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ARTICLE REVIEW

The Diagnostic Importance of Salivary Microflora

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Abstract: As a source of DNA, saliva has several advantages over blood. These advantages include easier sample collection, participants' ability to carry out the sampling process independently, and stability. Because it is related to each individual's oral health status, the study of the normal microflora of human saliva is becoming increasingly popular. We searched the Embase/PubMed, Web of National Library of Medicine/National Center for Biotechnology Information (NIH/NCBI) databases for this article. Several studies have been conducted to explain the microflora associated with diabetes, cancer, rheumatic heart disease, and other diseases. Other factors that influence an individual's microflora composition include lifestyle, hygiene, age, and smoking. Microflora in saliva includes protozoa, bacteria, yeast, and viruses. The dominant Gram-positive cocci and rods found in healthy people's oral microflora are Actinomycetes spp. and Streptococcus spp. Entamoeba gingivalis and Trichomonas tenax are common protozoa found in saliva, while the yeast Candida albicans group's most common viruses are herpes and retrovirus. To identify normal salivary microflora, microbiological and biomolecular approaches can be used. Biomolecular identification of microflora; currently, the most commonly used markers are 16S rRNA (bacteria) and ITS rDNA.

Keywords: 16S rRNA, ITS rDNA, microbiology, normal microflora, saliva.

INTRODUCTION

Saliva is a slightly acidic to neutral liquid (pH 6-7) produced by the oral cavity's major and minor salivary exocrine glands. This fluid is in charge of mineral ion remineralization, lubricants, and digestive function [1,2]. The amount of salivary secretion produced is determined by the stimulus. Humans can produce 0.1-0.3 mL/min of saliva at rest, and salivary secretion can reach 7 mL/min when stimulated [3]. Water makes up the majority of saliva, with the remainder made up of protein, glycoprotein, minerals, electrolytes, and microflora. Saliva contains inorganic elements like NaCl, KCl, NHCO3, and

CaCO3, as well as a variety of proteins classified into several major groups like mucin, enzymes, serum albumin and globulins, epithelial cells, and lymphocytes [4].

Salivary microflora are microbes that live in saliva [5]. These microbes do not cause disease in their hosts, but if their growth is stable, they can be beneficial [6]. The normal microflora of saliva, which includes bacteria, protozoa, yeast, mycoplasma, and viruses [7]. Entamoeba gingivalis is a type of commensal protozoa found in saliva [8]. Gram-positive-coccus bacteria such as Streptococcus and Staphylococcus are the most common bacteria found in healthy people's saliva [9]. The most common yeast found in human saliva is Candida albicans [10]. Human microflora diversity is relatively constant, but certain factors, such as smoking, oral hygiene, and diseases, can alter the composition of salivary microflora [11]. Individuals with poor oral hygiene may be more susceptible to pneumonia as they age [12]. This increased risk is most likely due to the development of oral microflora (which does not occur under normal circumstances); this condition will result in pulmonary infections (an increase in pathogenic microbes) [13]. Meanwhile, the cigarette tobacco content will interfere with, and even harm, the balance of normal microflora in smokers' saliva, resulting in oral health problems [14]. Microbial species have been discovered in the saliva of people with oral squamous cell carcinoma. These species are predictive indicators in 80% of cancer cases [15]. Minerals can also affect the normal microflora composition of saliva and are linked to tooth decay because certain minerals are required for microbial metabolic processes to continue. Minerals can also affect the normal microflora composition of saliva and are linked to tooth decay because certain minerals are required for microbial metabolic processes to continue. Saliva contains essential minerals such as cobalt, copper, manganese, molybdenum, and zinc [16].

Salivary microflora research is gaining popularity because it is associated with health status and has advantages as a sample. Some of the advantages of using saliva as a sample for human health studies include the stability of DNA samples, the absence of the need to rush for extraction as with blood samples, the ease with which saliva can be collected independently by participants of all ages, and, of course, the convenience [17]. The goal of this review paper is to look at the normal microflora of saliva using microbiological and biomolecular reviews that are relevant to human health.

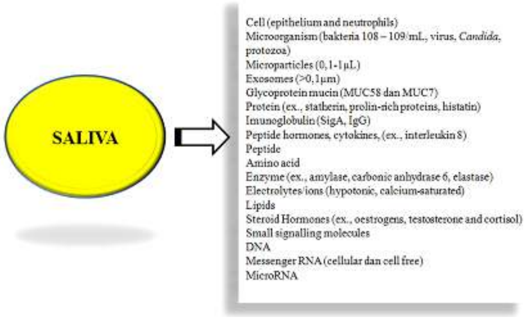

Figure 1. The composition of saliva
Source: [18]

H2O2, proteins, electrolytes, epithelial cells, neutrophils, and microorganisms are all found in saliva (bacteria, protozoa, yeast and viruses).

METHOD

The following databases were used for computerized searches: Embase/PubMed, Web of National Library of Medicine/National Center for Biotechnology Information (NIH/NCBI). Microflora, microbiome, saliva, saliva microbiology, protozoa, 16S rRNA, ITS rDNA, oral hygiene, microflora, and disease were used as search terms.

MICROBIOLOGY OF SALIVA

A normal microflora is a balanced microorganism that lives in one human organ [18]. The type of organism in each organ varies depending on the habitat environment, and the number is relatively constant / balanced, and it is known to have systemic effects on the body, including epithelial cells [19]. The mouth, after the intestines, has the most common microflora in humans. The most common groups of microbes found in healthy mouths are Firmicutes, Proteobacteria,Bacteriodetes, Actinobacteria, and Fusobacteria [20]. Changes in pH, gene mutations, and microbe interactions can cause these microbes to differ between individuals. The most common genera found in saliva are Streptococcus (17.5%), Prevotella (15.5%), Veillonella (15.3%), Neisseria (12.7%), and Haemophilus (10%) [9]. The normal microflora of the mouth found in healthy people consists primarily of Gram-positive bacteria cocci and rods, Actinomycetes spp. in addition to Streptococcus spp. [9].

Saliva can serve as a home for microflora due to the proteins and glycoproteins found in it. Almost all microorganisms found in saliva get their nutrition from saliva 21. Furthermore, saliva has a relatively stable pH of 6.5-7, which is the pH that almost all bacteria prefer [9]. Humans coexist with normal microflora. The interaction of microflora and humans as hosts is essential for the normal operation of physiological processes, metabolism, immunity, and neurological functions in the body. The relationship between microflora and the human body (host) has been linked to systemic diseases in adults [22].

In addition to bacteria, saliva contains microflora such as protozoa, yeast, and viruses. Two common protozoan populations found in saliva are saprophytes Entamoeba gingivalis and Trichomonas tenax. Candida albicans is the most common yeast found. Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium, and Cryptococcus are among the 85 fungi found in saliva [23]. The most common protozoa found in saliva are Entamoeba gingivalis and Trichomonas tenax [9]. Viruses are microbes that, unlike bacteria, are uncommon in the oral cavity.

In the absence of a stimulus, the study of viruses in the oral cavity is currently focused on saliva, and the virus type is bacteriophage in general. It has been reported that 1mL of saliva can contain up to 108 virus-like particles [9]. The most common eukaryotic viruses are herpes virus, retrovirus, papillomavirus, coronavirus, and poxvirus [24]. Individual differences in microflora status are possible and are caused by a variety of factors including nutrition, metabolism, detoxification, hormone status, immune tolerance, and inflammation [25].

The composition of the microflora is also affected by an individual's health status

[26]. Oral hygiene has a direct impact on salivary microflora [27]. Brushing teeth, as opposed to poor oral hygiene, prevents microbe demineralization and maintains the balance of salivary microflora [28]. Normal saliva microflora is linked to metabolic syndrome, atherosclerosis, cancer, intestine inflammation, and obesity, in addition to oral hygiene (Table 1).

Genetics, oral hygiene, diet, lifestyle, socioeconomic status, and geographical location are all factors that can influence the relationship between normal salivary microflora and systemic diseases [29]. Smoking habits can have a direct impact on the composition of salivary microflora. The 16S rRNA gene was used in one study to identify the composition of salivary microflora, and it was discovered that smokers had an increase in pathogenic microflora associated with oral diseases [30]. Prevotella, a type of microbe found in the saliva of individual smokers, has also been linked to cancer and inflammatory diseases [14]. The increase in such microbes in smokers as a result of the smoky environment is highly preferred and beneficial to Prevotella growth [14]. Porphyromonas microbes, on the other hand, have declined because nicotine inhibits their growth [31].

IDENTIFICATION OF SALIVARY MICROFLORA

One method for identifying salivary microflora is microbial culture with agar isolation and characterization. When compared to the sequencing method, however, the method produces a relatively low percentage result [25].

Small subunits of ribosomal RNA (16S rRNA) are currently being used to identify bacterial microorganisms using genetic material amplification and sequencing techniques [25]. Nucleic acids derived from bacterial ribosomes are bacteria components that encode proteins based on genetic information and can clearly distinguish bacteria from one another. This is because 16S rRNA has a large sustainable area and is shared by all bacteria. As a result, in the Human Genome Project [32], amplification using the 16S rRNA gene remains the gold standard for identifying bacteria. This technique has a higher accuracy rate (97%) than Matrix Assisted Laser Ionization Mass Spectrometry - Time of Flight (MALDI-TOF), which has an accuracy rate of 85%-89% [25].

The 16S rRNA gene is 1500 bp long and contains nine hypervariable regions. This area is appropriate for bacterial isolate classification. Currently, the 16S rRNA gene is used to identify bacteria in marine, soil, and human samples. The region chosen, the sequencing technology used, and the bioinformatics techniques and personnel employed can all influence the success rate of identification with the 16S rRNA gene [33].

To date, the majority of bacterial and archaea taxonomic identification activities have used the amplicon sequencing technique with the 16S rRNA gene as a marker of the V3-V4 region [34]. Meanwhile, the 18S rRNA gene marker is being used to identify eukaryotic microbes like fungi and protozoa. Both markers are effective for identification, with an accuracy of 97%. PCR amplification and sequencing with the 16S rRNA gene, on the other hand, are less effective. Metagenomic analysis, which obtains and studies the total DNA of the entire microflora using next generation sequencing, is a more promising technique for identifying salivary microflora [23].

Unlike the 16s rRNA gene, which is commonly used for bacterial identification, the Internal Transcribed Spacer ITS rDNA gene is commonly used for fungi identification. With 4S and 45S ribosomal subunit regions as conserved regions, ITS rDNA can be used to identify organisms with higher taxonomic levels (eukaryotic) such as fungi, plants, fruits, and humans [35].

Internal Transcribed Spacer (ITS) is a fungi-specific barcode marker used in the study of fungi systematics and ecology. ITS markers are divided into two types: ITS1 and ITS2. A more effective method for fungi identification is currently being debated and will necessitate additional research. The ITS2 marker is better suited for determining fungi taxonomy [36].

SALIVARY MICROFLORA AND HUMAN HEALTH

Human health is linked to microflora. Bacteria in each organ differ in composition. For example, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes can be found in the respiratory organs, the mouth (Fusobacteria), the skin (Cyanobacteria), the intestines (Lactobacillae, Streptococci, and Enterobacteria), and the vagina (Lactobacilli) [20]. If microbes commonly found in one organ are found in another, it may indicate that the individual is experiencing health problems due to a change in microbe composition. Koren et al. discovered that several common oral bacteria genera, including Streptococcus, Veillonella, and Neisseria, are found in atherosclerosis plaques and are associated with cardiovascular disease [37].

Obese people have different normal microflora. The salivary microflora of obese people is dominated by Streptococcus. Such bacteria grow in an acidic and glucose-rich environment, whereas Staphylococcus is found to be less [38,39]. Rothia species increased in the saliva of RHD patients, while Prevotella, Haemophilus, Veillonella, Campylobacter, and Actinomyces decreased [40].

The relationship between normal salivary microflora and systemic diseases is still being studied today (Table 1).

Diabetes is one of the systemic diseases associated with salivary microflora. The diversity of microflora in diabetic patients' mouths has changed, with an increase in pathogenic microbes Porphyromonas gingivalis [41]. This increase is linked to periodontitis and a deterioration in diabetics' glycemic control [42]. Meanwhile, toxic substances in cigarettes (nicotine and tobacco) kill bacteria that affect the smoker's body and promote the growth of oral pathogens and biofilm formation [43]. The commensal microbes that have increased are Streptococcus sobrinus and Eubacterium brachy, while Streptococcus sanguinis and S. Parasanguinis has decreased [31].

Steptococcus was discovered to be more prevalent in children and adults with caries than in those without [44]. The identification of normal salivary microflora can be used to predict a person's health status, which will be useful in personalized medicine [34].

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Table 1. Relationship between Normal Microflora and Individual Health Status

Normal Microflora	Health Status	Reference
Streptococcus oralis (+) Megasphaera (-) Micronuciformis Staphylococcus (-)	Obesity	26 39
Capnocytophaga gingivalis, Prevotella melaninogenica, and *Streptococcus mitis (+)*	Cancer	15
Blautia wexlerae, Lactobacillus fermentum, Nocardia coeliaca dan Selenomonas artemidis (+)	Diabetes	45
Haemophilus (+)	Eosinophilic esophagitis (EoE)	46
Haemophilus (+); Prevotella, Selenomonas, Actinomyces, Porphyromonas, and Fusobacterium (-)	Autism spectrum disorders (ASD)	47
Actinobacteria , *Bacilli* , *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* (+)	Periodontitis	9 48
Enterobacteriaceae dan *Enterococcaceae* (+)	Hepatic Encephalopaty	49
Streptococcus sobrinus, *Eubacterium brachy, Prevotella dan Megasphaera* (+)*; NeisseriaOribacterium, Capnocytophaga, Porphyromonas, Streptococcus sanguinis* dan *S. Parasanguinis* (-)	Smoker	14 31
Streptococcus and Rothia (+) *Prevotella, Haemophilus, Veillonella, Campylobacter,* and *Actinomyces* (-)	*Rheumatic Heart Disease*	40

*(+): positive corelation; (-): negative corelation

CONCLUSION

Human saliva microflora is linked to a person's health status, such as diabetes, cancer, rheumatic heart disease, and others. The composition of salivary microflora can be influenced by factors such as lifestyle, hygiene, age, and smoking. So, in the future, research into microflora saliva can be used to personalize medicine.

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