charlson, E. S. *et al.* Disordered Microbial Communities in the Upper Respiratory Tract of Cigarette Smokers. *PLoS ONE* 5, e15216 (2010).

89. Hemalatha, R. Diet and Gut Microbiota in Human Health. *Proceedings of the Indian National Science Academy* 82, (2016).

90. MetaHIT Consortium (additional members) *et al.* Enterotypes of the human gut microbiome. *Nature* 473, 174–180 (2011).

91. Barko, P. C., McMichael, M. A., Swanson, K. S. & Williams, D. A. The Gastrointestinal Microbiome: A Review. *J Vet Intern Med* 32, 9–25 (2018).

92. Valdes, A. M., Walter, J., Segal, E. & Spector, T. D. Role of the gut microbiota in nutrition and health. *BMJ* k2179 (2018) doi:10.1136/bmj.k2179.

93. Aziz, R. A hundred-year-old insight into the gut microbiome! *Gut Pathog* 1, 21 (2009).

94. Flint, H. J., Scott, K. P., Louis, P. & Duncan, S. H. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* 9, 577–589 (2012).

95. Salminen, S. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut* 53, 1388–1389 (2004).

96. Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z. & Dominguez-Bello, M. G. The infant microbiome development: mom matters. *Trends in Molecular Medicine* 21, 109–117 (2015).

97. Azad, M. B. *et al.* Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *CMAJ* 185, 385–394 (2013).

98. Tanaka, M. & Nakayama, J. Development of the gut microbiota in infancy and its impact on health in later life. *Allergology International* 66, 515–522 (2017).

99. Su, Q. & Liu, Q. Factors Affecting Gut Microbiome in Daily Diet. *Front. Nutr.* 8, 644138 (2021).

100. Conlon, M. & Bird, A. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. *Nutrients* 7, 17–44 (2014).

101. Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J. & Queipo-Ortuño, M. I. Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry* 24, 1415–1422 (2013).

102. Seo, Y. S., Lee, H.-B., Kim, Y. & Park, H.-Y. Dietary Carbohydrate Constituents Related to Gut Dysbiosis and Health. *Microorganisms* 8, 427 (2020).

103. Turnbaugh, P. J. *et al.* The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. *Sci. Transl. Med.* 1, (2009).

104. Walter, J. Murine Gut Microbiota—Diet Trumps Genes. *Cell Host & Microbe* 17, 3–5 (2015).

105. Weickert, M. O. & Pfeiffer, A. F. Impact of Dietary Fiber Consumption on Insulin Resistance and the Prevention of Type 2 Diabetes. *The Journal of Nutrition* 148, 7–12 (2018).

106. Semova, I. *et al.* Microbiota Regulate Intestinal Absorption and Metabolism of Fatty Acids in the Zebrafish. *Cell Host & Microbe* 12, 277–288 (2012).

107. Min, Y. *et al.* Sex-specific association between gut microbiome and fat distribution. *Nat Commun* 10, 2408 (2019).

108. Wan, Y. *et al.* Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut* 68, 1417–1429 (2019).

109. Wycherley, T. P. *et al.* A High-Protein Diet With Resistance Exercise Training Improves Weight Loss and Body Composition in Overweight and Obese Patients With Type 2 Diabetes. *Diabetes Care* 33, 969–976 (2010).

110. Koeth, R. A. *et al.* Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 19, 576–585 (2013).

111. Zhao, J., Zhang, X., Liu, H., Brown, M. A. & Qiao, S. Dietary Protein and Gut Microbiota Composition and Function. *CPPS* 20, 145–154 (2018).

112. Biesalski, H. K. Nutrition meets the microbiome: micronutrients and the microbiota: Nutrition meets the microbiome. *Ann. N.Y. Acad. Sci.* 1372, 53–64 (2016).

113. Sofi, F., Macchi, C., Abbate, R., Gensini, G. F. & Casini, A. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr.* 17, 2769–2782 (2014).

114. Takahashi, S., Tomita, J., Nishioka, K., Hisada, T. & Nishijima, M. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing. *PLoS ONE* 9, e105592 (2014).

115. Callahan, B. J., Sankaran, K., Fukuyama, J. A., McMurdie, P. J. & Holmes, S. P. Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses. *F1000Res* 5, 1492 (2016).

116. Kêkê, L. M. *et al.* Body mass index and childhood obesity classification systems: A comparison of the French, International Obesity Task Force (IOTF) and World Health Organization (WHO) references. *Revue d'Épidémiologie et de Santé Publique* 63, 173–182 (2015).

117. Adler, C. J. *et al.* Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat Genet* 45, 450–455 (2013).

118. Eaton, S. B. The ancestral human diet: what was it and should it be a paradigm for contemporary nutrition? *Proc. Nutr. Soc.* 65, 1–6 (2006).

119. Oba, P. M., Holscher, H. D., Mathai, R. A., Kim, J. & Swanson, K. S. Diet Influences the Oral Microbiota of Infants during the First Six Months of Life. *Nutrients* 12, 3400 (2020).

120. Cronin, P., Joyce, S. A., O'Toole, P. W. & O'Connor, E. M. Dietary Fibre Modulates the Gut Microbiota. *Nutrients* 13, 1655 (2021).

121. Garcia, S. S. *et al.* Targeting of *Streptococcus mutans* Biofilms by a Novel Small Molecule Prevents Dental Caries and Preserves the Oral Microbiome. *J Dent Res* 96, 807–814 (2017).

122. Forssten, S. D., Björklund, M. & Ouwehand, A. C. Streptococcus mutans, Caries and Simulation Models. *Nutrients* 2, 290–298 (2010).

123. Govoni, M., Jansson, E. Å., Weitzberg, E. & Lundberg, J. O. The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 19, 333–337 (2008).

124. Brandtzaeg, P. Secretory immunity with special reference to the oral cavity. *Journal of Oral Microbiology* 5, 20401 (2013).

125. Amerongen, A. N. & Veerman, E. Saliva the defender of the oral cavity. *Oral Diseases* 8, 12–22 (2002).

126. Könönen, E. & Gursoy, U. K. Oral Prevotella Species and Their Connection to Events of Clinical Relevance in Gastrointestinal and Respiratory Tracts. *Front. Microbiol.* 12, 798763 (2022).

127. Precup, G. & Vodnar, D.-C. Gut *Prevotella* as a possible biomarker of diet and its eubiotic versus dysbiotic roles: a comprehensive literature review. *Br J Nutr* 122, 131–140 (2019).

128. Najeeb, S., Zafar, M., Khurshid, Z., Zohaib, S. & Almas, K. The Role of Nutrition in Periodontal Health: An Update. *Nutrients* 8, 530 (2016).

129. Olsen, I. & Yamazaki, K. Can oral bacteria affect the microbiome of the gut? *Journal of Oral Microbiology* 11, 1586422 (2019).

130. Costabile, A. *et al.* Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *Br J Nutr* 99, 110–120 (2008).

131. Baker, J. L. & Edlund, A. Exploiting the Oral Microbiome to Prevent Tooth Decay: Has Evolution Already Provided the Best Tools? *Front. Microbiol.* 9, 3323 (2019).

132. Cugini, C., Klepac-Ceraj, V., Rackaityte, E., Riggs, J. E. & Davey, M. E. *Porphyromonas gingivalis* : keeping the pathos out of the biont. *Journal of Oral Microbiology* 5, 19804 (2013).

133. Atarbashi-Moghadam, F., Moallemi-Pour, S., Atarbashi-Moghadam, S., Sijanivandi, S. & Baghban, A. Effects of raw vegan diet on periodontal and dental parameters. *Tzu Chi Med J* 32, 357 (2020).

134. Posch, G. *et al.* Glycobiology Aspects of the Periodontal Pathogen Tannerella forsythia. *Biomolecules* 2, 467–482 (2012).

135. Nagarajan, M., Prabhu, V. R. & Kamalakkannan, R. Metagenomics: Implications in Oral Health and Disease. in *Metagenomics* 179–195 (Elsevier, 2018). doi:10.1016/B978-0-08-102268-9.00009-4.

136. Koga, A. *et al.* The Association between Tannerella forsythia and the Onset of Fever in Older Nursing Home Residents: A Prospective Cohort Study. *IJERPH* 19, 4734 (2022).

137. Chen, C. K. C. & Wilson, M. E. *Eikenella corrodens* in Human Oral and Non-Oral Infections: A Review. *Journal of Periodontology* 63, 941–953 (1992).

138. Chen, C.-K. C., Dunford, R. G., Reynolds, H. S. & Zambon, J. J. *Eikenella corrodens* in the Human Oral Cavity. *Journal of Periodontology* 60, 611–616 (1989).

139. Yamazaki, Y., Ebisu, S. & Okada, H. Eikenella corrodens adherence to human buccal epithelial cells. *Infect Immun* 31, 21–27 (1981).

140. Chen, C. K., Sunday, G. J., Zambon, J. J. & Wilson, M. E. Restriction endonuclease analysis of Eikenella corrodens. *J Clin Microbiol* 28, 1265–1270 (1990).

141. Nobel, Y. R. *et al.* Increasing Dietary Fiber Intake Is Associated with a Distinct Esophageal Microbiome. *Clinical and Translational Gastroenterology* 9, e199 (2018).

142. Jockel-Schneider, Y. *et al.* Nitrate-rich diet alters the composition of the oral microbiota in periodontal recall patients. *Journal of Periodontology* 92, 1536–1545 (2021).

143. Velmurugan, S. *et al.* Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. *The American Journal of Clinical Nutrition* 103, 25–38 (2016).

144. Vanhatalo, A. *et al.* Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. *Free Radical Biology and Medicine* 124, 21–30 (2018).

145. Zamakhchari, M. *et al.* Identification of Rothia Bacteria as Gluten-Degrading Natural Colonizers of the Upper Gastro-Intestinal Tract. *PLoS ONE* 6, e24455 (2011).

146. Ercolini, D. & Fogliano, V. Food Design To Feed the Human Gut Microbiota. *J. Agric. Food Chem.* 66, 3754–3758 (2018).

147. Tennert, C. *et al.* An oral health optimized diet reduces the load of potential cariogenic and periodontal bacterial species in the supragingival oral plaque: A randomized controlled pilot study. *MicrobiologyOpen* 9, (2020).

148. *Fusobacterium nucleatum* in Periodontal Health and Disease. *Current Issues in Molecular Biology* (2011) doi:10.21775/cimb.013.025.

149. Fardini, Y. *et al.* Fusobacterium nucleatum adhesin FadA binds vascular endothelial cadherin and alters endothelial integrity: VE-cadherin is a novel receptor for F. nucleatum. *Molecular Microbiology* 82, 1468–1480 (2011).

150. Mehta, R. S. *et al.* Association of Dietary Patterns With Risk of Colorectal Cancer Subtypes Classified by *Fusobacterium nucleatum* in Tumor Tissue. *JAMA Oncol* 3, 921 (2017).

151. Pignatelli, P. *et al.* The Potential of Colonic Tumor Tissue Fusobacterium nucleatum to Predict Staging and Its Interplay with Oral Abundance in Colon Cancer Patients. *Cancers* 13, 1032 (2021).