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RECENT INSIGHTS ABOUT ORAL MICROBIOTA AND SYSTEMIC DISEASES

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Abstract – The last two years have been characterized by the COVID-19 pandemic, which required a massive use of forces to fight it. Due to this issue, the number of scientific articles published on the subject of the relationship between oral microbiota and systemic diseases has undergone a slight contraction. Nevertheless, the continuous development of improved microbiological analysis techniques, most of which include molecular and cultural methods, have allowed researchers to discover or hypothesize some new associations between oral microbiota dysbiosis and an increasing amount of local and systemic diseases. The purpose of this review is to present the scientific evidence, published from April 2021 to March 2022, on the relationships between oral microbiota and systemic pathologies.

Keywords: Oral microbiota, Metagenomics, Culturomics, Systemic diseases.

INTRODUCTION

Human microbiome is considered as the aggregate of all bacterial, viruses and fungal species that live on or within human tissues and fluids. Bacteria have been found to reside in a plethora of anatomical sites including the skin, mammary glands, seminal fluid, uterus, ovarian follicles, lung, saliva, oral mucosa, conjunctiva, biliary tract, and gastrointestinal tract. The term "human microbiome" is currently used to define the microbial genomes whereas the term "microbiota" is more commonly used to refer microbial species, which make up the microbial community of an organ, system or biological fluid[1].

Man is colonized by approximately a number of bacterial cells equal to those that make up the entire human body, this element explains how eubiosis plays a key role in maintaining homeostasis[2].

The relationships that these bacterial cells maintain with human cells are not yet fully understood, especially in environments, such as the oral cavity where they reach considerable quantities. Such ease of colonization is due to the fact that the environment present in the human mouth is suited to the growth of characteristic microorganisms found there. In fact, it provides a source of water and nutrients, as well as a moderate temperature and a high number of niches that provide many different environmental conditions. Among the over 300 bacterial species known to be hosted in human mouth, indeed, a great variety of aerobic, microaerophilic and anaerobic species can be found[3].

The aim of this review is to depict the current evidence, published from April 2021 to March 2022, on the relationships between the oral microbiota and systemic diseases. The electronic search was performed on PubMed using the combination of the free text words "oral micro-

biota" and "systemic diseases". Such two words have been linked by the Boolean operator AND. The search retrieved 222 results, from which only 14 papers were included as reported in the flow diagram (Figure 1). No restrictions were applied regarding the article type. Papers were included if they presented data about any type of microbiological analyses of human samples taken from the oral cavity. Grey literature and papers presenting results about animals or published before April 2021 or in languages other than English were excluded.

The evidence from the review was divided into seven main topics. In Table I, a summary of all the included studies is given.

ORAL MICROBIOTA AND ONCOLOGICAL DISEASES

Two studies[4,5] have explored the possible connections between oral microbiota dysbiosis and oncological diseases. More specifically the review of Oldenburg et al[4] dealt with the oral

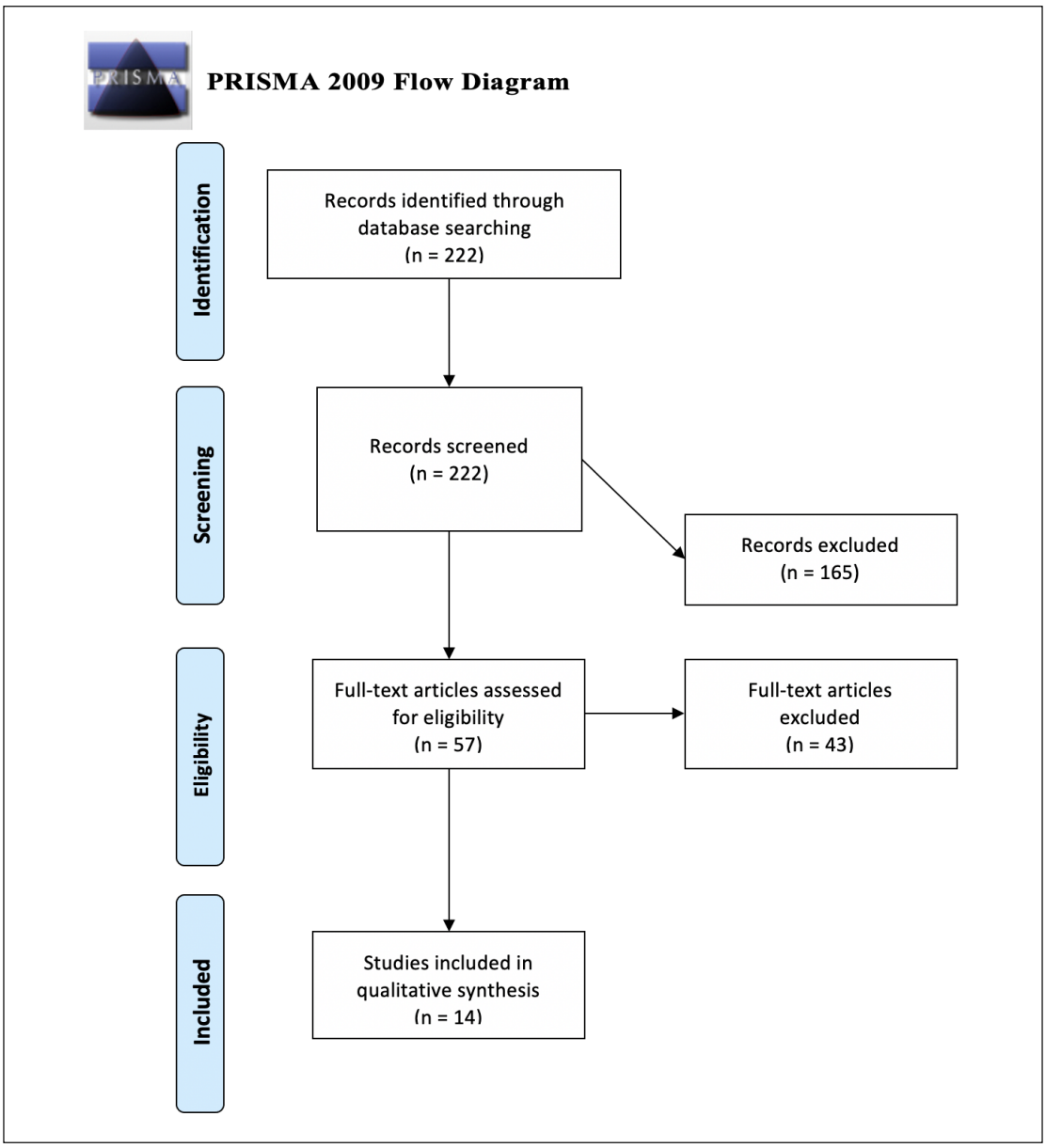

Figure 1. Flow diagram of the search strategy. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta- Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.

TABLE 1. SUMMARY OF ALL INCLUDED STUDIES. EVERY FIRST AUTHOR OF THE INCLUDED STUDIES IS GIVEN ALONG WITH THE MAIN AND SPECIFIC TOPIC ON WHICH ITS RESEARCH FOCUSED.

First Author	Main Topic	Specific Topic	Sample type	Type of metagenomic sequencing
Oldenburg M[4]	Oncologic diseases	Paediatric Leukemia	Supragingival plaque	16S
Shi J[5]	Oncologic diseases	Lung cancer	Mouth rinse	16S
Cruz de Jesus V[6]	Autoimmune diseases	Rheumatoid Arthritis	Oral swab	16S
Khasnobish A[7]	Autoimmune diseases	IgA-nephropathy	Saliva	16S
Gare J[8]	Autoimmune diseases	Pre-eclampsia	Supragingival plaque	16S
Fleury V[11]	Neurologic and psychologic diseases	Parkinson's disease Subgingival plaque	Saliva and	16S
Yang I[12]	Neurologic and psychologic diseases	Alzhemeir disease tongue and palate	Swabs from gingiva,	16S
Wingfield B[13]	Neurologic and psychologic diseases	Depression	Saliva	16S
Martínez M[14]	Neurologic and psychologic diseases	Anxiety, mood and trauma- and stress-related disorders	Saliva, subgingival and supragingival plaque	16S
Gasmi Benahmed A[15]	Metabolic diseases	Obesity supragingival plaque	Saliva, subgingival and	16S
Del Giudice C[18]	Cardiac diseases	Infective Endocarditis supragingival plaque and oral swabs	Saliva, subgingival,	16S
Schulz-Weidner N[19]	Cardiac diseases	Congenital Heart Disease	Samples from decayed teeth	16S
Melo Lettieri G[20]	Rare Syndromes	Papillon-Lefèvre Syndrome	Saliva	16S
Ma S[21]	Viral Infections	COVID-19	Oropharyngeal swabs	Shotgun

microbiota analysis of children affected by Acute Lymphoblastic Leukemia (ALL); the authors found that if ALL patients were compared with healthy controls, *Firmicutes* and *Fusobacteria* were significantly different. ALL patients had an increased abundancy of *Firmicutes*, while *Fusobacteria* abundance was decreased. More in detail *Granulicatella* and *Veillonella*, both belonging to *Firmicutes*, were more abundant in ALL patients. They conclude that ALL patients had a reduced microbial diversity and lower richness.

The other investigation was conducted by Shi et al[5] that studied the association of oral microbiota with lung cancer risk analysing 156 incident lung cancer cases and 156 individually matched controls. All bacterial analyses were conducted through metagenomic approach. No significant differences were observed for alpha or beta diversity between lung cancer cases and controls but some families (*Lachnospiraceae, Peptostreptococcaceae* and *Erysipelotrichaceae*) and *Parvimoas micra* were associated with a decreased risk of developing lung cancer.

ORAL MICROBIOTA AND AUTOIMMUNE DISEASES

Literature about connections between oral microbiota and autoimmune disease was slightly more represented than the previous topic and involved various diseases.

Cruz de Jesus et al[6] studied the influence of oral microbiota on rheumatoid arthritis (RA) through metagenomic analysis of oral bacterial microflora samples taken from 35 patients with RA and 64 non-RA controls. The authors demonstrated differences in the relative

abundance of some taxa between groups. At the genus level, *Streptococcus, Rothia,* and *Leptotrichia* were most abundant in RA, whereas *Fusobacterium, Porphyromonas, Aggregatibacter,* and *Capnocytophaga* were relatively enriched in non-RA. At the species level, *Streptococcus salivarius*, *Rothia mucilaginosa*, *Prevotella spp*., *Leptotrichia spp*., and *Selenomonas fueggei* were more abundant in RA, whereas other *Prevotella* species, including *Prevotella melaninogenica*, *Bacteroidetes*, *Fusobacterium periodonticum*, *Granulicatella elegans*, and *Porphyromonas endodontalis*, among others, were more abundant in non-RA controls.

A cohort of 43 Japanese patients suffering from IgA-nephropaty (IgAN) was enrolled by Khasnobish et al[7] for the metagenomic analysis of salivary microbiota with the aim of finding some peculiar bacterial associations. Comparisons were made with 65 healthy controls (HC). The relative mean abundance of *Bacteroidetes* was significantly lower, whereas that of *Proteobacteria* was significantly higher in the IgAN group than in HC group. The taxonomic assignment at the genus level found that *Neisseria* was significantly more abundant in the IgAN group than in the HC group whereas *Prevotella, Megasphaera, Peptostreptococcus,* and *Solobacterium* were significantly less abundant in the IgAN group than in the HC group.

The review conducted by Gare et al[8] reported evidence about the possible association between oral microbiota dysbiosis and pre-eclampsia. Such association was based on the fact that while in normotensive women, no bacteria could be detected by PCR in placental samples, a not negligible percentage (12.7%) of samples from women with pre-eclampsia had bacteria. Such bacteria included some common oral ones as: *Dialister, Porphyromonas, Prevotella*, and *Variovorax*[9]. Moreover, the comparative metagenomic analysis on 320 placental specimens revealed that the placental microbiome was closely related to the supragingival plaque[10], having 50% of placenta samples from pregnant women suffering of pre-eclampsia periodontopathogens, such as *Aggregatibacter actinomycestemcomitans, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsynthia*, and *Treponema denticola* in their placentas.

ORAL MICROBIOTA AND NEUROLOGIC AND PSYCHOLOGIC DISEASES

The chapter of neurologic and psychologic diseases has been deeply investigated by four different research groups that addressed their interests towards various diseases.

Subgingival and salivary samples were taken by Fleury et al[11] from a population of patients suffering from Parkinson's disease (PD) and from a population of healthy controls. Quantitative PCR and metagenomic analyses revealed that PD patients presented with a lower alpha-diversity. The genus *Lactobacillus* and *Scardovia* (*Actinobacteria*) had higher relative abundance in PD patients. Moreover, *Actinomyces, Veillonella*, *Kingella oralis* and *Streptococcus mutans* were more abundant in PD patients. The taxa that had significantly lower relative abundance in PD patients included *Leptotrichia* and *Treponema*.

The eventual connection with the precursor condition of Alzhemeir disease (mild cognitive impairment – MCI) was investigated by Yang et al[12]. Oral swabs from different areas of mouth (keratinized gingiva, tongue, palate) were taken for the metagenomic analyses in both groups (34 patients suffering from MCI and 34 healthy controls). The most predominant phyla in both groups were *Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria*, and *Actinobacteria* but no differences in terms of alpha, beta diversity and considering some specific genus or species were found.

The possible relationship between oral microbiota and depression, anxiety, mood and trauma- and stress-related disorders were analysed by Wingfield et al[13] and by Martínez et al[14].

The first research group focused its attention on depression. Differential abundance testing of prevalent taxa found that 21 bacterial taxa were differentially abundant in the depressed cohort relative to the controls: two were significantly more abundant in depressed subjects, and 19 were significantly less abundant. *Prevotella nigrescens* and *Neisseria* genera were significantly more abundant in the depressed cohort. Among the most represented *Haemophilus, Rothia, Treponema, Schaalia, Solobacterium, Lepotrichia, Fusobacterium,* and *Veillonella* were less abundant in the depressed cohort.

From their review, Martínez et al[14] found that *Streptococcus, Leptotrichia* and *Fusobacterium* taxa were positively associated with anxiety, depressive, and trauma- and stressor-related disorders whereas *Prevotella* and *Neisseria* taxa were negatively associated. A potential bias of this review should be considered as the authors only included studies with populations affected by periodontal disease so a not-negligible part of commensal communities could have been underestimated. Additional bias could be the criteria for the diagnosis of the periodontal disease condition not always standardized.

ORAL MICROBIOTA AND METABOLIC DISEASES

The review of Benahmed et al[15] tried to elucidate the relationship between oral microbiota composition and obesity. The authors found that likewise gut microbiome, the oral microbiome also shows differences in the overall composition between obese and lean subjects. They observed that with an increase in BMI, the diversity in bacterial species in saliva and the sublingual plaque decreases[16]. Non-obese individuals have a higher population of *Firmicutes, Bacteroidetes,* and *Spirochaetes* in the sublingual plaque. However, obese individuals showed a higher proportion of *Proteobacteria, Chloroflexi,* and *Firmicutes*. Moreover, the obese individuals showed an absence of the phylum *Bacteroidetes*, thus showing a higher *Firmicutes*/*Bacteroidetes* ratio in comparison to the non-obese individuals. This ratio is of paramount importance because a higher one has also been observed in the gut microbiome of obese individuals[17]. Such findings have established that *Firmicutes*/*Bacteroidetes* in the oral microbiome might be a very important contributing factor in the development of obesity.

ORAL MICROBIOTA AND CARDIAC DISEASES

The review of Del Giudice et al[18] analysed the current evidence about connections between oral microbiota and infective endocarditis (IE). The authors reported that *Enterococcus faecalis, Haemophilus spp., Kingella kingae, Rothia dentocariosa, Staphylococcus aureus, Streptococcus bovis, Streptococcus sanguinis, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Clostridium septicum* and *Eikenella corrodens* were found to be more representative in oral samples of patients affected by IE. Since a large part of the abovementioned bacteria belong to the periodontal pathogens group such evidence supports the theory of a strict connection between periodontal disease and IE.

The research group of Schulz-Weidner et al[19] conducted a peculiar study in which they analysed the bacterial composition of samples taken from carious lesions of children affected by congenital heart disorders (CHD) and compared such findings with samples from a control group of healthy children (HCG). The aim was to describe and compare the carious microbiome regarding the composition, diversity, and taxonomic patterns in these two groups. Twenty children with CHD and an HCG aged between two and six years participated. Microbiome analysis was conducted through a metagenomic approach. The authors found comparable amounts of *Lactobacillus, Neisseria,* and *Streptococcus* in the CHD and HCG samples whereas *Veillonella* was twice as much in the HCG than in children with CHD. Additionally, *Olsenella* and *Rothia* were increased in the HCG. In contrast, the average for *Actinomyces* in the CHD group was twofold higher than in the HCG. Furthermore, *Fusobacterium* was more abundant in the CHD samples.

ORAL MICROBIOTA AND RARE SYNDROMES

In the last year the research group of Lettieri et al[20] presented the results of their oral microbiota metagenomic analysis of three cases of an extremely rare syndrome: the Papillon-Lefèvre Syndrome. In their case series the authors presented data from three sisters affected. The salivary microbiome presented different profiles with different proportions of *Fusobacteria, Firmicutes, Proteobacteria* and *Bacteroidetes* among the sisters. Of the three sisters, only one was toothed and her microbiome presented high abundances of *Tannarella, Treponema,*

Campylobacter, and *Aggregatibacter* when compared with the other two sisters. *Streptococcus* dominated the microbiome of one toothless sister (32% of the total microbiome), whereas *Haemophilus* reached a relative abundance higher than 10%; the other toothless sister had higher abundances of *Lactobacillus* and *Porphyromonas* (all abundances below 10% of total microbiome). Even with some minor differences a core microbiome could be identified, including the organisms *Prevotella, Fusobacterium* (high abundance in all samples, and dominant in the toothed sister), *Streptococcus, Aggregatibacter, Haemophilus, Actinomyces, Campylobacter* and *Peptococcus.*

ORAL MICROBIOTA AND VIRAL INFECTIONS

After the tremendous SARS-CoV-2 pandemic, scientists from all over the world have been forced to deeply investigate all the virus characteristics in order to brew an effective method to put a stop to it. Among all the research areas, Ma et al[21] used deep sequencing and metagenomic analysis of the oropharyngeal microbiome in 31 COVID-19 patients including 29 flu patients and 28 healthy individuals as controls to identify unique characteristics of COVID-19. The authors examined the association between microbiota and disease severity. Among all the identified species, *Klebsiella spp.*, *Acinetobacter spp.* and *Serratia spp.* were among the species most positively correlated with COVID-19 severity. *Streptococcus spp.* and *Peptoniphilus spp.* were found to be the two species most negatively associated with COVID-19 disease severity. For these two species a sort of protective role in the oral cavity against COVID-19 worsening has been advocated by the authors.

CONCLUSIONS

With the advent of COVID-19 pandemic a relatively small amount of new clinical studies investigating the microbiota role in systemic diseases has been registered in favour of narrative and systematic reviews about the topic. This was undoubtedly due to the difficulty of engaging in clinical and research activities that were not primarily aimed at treating patients with COVID-19 or discovering an effective vaccine against the causative agent, the SARS-CoV-2 coronavirus. Nevertheless, the scientific literature has provided new contributions on the important issue of the links between the oral microbiota and systemic diseases. In the last year, research has focused on the relationship between oral microbiota and oncological, autoimmune, metabolic, neurological and psychological pathologies. it has been found that dysbiosis is a variable characteristic of all the aforementioned conditions; this evidence supports the strengthening of scientific research on this issue aiming at the development of therapies for balancing oral dysbiosis as a supportive therapy for the treatment of such systemic pathologies. With the overcoming of the pandemic, it is desirable to return to producing clinical studies aimed at studying this issue with a large clinical sample and characterized by high methodological rigor and standardization of methods of analysis. This will allow the comparison of data between different studies and the improvement of therapies for patients suffering from diseases caused by oral dysbiosis both in the oral cavity itself and in the various districts of the organism.

Conflict of Interest

The authors declare no conflict of interest.

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