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ORAL MICROBIOME AND OBSTRUCTIVE SLEEP APNEA – A SYSTEMATIC REVIEW

Dissertação apresentada à Universidade Católica Portuguesa
para obtenção do grau de Mestre em Medicina Dentária

Por:
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Orientador: Professora Doutora Maria José Correia
Coorientador: Professora Doutora Susana Falardo e Professor Doutor Pedro Lopes

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Aos meus pais e à minha irmã.

AGRADECIMENTOS

À minha orientadora, Professora Doutora Maria José Correia, não só por todo o apoio no desenvolvimento deste projeto, mas também pela pessoa que é, por todos os ensinamentos dos últimos 5 anos, pelo rigor científico e ajuda mesmo nos dias mais preenchidos.

À minha coorientadora Professora Doutora Susana Falardo, pela disponibilidade constante, respostas imediatas às minhas dúvidas, conhecimento científico excecional, ajuda e rigor.

Ao meu coorientador, Professor Doutor Pedro Lopes, por todos os ensinamentos ao longo dos últimos 4 anos, pelo apoio, incentivo e constante disponibilidade. Obrigada, ainda, por ter acreditado em mim, nas minhas capacidades e pelas palavras amigas sempre presentes.

Aos meus pais e à minha irmã por serem o meu pilar constante nos bons e maus momentos. Estou-vos eternamente grata e tudo o que consegui é graças a vocês.

À Francisca, a minha binómia, amiga desde o primeiro dia e para o resto da vida. Tornaste estes últimos 5 anos mais bonitos e felizes. 315 é eterno tal como esta amizade.

A todos os docentes pela partilha de conhecimento, e funcionários que diariamente tornaram os dias na faculdade mais leves.

A todas as pessoas que fizeram parte do meu percurso académico e acompanharam esta etapa tão desafiante, mas gratificante e bonita, o meu bem-haja, nada disto seria igual sem vocês na minha vida.

Resumo

Introdução: A apneia obstrutiva do sono é responsável por uma menor saturação de oxigénio e aumento do dióxido de carbono. Os pacientes diagnosticados com esta condição são na sua maioria respiradores orais. Estes fatores podem ser responsáveis por alterações no microbioma oral dos mesmos.

Objetivos: Avaliar a evidência científica disponível sobre as possíveis alterações no microbioma oral (identificado por métodos moleculares) dos pacientes diagnosticados com apneia obstrutiva do sono.

Material e métodos: Esta revisão sistemática utilizou as bases de dados PubMed, Web of Science, Scopus e Cochrane para identificação de artigos relevantes. Dois autores independentes realizaram a seleção dos estudos. A extração dos dados foi realizada seguindo o método PRISMA, respondendo à questão PICO *"In patients diagnosed with obstructive sleep apnea, compared to patients without the same diagnosis, what changes occur in the oral microbiome?"*

Resultados: Foram selecionados no total 10 artigos, 7 em população adulta e 3 em população pediátrica. Seis estudos utilizaram zaragatoas de diversas áreas da cavidade oral e 4 estudos utilizaram saliva como amostra microbiana. De forma geral, foram encontradas diferenças entre o microbioma oral de pacientes com apneia obstrutiva do sono e indivíduos saudáveis, no entanto, estas diferenças nem sempre eram significativas.

Conclusão: Dada a escassa literatura existente, não é possível concluir de forma definitiva sobre o impacto da apneia obstrutiva do sono no microbioma oral. No entanto, as condições favorecidas por estes pacientes parecem favorecer o crescimento de bactérias anaeróbias e periodontopatogénicas.

Palavras-chave: Apneia obstrutiva do sono; Hipoxia intermitente; Hipercapnia; Microbioma Oral; Bactérias Orais.

Abstract

Introduction: Obstructive sleep apnea is responsible for a decrease in oxygen saturation and an increase in carbon dioxide. Patients diagnosed with this condition are mostly mouth breathers. These factors may be responsible for changes in their oral microbiome.

Objective: To assess the available scientific evidence on possible changes in the oral microbiome (identified by molecular methods) of patients diagnosed with obstructive sleep apnea.

Materials and Methods: This systematic review used Pubmed, Web of Science, Scopus and Cochrane databases to identify relevant articles. Two independent authors selected the studies. Data extraction was carried out using the PRISMA method, answering the PICO question "In patients diagnosed with obstructive sleep apnea, compared to patients without the same diagnosis, what changes occur in the oral microbiome?"

Results: Ten articles were selected, seven on the adult population and three on the pediatric population. Six studies used swabs from different areas of the oral cavity and 4 studies used saliva as microbial sample. In general, differences were found between the oral microbiome of patients with obstructive sleep apnea and healthy individuals, however, these differences were not always significant.

Conclusion: Given the limited existing literature, it is not possible to definitively conclude on the impact of obstructive sleep apnea on the oral microbiome. However, the conditions favored by these patients seem to favor the growth of anaerobic and periodontopathogen bacteria.

Keywords: Obstructive sleep apnea; Intermittent hypoxia; Hypercapnia; Oral microbiome; Oral bacteria.

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LIST OF ABBREVIATIONS

AHI	Apnea-hypoapnea index
CFU	Colony - forming unit
GERD	Gastroesophageal reflux disease
HTN	Hypertension
JBI	Joanna Briggs Institute
OSA	Obstructive sleep apnea
OSF	Open science framework
PCR	Polymerase chain reaction
PICO	Population Intervention Comparison Outcome
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-analysis
PSG	Polysomnography

INTRODUCTION

1. INTRODUCTION

1.1. Oral microbiome

Antony Van Leeuwenhoek was the first person to study diverse forms of microbes including bacteria, fungi, and protozoa, that were present in water, mud, and in dental plaque from tooth surface (1, 2). He used his own microscope, curiously built by himself, being the very first microscope of all time (3). Over the years, especially in the last 20 years, the study and subsequent knowledge of the human microbiome have grown exponentially using very different methods (2). Techniques and knowledge on the human microbiome have exploded in the recent years and, it is now clear that both study of the oral and intestinal microbiome can bring important scientific advantages and medical treatments (2).

The microbiome is present in numerous areas of the body, but the largest in terms of biomass, including more than 30 trillion bacteria, is in the gut. It is known that the gut microbiome takes part in numerous vital processes including digestion, development and regulation of the immune system, energy homeostasis and metabolism, synthesis of vitamins and nutrients (4). A balanced microbiome leads to numerous health benefits, but when the balance between the microbiome and the host is lost dysbiosis occurs both locally and in other areas of the body (4).

The oral cavity is the most diverse microbiome of the human body and establishes a pathway between the external environmental and the respiratory / digestive system (5). The composition of the human microbiomes is different and those differences started being explored in a systematic way with the Human Microbiome Project (6). The microbiome of the human oral cavity includes different anatomic structures, comprising teeth, tongue, hard and soft palates, and gingival sulcus (3). It provides the right temperature, humidity, pH, and nutrition for microorganisms to colonize but the very different structures provide different conditions and therefore harbour very different communities (5).

Eubiosis is the scientific name given to the relationship between the microbiome and the host, where there is a dynamic balance between the two. It is therefore a healthy state in which there is high diversity and uniformity (2). In contrast, dysbiosis occurs when there is an alteration in the dynamic microbiome-host balance that can cause a range of oral infectious diseases, including dental caries, apical periodontitis, periodontal disease, pericoronitis, fungal infections, among others. The oral microbiome is also connected to various diseases affecting the entire body, such as cardiovascular disease, diabetes, respiratory problems, rheumatoid arthritis, pancreatic, colorectal, and oesophageal cancer, stroke, and adverse pregnancy outcomes (2, 5).

One of the methods used to study the oral microbiome is by collecting and analysing saliva samples, for example (1). The microbial community present on this saliva samples can differ from individual to individual due to endogenous and exogenous factors. Lifestyle and human experiences can rapidly and profoundly alter the stability of microbial communities associated with the host. In other words, the host lifestyles, physiology, genotype, pathobiology, environment, immune system, transient community members and socioeconomic status are generally considered to be important factors in the multifactorial context of oral and systemic diseases (5). When there are significant changes in the temperature of the oral cavity, as well as the pH and oxygen content, for example due to conditions such as obstructive sleep apnea, changes in the oral microbiome are a possibility (7). Another example are the changes in the dietary patterns that can result in changes of the oral microbiome and disease. Fats, sugars, and vitamins have a significant role in shaping the oral microbiome. Other factors that can change the oral microbiome are the use of antibiotic, pregnancy, age, personal hygiene, and drinking alcohol (5, 8).

Throughout the transition from early life to adulthood, notable variations occur within the microbiome, resulting in a significant more diverse and richer microbiome. In the first month’s species like *Escherichia coli*, *Staphylococcus*, *Pseudomonas, Lactobacillus* and *Streptococcus* are prevalent. When the transition from infants into more mature stages occurs, a more mature oral microbiome appears, and likely increased exposure to the external environment, such as *Veillonella, Fusobacterium, Neisseria, S.mutans, Treponema, Rothia* and *Prevotella* (9).

The oral cavity harbours communities of commensal, symbiotic and pathogenic microorganisms such as bacteria, viruses, archaea, fungi, and protozoa. Research done within the scope of the Human Microbiome Project inspired and oriented several studies which have tried to pinpoint which microbial communities are associated with health. However the picture is not fully clear since it is difficult to compare the results of the different studies and microbiome variation is great between individuals and even in the same individual over time (6).

What is becoming more evident is that the oral microbiome can undergo changes, which seem to be similar because of certain conditions. In fact, conditions such as obstructive sleep apnea, in which many of the patients are mouth breathers, causing less salivary flow which can lead to dry mouth, as well as intermittent hypoxia/ hypercapnia processes, can cause changes in the oral microbiome of patients (10). It has been suggested that oral dryness influences bone remodeling, which is triggered by the hypoxia process, and an increase in CO_2 levels, thus reducing the immune system's response to infections, and favoring a wider diversity of pathogenic microorganisms (11).

1.2. Obstructive Sleep Apnea and Hypopnea Syndrome

Obstructive sleep apnea (OSA) is a condition or disorder with a high prevalence in adults and, if left untreated, can have serious consequences. It's the most common sleep-disordered breathing condition and, although it affects both men and women, its prevalence is higher in men and in post-menopausal women (12, 13). It also affects children between the ages of 2 and 8 (12). Defined by the *American Academy of Sleep Medicine*, this disorder is periodic and repetitive, and can cause partial (hypopneas) or complete (apneas) collapse of the upper airway during sleep time (12, 14, 15). OSA is responsible for lower oxygen saturation, known as intermittent hypoxia, hypercapnia, sleep disruption, increased sympathetic nervous activity and altered immunity, and all of this has a negative impact on people's lives (12, 16).

A study carried out in 2019 on the global prevalence of obstructive sleep apnea concluded that around 1 billion people between the ages of 30-69 years old could suffer from this condition. This condition is highly associated with obesity and increasing age. Due to the ongoing obesity epidemic, these numbers are expected to rise in the future. In addition, average life expectancy is increasing, so the prevalence of obstructive sleep apnea will also rise in the coming years (17).

The pathophysiology of OSA has complex and multifactorial mechanisms and results from the interaction between anatomical and non-anatomical factors.

Regarding anatomical factors, there is no doubt that the primary cause of OSA is a congested, narrow, or naturally congested airway, known as impaired upper airway anatomy (18). The vast majority of existing therapies for OSA aim to reverse these anatomical problems. Obesity is one of the risk factors for a narrow upper airway due to the accumulation of adipose tissue in the areas surrounding the airway, such as the lateral walls of the pharynx, the soft tissues of the neck and on the tongue insertion. For this reason, the pharyngeal airspace is more susceptible to reduce (18).

In addition, lung volume is reduced by the accumulation of fat around the abdomen and the compression of the diaphragm. Non-anatomical factors include narrowing of the airway (low ventilatory response known as low arousal threshold), unstable regulation of breathing (high amplitude response known as high loop gain) and poor pharyngeal dilator muscle function (19, 20).

The diagnosis of OSA is often overlooked by health professionals. Correct diagnosis and treatment are essential and involve several medical specialties (16). OSA screening includes the patient's body mass index, blood pressure and neck circumference. Anatomical structures such as the soft palate, uvula and palatine tonsils should also be assessed, using the Mallampati, Friedman and Broadsky classifications (21).

It is important to question the patient and their bed partner about possible signs and symptoms such as snoring, sleepiness and witness apneas,. In adults, it is also important to complete the Epworth Sleepiness Scale, Berlin, and/or Stop-bang questionnaires (21).

A detailed diagnosis of the patient's entire medical history is important, including medication, comorbidities, and family history (21). The gold standard exam to diagnose OSA is the polysomnography (PSG) by an apnea-hypopnea index (AHI), which is the number of apneas/hypopneas per hour of sleep. Home sleep apnea testing is also an alternative method but has some limitations (22, 23).

According to the *International Sleep Academy*, there are two ways of diagnosis. The first one is if the patient presents symptoms (snoring, sleepiness, unrefreshing sleep, insomnia, tiredness, waking up with choking/gasping) or certain comorbidities (type 2 diabetes mellitus, coronary artery disease, congestive heart failure, hypertension, cognitive dysfunction, atrial fibrillation) associated with five or more obstructive respiratory events per hour of sleep (24). In other words, we can have a diagnose of OSA, if the patient has five or more events/h combined with any of the clinical symptoms/ certain comorbidities previously mentioned (22). The second one is when there are fifteen or more obstructive respiratory events per hour of sleep even without any apparent symptoms (22). Severity of OSA can be classified according to AHI has mild, if there are only 5-15 events per hour, moderate if more than 15 and less than 30, and severe if there are more than 30 events per hour (25).

It is also important to understand the diagnosis of this condition in children, as it differs from the diagnosis in adults. The most frequent complaint from children's parents is snoring, but during sleep there are also reports of frequent awakenings, gasping and pauses in breathing. Diaphoresis and enuresis are also common. Daytime sleepiness is not very common in children, but hyperactivity, attention deficit and aggression are (26). The severity of this condition in this age group is measured according to AHI, the most used parameter. The classification is as follows: from 1 to less than 5 events per hour, we have mild OSA, between 5 and 10 events per hour, moderate OSA and lastly, more than 10 events per hour severe OSA. The aetiology is multifactorial and involves several risks. The most common is adenotonsillar hypertrophy (27).

There are several risk factors associated with obstructive sleep apnea. Some of the most important are obesity, male gender, advancing age, craniofacial alterations, and ethnicity (28).

Men are more prone to OSA, mainly due to anatomical differences. They tend to have more fat deposits in the neck and abdomen and thus as greater predisposition to upper-airway collapsibility (28). On the other hand, in women, there is a considerable increase in the prevalence of this condition after the menopause (13).

Regarding age, it is known that the prevalence of OSA tends to increase with age, since the respiratory system of elderly people changes with age. Some of these examples are low lung volume, poor upper airway muscle function, and low arousal threshold (29). On the other hand, recent studies have shown that the severity of the disease tends to decrease with age (28).

Obesity is the main risk factor for developing obstructive sleep apnea, since it is estimated that around 58% of cases are due to this condition. Studies have shown that men aged between 30-49 years old with a body mass index of 30-39 kg/m2, the prevalence of OSA was indeed high (30). The accumulation of fat in the tissues surrounding the upper airway, increasing neck circumference, seems to result in a smaller lumen and greater collapsibility of the upper airway, predisposing to apnea (30).

Craniofacial alterations such as increased amygdala, uvula and tongue base, retrognathia, micrognathia, high arched palate is also associated with OSA (16, 30).

Other risk factors that are known to have an influence are alcohol consumption, smoking, family history and night-time nasal congestion (14).

Clinical manifestations and variations are important to recognize so that a correct diagnosis and subsequent treatment can be made (30). Symptoms can vary depending on gender, age, and whether they occur during the day or at night (31). At night, one of the most common symptoms is loud snoring, caused by increased resistance of the upper airway, choking or gasping episodes, dry mouth, excessive sweating and nicturia (23, 30, 31). Also, bed partners reported breathing pauses (24).

During the day, patients complain of excessive sleepiness, fatigue, morning headaches and irritability, which interferes with their quality of life (22, 24, 31).

Due to these factors, there is evidence of a decreased productivity in the workplace and an increase in motor vehicles accidents (22). Women can also experience mood disturbance, insomnia, anxiety, and reflux (24).

Continuous obstruction of the upper airways results in intermittent hypoxia, the defining feature of OSA, leading to oxidative stress and hypercapnia. In addition, consequently there is an increase in sympathetic activity and in lipid production, systemic inflammation, and endothelial dysfunction. All these processes are associated with various comorbidities. There is a clear association between OSA with the development of congestive heart failure, coronary artery disease, cardiac arrhythmias, hypertension, stroke, type II diabetes mellitus and non-alcohol fatty liver disease (30, 32, 33).

Sympathetic activity due to hypoxia leads to changes in the glucose metabolic cycle, which results in high levels of cortisol and growth hormone levels, deregulating insulin sensitivity leading to diabetes (23).

Leptin and ghrelin are hormones that inhibit and stimulate appetite, respectively. In OSA patients, where sleep deprivation occurs, leptin levels are decreased resulting in decreased satiety, and ghrelin levels are increased, leading to an increased feeling of hunger. This contributes to a higher risk of obesity (34).

Gastroesophageal reflux disease (GERD), in which acid refluxes from the stomach into the esophagus, is also associated with OSA due to the increased respiratory effort and coughing caused by the respiratory conditions (35).

Patients with risk factors for obstructive sleep apnea should undergo properly diagnosis and examination. Failing to do so may contribute to therapeutic failure in the treatment of these comorbidities (36).

There is currently a systematic review about oral microbiome in obstructive sleep apnea patients (10), but the difference between our study and this one, is that ours uses only molecular methods for assessing the oral microbiome, while the other uses both culture and molecular methods. In addition, the other study essentially focuses on the relationship between obstructive sleep apnea and periodontal disease.

1.3. Aim of the study

The aim of this study is to carry out a systematic review of the changes that occur in the oral microbiome of patients diagnosed with obstructive sleep apnea.

MATERIALS AND METHODS

2. MATERIALS AND METHODS

A review of the literature available in the following electronic databases was conducted: Pubmed, Web of science, Cochrane, and Scopus, to identify relevant articles. It was conducted following the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines to answer an intervention question determined according to the Population, Intervention, Comparison and Outcomes (PICO) strategy. It has also been registered in OSF Database with the following Registration DOI: osf.io/hec84

2.1. Search strategy

The search strategies were based on the PICO(S) question: **In patients diagnosed with obstructive sleep apnea, compared to patients without the same diagnosis, what changes occur in the oral microbiome?**

The defined PICO(S) question is the following:
P(Population): patients, adults or pediatrics, diagnosed with obstructive sleep apnea; **I**(Intervention): analysis of the oral microbiome; **C**(Comparison): patients with/without a diagnosis of obstructive sleep apnea; **O**(Outcomes): Alterations of the composition of the oral microbiome; **S** (Study Type): cross-sectional, cohort.

The following search equations were used for the different databases, and the following results were obtained:

PubMed/Medline: ((obstructive sleep apnea OR intermittent hypoxia OR hypercapnia) AND (oral microbiome OR oral bacteria)) - 40 results.

Web of Science: AB= (((((obstructive sleep apnea OR intermittent hypoxia OR hypercapnia)) AND (oral microbiome OR oral bacteria)))) - 19 results.

Scopus database: TITLE-ABS-KEY= (obstructive AND sleep AND apnea OR intermittent AND hypoxia OR hypercapnia AND oral AND microbiome OR oral AND bacteria) - 0 results.

Cochrane: Obstructive sleep apnea OR intermittent hypoxia OR hypercapnia AND “oral microbiome” OR “oral bacteria” - 0 results.

2.2. Eligibility Criteria

To present results of interest for this review and subsequently answer the PICOS question, it was necessary to define the inclusion and exclusion criteria. All the titles of the studies obtained were initially screened based on the following inclusion criteria: (1) studies in humans, (2) in English, (3) published between 2003-2023, (4) full text available, (5) no systematic reviews. All *in vitro* studies were excluded. After the initial assessment, the articles that effectively met the inclusion criteria were read in full. Articles with at least one of the following exclusion criteria were excluded: (1) case-reports (2) articles without information about microorganisms, (3) studies on other types of apneas than obstructive, (4) studies in which patients were not diagnosed with obstructive sleep apnea using polysomnography, (5) studies using culture methods for the microbiome evaluation, (6) studies in which the individuals in the control were patients diagnosed with obstructive sleep apnea.

The articles selected were read in full to ensure that they were only studies about obstructive sleep apnea with information on microorganisms and to ensure that the study of the oral microbiome was carried out using only molecular methods. Additionally, three articles were also added manually.

2.3. Study selection and data extraction

After the initial search of the literature in the various databases by one reviewer, two independent researchers (IA and MC) analyzed the title and abstract of all the articles to select the relevant studies. Any disagreement between the two reviewers was discussed with a third reviewer (PL). The Rayyan tool was used to organize and sort the selected the studies.

Data extraction began was summarized in Excel tables where the following aspects were recorded: study design, number of subjects, age of participants, control group, biological sample, microbiota assessment method, diagnosis of obstructive sleep apnea, results, and conclusions.

RESULTS

3. RESULTS

The results of the search and selection are summarized in Figure 1. A total of 37 potentially relevant articles were screened.

After reading the titles and abstracts, 17 articles were eliminated because they did not fulfil the inclusion criteria, namely for 1) being a systematic review, 2) being a study in animals, 3) having the wrong outcome and 4) having the wrong study duration. Thus, 20 articles were read in full to ensure that they referred to obstructive sleep apnea and provided information on microorganisms. Only 10 articles were included because 6 articles were further excluded for having the wrong OSA diagnosis and group control, and 4 for having culture based microbial identification.

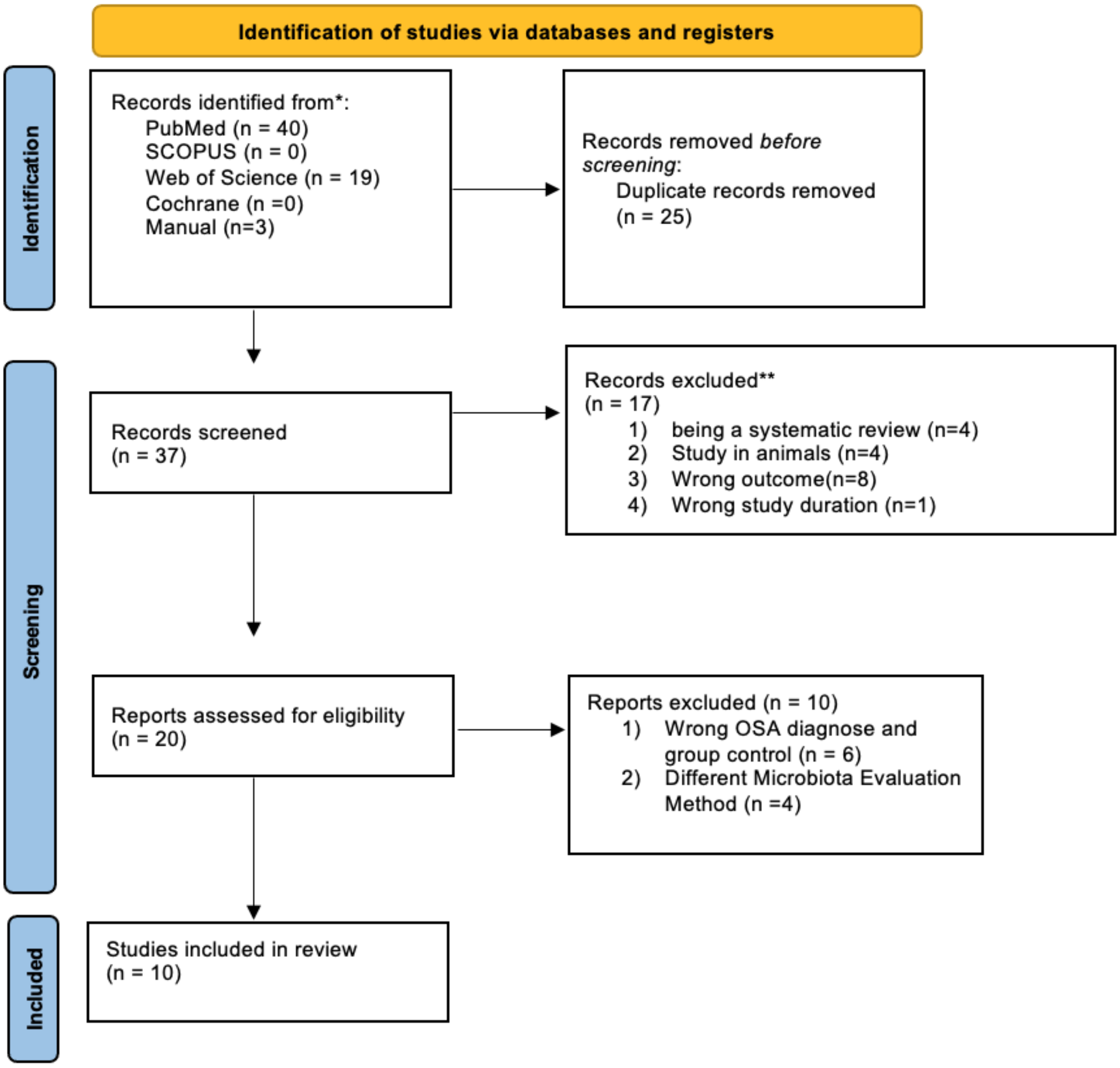

Figure 1. Article selection according to PRISMA guideline

3.1. Studies characteristics

Table I shows all the characteristics of the selected studies for this systematic review. All articles were published between 2018-2023, 8 were cross sectional studies, (12, 37-43) and only 2 cohort studies (44, 45). As far as the target population of the different studies is concerned, most of them study adult populations (12, 39, 40, 42-45) and a minority pediatric populations (37, 38, 41). As imposed by the selection criteria all the studies used molecular methods to assess the microbiome and diagnosed obstructive sleep apnea through polysomnography.

The total number of patients involved in the studies is 711, with 228 control patients and 430 diagnosed with obstructive sleep apnea. The patients diagnosed with this condition have various levels of severity of OSA and, in addition, some studies assess the association with diseases such as hypertension (98 patients) (12, 40).

As already mentioned, the diagnosis of obstructive sleep apnea was made using polysomnography and the apnea-hypopnea index (AHI). The values used as a reference in the studies to assess severity depended on the population. In adults, values above 5 and up to and including 15 events/h were considered mild, above 15 events/h moderate to severe. In children, values of 1-5 events/h were considered mild, 5-10 events/h moderate and more than 10 events/h severe.

Regarding the type of samples used for microbial analysis, saliva samples or oral/nasopharyngeal mucosal swabs were used. Four studies used saliva samples (12, 40, 41, 43) and 6 mucosal swabs (37-39, 42, 44, 45).

The molecular evaluation method was based on the sequencing of 16S rRNA gene amplicons. Seven studies used the V3-V4 region (12, 37, 40, 41, 43-45), while 1 study used the V1-V3 region (38) and one of the studies does not specify the region used (39). One of the studies used metagenomic analyses of the whole genome (42).

Table I. Characteristics of the selected studies

Author, year	**Article Title**	**Study Design**	**Type of patients**	**Nr of subjects**	**Age of participants**	**Biological Sample**	**Microbiota Evaluation Method**	**Outcomes**
Yanlong Chen et al, 2021 (12)	Analysis of Salivary Microbiome and Its Association with Periodontitis in patients with obstructive sleep apnea	Cross-sectional	Adults	103 (27 control, 27 OSA, 23 HTN, 26 OSA + HTN)	24-35	Saliva samples	16S rRNA gene sequencing (V3-V4 region)	• OSA group had lower microbial richness than the controls. • No significant difference in microbial diversity. • Several gram-positive bacteria and *Prevotella* more abundant in OSA.
Xiaoman Zhang et al, 2023 (37)	Changes in the oral and nasal microbiota in pediatric obstructive sleep apnea	Cross-sectional	Pediatric	60 (30 control, 30 OSA)	3-12	Swabs (tongue base, soft palate, palatine tonsils and adenoid tonsils, both anterior nares)	16S rRNA gene sequencing (V3-V4 region)	• Significant differences in β-diversity between OSA group and control. • α-diversity values similar between OSA group and control • Higher abundances of *Haemophilus, Fusobacterium* and *Porphyromonas* in adenoids and tonsils of OSA group.
Peizeng Jia et al, 2020 (43)	Analysis of the Salivary Microbiome in Obstructive Sleep apnea syndrome patients	Cross-sectional	Adults	24 (9 control, 15 OSA)	40-47	Saliva samples	16S RNA gene sequencing (V3-V4 region)	• No significant differences in α and β-diversity between OSA and the control group. • Only *Scardovia* species were significantly more abundant in OSA group.

Table I cont. Characteristics of the selected studies

Author, year	***Article Title***	***Study Design***	***Type of patients***	***Nr of subjects***	***Age of participants***	***Biological Sample***	***Microbiota Evaluation Method***	***Outcomes***
Huajun Xu et al, 2018 (38)	Pediatric Obstructive Sleep Apnea is Associated With Changes in the Oral Microbiome and Urinary Metabolomics Profile: A Pilot Study	Cross-sectional	Pediatric	60 (30 control, 30 OSA)	5-8	Swabs (buccal mucosa)	16S rRNA gene sequencing (V1-V3 region)	• Slightly higher α-diversity in OSA group than in controls. • Composition of the oral microbiome of OSA patients was significantly altered compared to the control group, especially with *Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria* and *Actinobacteria.*
Yang, Wenbo et al, 2019 (39)	Oropharyngeal Microbiome in Obstructive sleep Apnea: Decreased Diversity and Abundance	Cross-sectional	Adults	51 (25 control, 26 OSA)	30-65	Swabs (oropharyngeal)	16S rDNA gene sequencing	• OSA group showed a significant decrease in bacterial biodiversity compared to control group • Most notable decrease in the moderate OSA group. • Proportion of *Neisseria* was slightly higher in the severe OSA group.
Xuehui Chen et al, 2022 (40)	Altered Salivary Microbiota in Patients with Obstructive Sleep Apnea Comorbid Hypertension	Cross-sectional	Adults	103 (27 control, 27 OSA, 23 HTN, 26 OSA + HTN)	>18	Saliva samples	16S rRNA gene sequencing (V3-V4 region)	• α-diversity of patients with OSA decreased compared to control group. • β-diversity analysis revealed significant differences in the microbial communities in patients with OSA comorbid HTN compared to patients with OSA or HTN.

Table I cont. Characteristics of the selected studies

Author, year	***Article Title***	***Study Design***	***Type of patients***	***Nr of subjects***	***Age of participants***	***Biological Sample***	***Microbiota Evaluation Method***	***Outcomes***
Xin Huang et al, 2022 (41)	Characteristics of salivary microbiota in children with obstructive sleep apnea: A prospective study with polysomnography	Cross-sectional + longitudinal study	Pediatric	58 (22 control, 36 OSA)	7-9	Saliva samples	16S rRNA gene sequencing (V3-V4 region)	• Higher α-diversity and observed species in OSA group compared to control group, with significant β-diversity separation. • *Prevotella*, *Actinomyces*, *Bifidobacterium*, *Escherichia, Lactobacillus* were more abundant in the OSA group.
Yinghui Gao et al, 2023 (42)	Whole genome metagenomic analysis of the oral microbiota in patients with obstructive sleep apnea	Cross-sectional	Adults	21 (7 control, 7 OSA)	25-57	Swabs (buccal mucosa)	Whole genome metagenomic analysis (DNA extraction)	• No significant differences in the number of oral species between OSA and control group • No significant clustering of oral microbial communities between samples from control and OSA groups. • Relative abundances of *Prevotella*, *Alloprevotella*, *Bacteroidetes*, *Veillonella_tobetsuensi*s, *Candidatus saccharimonas*, and *Leptotrichia* were lower in the group with severe OSA
Seung-No Hong et al, 2022 (44)	Association of obstructive sleep apnea severity with the composition of the upper airway microbiome	Cohort	Adults	92 (38 control, 54 OSA)	62-67	Swabs (nasopharyngeal)	16S rRNA gene sequencing (V3-V4 region)	• Higher α-diversity in OSA patients than in controls but no significant changes. • Specific anaerobes negatively correlated with the lowest oxygen saturation level during sleep

Table I cont. Characteristics of the selected studies

Author, year	***Article Title***	***Study Design***	***Type of patients***	***Nr of subjects***	***Age of participants***	***Biological Sample***	***Microbiota Evaluation Method***	***Outcomes***
Chih-Yuan Ko et al, 2019 (45)	Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension	Cohort	Adults	139 (13 control, 35 OSA mild, 91 OSA moderate-severe)	28-59	Swabs (buccal mucosa, tongue, soft palate, hard palate)	16S rRNA gene sequencing (V3-V4 region)	• No significant differences in community richness and diversity between group control and OSA. • Higher relative abundances of *Porphyromonas* and *Aggregatibacter* in patients with OSA of varying severity compared with control group. • Evidence of changes in the oral microbiome in patients with OSA.

In pediatric studies in which the biological sample used was saliva (41), there was a greater microbial diversity compared to the control group, as well as a significant difference between species composition and abundance.

Also in pediatric studies, but with biological samples from swabs of the buccal mucosa (38) and from tongue base, soft palate, palatine tonsils and adenoid tonsils (37), in both the oral microbiome of patients diagnosed with OSA showed differences in the composition when compared with control groups.

Regarding the adult population, in saliva samples (12, 40, 43), two of the studies (12, 40) concluded that there were changes in the salivary microbiome of OSA patients, while in another study (43) no significant changes were found in the relative abundance of bacteria between the control group and the OSA group. On the swab samples (39, 42, 44, 45), two of the studies (39, 42, 45) have concluded that there was a decrease in bacterial biodiversity in OSA group and no significant differences in community richness and diversity between OSA group and control, respectively. Another study (42), also concluded that there was no significant difference in the number of oral species among the groups. The last study (44), concluded that there was a higher α-diversity in OSA patients than in controls but no significant changes.

In summary, results regarding diversity and species composition were very heterogeneous. The reasons for this lack of consensus will be explored in the discussion section.

3.2. Methodological quality analysis

Assessment of the risk of bias was done by using quality analysis tools of the Joanna Briggs Institute (JBI). JBI is an international research organization based in the Faculty of Health and Medical Sciences at the University of Adelaide, South Australia. The purpose is to assess the methodological quality of the study where the results will be placed in a table, analyzing the various parameters that this tool provides to assess the articles included in the study.

Two tables were filled in depending on the type of study, one for cross-sectional studies and one for cohort studies. After completing the questionnaire for cross-sectional studies for each article, it was found that all the parameters of the 8 articles analyzed were positive (Table II).

Regarding the 2 cohort studies (Table III), the vast majority of the parameters are positive, excluding in the articles by Seung-No Hong, 2022 (44) / Chih-Yuan Ko, 2018 (45), the following: if the follow-up was complete, and if not, the reasons for the loss of follow-up were described and explored, and strategies were used to deal with incomplete follow-up. Furthermore, in the article by Chih-Yuan Ko, 2018 (45), the parameters on whether confounding factors were identified and whether there were strategies to resolve them, and if the groups/participants were free of the outcome at the start of the study (or at the time of exposure) were also negative.

Table II. Assessment of the study quality of cross-sectional studies using the JBI tool

Article	**1.Were the criteria for inclusion in the sample clearly defined?**	**2. Were the study subjects and the setting described in detail?**	**3. Were the exposure measured in a valid and reliable way?**	**4. Were objective, standard criteria used for measurement of the condition?**	**5. Were confounding factors identified?**	**6. Were strategies to deal with confounding factors stated?**	**7. Were the outcomes measured in a valid and reliable way?**	**8. Was appropriate statistical analysis used?**
Yanlong Chen, 2021 (12)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Xiaoman Zhang, 2023 (37)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Peizeng Jia, 2020 (43)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yang Wenbo, 2019 (39)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Huajun Xu, 2018(38)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Xuehui Chen, 2021(40)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Xin Huang, 2022 (41)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yinghui Gao, 2023 (42)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table III. Assessment of the study quality of cohort studies using the JBI tool

Article	***1. Were the two groups similar and recruited from the same population?***	***2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?***	***3. Was the exposure measured in a valid and reliable way?***	***4. Were confounding factors identified?***	***5. Were strategies to deal with confounding factors stated?***	***6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?***	***7. Were the outcomes measured in a valid and reliable way?***	***8. Was the follow up time reported and sufficient to be long enough for outcomes to occur?***	***9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?***	***10. Were strategies to address incomplete follow up utilized?***	***11. Was appropriate statistical analysis used?***
Seung-No Hong, 2022 (44)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Chih-Yuan ko, 2019 (45)	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No	Yes

3.3. Differences in the oral microbiome of OSA patients and control group

Table IV refers to the microbial changes observed in pediatric studies in relation to genus and species and Table V the corresponding results for studies featuring adults.

Table IV. Microbial changes in pediatric studies

Author, year	***Article Title***	***Biological Sample***	***Genus Non-OSA***	***Genus OSA***	***Species Non-OSA***	***Species OSA***
Xiaoman Zhang et al, 2023 (37)	Changes in the oral and nasal microbiota in pediatric obstructive sleep apnea	Swabs (tongue base, soft palate, palatine tonsils and adenoid tonsils, both anterior nares)	Adenoids - *Actinobacillus, Burkholderiales (↑), Ruminococcaceae_UCG_005 (↑), Eikenella, Romboutsia (↑);* Palate- *Lachnoanaerobaculum (↑), Abiotrophia (↑), Trichococcus (↑), Rothia (↑), Actinomyces (↑);* Tongue –*Abiotrophia (↑), Trichococcus (↑), Lautropia (↑), Alloprevotella (↑), Streptococcus (↑);* Tonsils – *Trichococcus (↑)*	**Adenoids** – *Haemophilus (↑), Fusobacterium (↑), Porphyromonas (↑), Prevotella (↑), Treponema (↑), Agathobacter (↑), Parvimonas (↑), Campylobacter (↑), Faecalibacterium (↑) ;* Nares- *Haemophilus (↑), Porphyromonas (↑), Capnocytophaga (↑);* **Palate-** *Haemophilus (↑), Actinobacillus (↑), Porphyromonas (↑), Fusobacterium (↑), Prevotella (↑), Streptobacillus (↑), Campylobacter (↑);* **Tongue**- *Porphyromonas (↑), Fusobacterium (↑), Haemophilus (↑), Capnocytophaga (↑), Prevotella (↑);* **Tonsils** – *Fusobacterium (↑), Haemophilus (↑), Porphyromonas (↑), Moraxella (↑), Neisseria (↑), Aggregatibacter (↑), Treponema (↑), Prevotella (↑), Parvimonas (↑), Streptobacillus (↑), Campylobacter (↑), Collinsella (↑)*		

Table IV cont. Microbial changes in pediatric studies

Author, year	***Article Title***	***Biological Sample***	***Genus Non-OSA***	***Genus OSA***	***Species Non-OSA***	***Species OSA***
Huajun Xu et al, 2018 (38)	Pediatric Obstructive sleep apnea is associated with changes in the oral microbiome and urinary metabolomics profile: a pilot study	Swabs (buccal mucosa)	*Thermus (↑), Pseudomonas (↑), Lautropia (↑), Achromobacter (↑)*	*Veillonella (↑), Prevotella (↑), Mogibacterium (↑), Campylobacter (↑), Butyrivibrio (↑)*		*debontii, dispar, firmus, melaninogenica, parainfluenzae, boronitolerans, mizutaii, and cinerea*
Xin Huang et al, 2022 (41)	Characteristics of salivary microbiota in children with obstructive sleep apnea: a prospective study with polysomnography	Saliva	*Haemophilus (↑), Fusobacterium (↑)*	*Prevotella (↑), Actinomyces (↑), Bifidobacterium (↑), Eschericia (↑), Lactobacillus (↑), Enterococcus (↑), Atopobium (↑), Klebsiella (↑), Bacteroides (↑)*	*Haemophilus parainfuenzae (↑), Veillonella parvula (↑), Fusobacterium periodonticum (↑), Haemophilus sputorum (↑), and Veillonella rogosae (↑)*	*Prevotella pallens (↑), Prevotella salivae (↑), Prevotella sp_HMT_313 (↑), Prevotella sp_HMT_396 (↑), Prevotella scopos (↑)*

Table V. Microbial changes in adult studies

Author, year	***Article Title***	***Biological Sample***	***Genus Non-OSA***	***Genus OSA***	***Species Non-OSA***	***Species OSA***
Yanlong Chen et al, 2021 (12)	Analysis of Salivary Microbiome and Its Association with Periodontitis in patients with obstructive sleep apnea	Saliva	*Streptococcus (↑), Veillonella (↑)*	*Rothia (↑), Gemella (↑), Prevotella (↑)*	*P. melaninogenica (↑)*	*R. mucilaginosa* **(↑)**, *Aggregatibacter actinomycetemcomitans (↑), Prevotella intermedia (↑), Treponema denticola (↑)*
Peizeng Jia et al, 2020 (43)	Analysis of the Salivary Microbiome in Obstructive Sleep apnea syndrome patients	Saliva	*Peptococcus (↑), Peptostreptococcus (↑), Alloprevotella (↑), Granulicatella (↑)*	*Scardovia (↑)*		
Yang, Wenbo et al, 2019 (39)	Oropharyngeal Microbiome in obstructive sleep apnea: decreased diversity and abundance	Swabs (oropharyngeal)	*Glaciecola (↑), Tannerella (↑), Anaerovorax (↑), Halomonas (↑)*	*Neisseria (↑)* (OSA severe), *Prevotella* (moderate OSA) *(↑)*		
Xuehui Chen et al, 2022 (40)	Altered salivary microbiota in patients with obstructive sleep apnea comorbid hypertension	Saliva	*Haemophilus (↑)*	*Rothia (↑), Granulicatella (↑), Peptostreptococcus (↑)*		

Table V cont. Microbial changes in adult studies

Author, year	Article Title	Biological Sample	Genus Non-OSA	Genus OSA	Species Non-OSA	Species OSA
Yinghui Gao et al, 2022 (42)	Whole genome metagenomic analysis of the oral microbiota in patients with obstructive sleep apnea	Swabs (buccal mucosa)	Alloprevotella (↑), Prevotellaceae (↑), Candidatus (↑), Saccharimonas (↑), Bacteroidales (↑), Muribaculaceae (↑), Photorhabdus (↑), Leptotrichia (↑), Pseudopropionibacterium (↑)	Flavobacteriaceae (↑), Bibersteinia (↑), Desulfovibrio (↑), Capnocytophaga (↑), Isoptericola (↑), Butyricimonas (↑), Veillonella (↑), Eikenella (↑), Kingella (↑)	Prevotella (↑), Alloprevotella (↑), Bacteroides (↑), Veillonella tobetsuensis (↑), Candidatus Saccharimonas (↑)	
Seung-No Hong et al, 2022 (44)	Association of obstructive sleep apnea severity with the composition of the upper airway microbiome	Swabs (nasopharyngeal)		Peptoniphilus (↑), Finegoldia (↑), Anaerococcus (↑), Corynebacterium (↑)		Klebsiella pneumoniae (↑), Streptococcus parasanguinis (↑), Streptococcus mitis (↑)
Chih-Yuan ko et al, 2019 (45)	Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension	Swabs (Buccal mucosa, tongue, soft palate, hard palate)	Kingella (↑), Fusicatenibacter (↑), Mobiluncus (↑), f__Clostridiaceae 1 (↑), Fluviicola (↑), Clostridium III (↑), p__Actinobacteria (↑), o__Acidimicrobiales (↑), Parcubacteria_genera_incertae_sedis (↑), f__Leptotrichiaceae (↑), Lysobacter (↑), Pirellula (↑), f__Verrucomicrobiaceae (↑), Rheinheimera (↑), f__Geodermatophilaceae (↑), Methylobacillus (↑), Anaerococcus (↑), c__Acidobacteria_Gp4 (↑), Aciditerrimonas (↑), f__Parachlamydiaceae (↑), Ulvibacter (↑), Gp21 (↑), Gp7 (↑), and Latescibacteria_genera_incertae_sedis (↑)	Porphyromonas (↑), Aggregatibacter (↑) (OSA mild) Treponema (↑), Abiotrophia (↑), Hydrotalea (↑), Schlegelella (↑), Gemmiger (↑) (OSA moderate-severe)		

In pediatric studies and when the biological sample used was saliva (41), the genera that increased compared to the control group were: *Prevotella, Actinomyces, Bifidobacterium, Eschericia, Lactobacillus, Enterococcus, Atopobium, Klebsiella, Bacteroides.* In terms of species, the ones found in OSA group were: *Prevotella pallens, Prevotella salivae, Prevotella sp_HMT_313, Prevotella sp_HMT_396, Prevotella scopos.* In the control group, *Haemophilus* and *Fusobacterium* were the most numerous genera, and the species were*:* *Haemophilus parainfuenzae*, *Veillonella parvula*, *Fusobacterium periodonticum*, *Haemophilus sputorum*, and *Veillonella rogosae*.

Also in pediatrics studies, but using swabs as biological sample (37, 38), in Huajun Xu *et al.* (2018)(38) study, the increased genera in OSA group was: *Veillonella, Prevotella, Mogibacterium, Campylobacter* and *Butyrivibrio.* The genera increased in healthy group were: *Thermus, Pseudomonas, Lautropia* and *Achromobacter*.

In Xioman Zhang *et al.* (2023)(37) study the different genera were distinguished according to the area analyzed. Thus, the following bacteria were increased in the different areas: in the adenoids the genera *Haemophilus, Fusobacterium, Porphyromonas, Prevotella, Treponema, Agathobacter, Parvimonas, Campylobacter, Faecalibacterium*, in the nares, *Haemophilus, Porphyromonas, Capnocytophaga,* in the palate*, Haemophilus, Actinobacillus, Porphyromonas, Fusobacterium, Prevotella, Streptobacillus, Campylobacter, in the tongue, Porphyromonas, Fusobacterium, Haemophilus, Capnocytophaga, Prevotella* and in the tonsils*, Fusobacterium, Haemophilus, Porphyromonas***,** *Moraxella, Neisseria, Aggregatibacter, Treponema, Prevotella, Parvimonas, Streptobacillus, Campylobacter, Collinsella.* In the non-OSA group, the increased genera, in the adenoids, *Actinobacillus, Burkholderiales, Ruminococcaceae_UCG_005, Eikenella, Romboutsia,* in the palate, *Lachnoanaerobaculum, Abiotrophia, Trichococcus, Rothia* and *Actinomyces*, in the tongue, *Abiotrophia, Trichococcus, Lautropia, Alloprevotella, Streptococcus* and in the tonsils *Trichococcus.* There was no information on species.

In adults where saliva samples were used (12, 40, 43), in the study by Yanlong Chen *et al.* (2021)(12), there was an increase in the genera *Rothia, Gemella, Prevotella* and in the species *R.mucilaginosa, Aggregatibacter actinomycetemcomitans, Prevella intermedia* and *Treponema denticola* in OSA group. In the control group, *Streptococcus* and *Veillonella* were the increased genera and the specie *P. melaninogenica.*

In the study by Peizeng Jen *et al*. (2020)(43), only *Scardovia* was found to be increase, and in the healthy group *Peptococcus, Peptostreptococcus, Alloprevotella* and *Granulicatella*. This study had no information about species.

Finally, in the study by Xuehui Chen *et al.* (2022)(40), *Rothia, Granulicatella, Peptostreptococcus* are the genera that were found increased in the OSA group and *Haemophilus* in the non-OSA group.

In biological samples by swabs (39, 42, 44, 45), in the study by Yang Wenbo *et al.* (2019)(39), *Prevotella* and *Neisseria* are increased in OSA group, and *Glaciecola, Tannerella, Anaerovorax* and *Halomonas* in healthy group. In Yinghui Gao *et al.* (2022)(42), the genera Fl*avobacteriaceae, Bibersteinia, Desulfovibrio, Capnocytophaga, Isoptericola, Butyricimonas, Veillonella, Eikenella* and *Kingella*. Seung-No Hong *et al.* (2022)(44), the genera *Peptoniphilus, Finegoldia, Anaerococcus, Corynebacterium* and the species *Klebsiella pneumoniae, Streptococcus parasanguinis and Streptococcus mitis* are increased in OSA group.

Chih-Yuan Ko *et al.* (2019)(45), divided the patients according to the severity of the disease, so the increased genera in patients with mild OSA were the following: *Porphyromonas, Aggregatibacter* and in moderate-severe OSA: *Treponema, Abiotrophia, Hydrotalea, Schlegelella* and *Gemmiger*.

In summary, as it happened with the results from the diversity observed in OSA patients *versus* controls, there isn’t a strong support for stating that there are significant differences in the genera and species present in the samples from OSA and healthy patients. The genus *Prevotella* is referred to as increased 8 times in OSA patients (12, 37-39, 41), the genus *Fusobacterium* is referred 5 times as increased, 4 in OSA patients (37) and one (41) in healthy patients. *Treponema* is also one of the most mentioned genus referred as increased in OSA patients (37, 45).

Regarding the genera most referred as increased in healthy patients *Streptococcus* is mentioned twice (12, 37) and *Rothia* is also mentioned in healthy patients (37) and twice in OSA patients (12, 40).

DISCUSSION

4. DISCUSSION

The oral cavity plays a very important role in people's general health, with more than 700 microbial species, both commensal and opportunistic. There are various microbial habitats such as the tongue, saliva, palate, buccal mucosa, and others. It is known that the microbiomes of the same site in the body are more similar between different individuals than those of different sites in the same individual (5).

When there is an imbalance in the oral community, with an increase in pathogenic bacteria and a decrease in beneficial bacteria, we call it oral dysbiosis. This situation can occur due to different factors, some of which are oral and systemic diseases (46). Many patients diagnosed with obstructive sleep apnea suffer from oral dryness due to breathing through their mouths. They also suffer from the side effects of intermittent hypoxia and hypercapnia (11).

In this systematic review, with the aim of analyzing whether changes occur in the oral microbiome of patients diagnosed with obstructive sleep apnea, only 10 articles were considered eligible. In general, these studies found differences between the oral microbiome (determined by molecular methods) of patients diagnosed with this condition and control groups, in both pediatric and adult studies, even though these differences were not always statistically significant.

The different studies selected for this review reveal great heterogeneity in several aspects. As this is a relatively recent topic with few publications, the design of the studies varies and although we included cohort studies with cross-sectional studies accurate comparisons between the 2 types of studies is not wise.

Oral samples used to study the composition of oral microbiome can be collected quickly, practically, and minimally invasively, such as swabs or saliva (47). In these studies, most of the samples used were swabs. The location in the oral cavity where the samples were taken was not homogeneous between the studies. These were taken from different locations, such as the base of the tongue, the palate, the buccal mucosa, the adenoids, and the tonsils. Furthermore, in most studies, the microbial results described were general and not descriptive of each individual area. Saliva samples were also used, but in smaller numbers. This means that, as it happens with the study design, a significant comparison between the microbiome of different samples such oral biofilm (swabs) and saliva is not feasible. Several studies have shown that saliva and biofilm samples are different regarding the microbial composition even in samples collected from the same individual at the same time (47).

A standardized protocol for collecting oral samples to support research into the oral microbiome has yet to be established (47) and would indeed increase reproducibility of the results. Another source of interindividual differences in the microbiome is subject age. Several studies have shown that microbiomes of children is very different from adults and that microbiome of adults (48).

Only molecular methods were used to identify the microorganisms, although the vast majority used sequencing of the 16sRNA gene and the V3-V4 region, one study used the V1-V3, and another did not specify. There was also a study in which metagenomic analysis was done. These differences in identification methods may contribute to the discrepancies between some studies. Although this systematic review is about the oral microbiome, results included only bacteria, without assessing viruses, fungi or Archaea.

Regarding the analysis of study bias, the negative parameters were only on cohort studies, mainly based of the fact that there was no complete follow-up and the reasons for losing the follow-up were not described and explored. Furthermore, strategies for dealing with incomplete follow up were not used. In one of the studies, there was still a failure to identify confounding factors and strategies to deal with these factors and no information on whether the groups/participants were free of the outcome at the start of the study (or at the moment of exposure). However, it can be concluded that all the articles, despite the parameters mentioned above, are articles of scientific quality.

The results of this review show heterogeneity between the different studies, both in children and adults. In the case of pediatric studies, according to Xiaoman Zhang *et al.* (2023)(37) there was a significant difference in beta diversity and microbial profile, but in terms of alpha diversity the values were similar between the OSA and control groups. In this study, different locations in the oral cavity were analyzed using swabs, which made it possible to differentiate the microorganism from the various locations. The bacterial genera *Haemophilus, Porphyromonas, Fusobacterium* and *Prevotella* were found to be increased in all locations in the oral cavity (palate, tongue, adenoids, and tonsils) of patients diagnosed with OSA. Still on swabs samples*,* Huajun Xu *et al*. (2023)(38) concluded that there are changes in the functioning and composition of the oral microbiome of these patients, with an increase in the following genera: *Veillonella,* *Prevotella, Mogibacterium, Campylobacter and Butyrivibrio*. Compared to above mentioned study, only *Prevotella* and *Campylobacter* increased in common.

In Xin Huang *et al*. (2022)(41) study with saliva samples, both alpha and beta diversity were altered when compared to the control group. The increased genus coinciding with the two previous studies is only *Prevotella*. Interestingly, in this study, *Haemophilus* and *Fusobacterium* were increased in the control group, whereas in previous studies they were higher in OSA group.

We can conclude that in pediatric studies there were alterations in the oral microbiome of OSA patients, in terms of alpha diversity in 2 studies and beta diversity in all. There is great diversity at the microbial level, which may be due to the different biological sampling method and the heterogeneity in the location of these samples. It is important to note that in all pediatric studies, one of the exclusion criteria was the presence of oral disease, either caries or periodontal disease. Furthermore, in the studies by Xioman Zhang *et al.* (2023)(37) and Xin Huang *et al*. (2022)(41) the patients submitted to the study had a diagnosis of adenoid hypertrophy, which is the main risk factor for obstructive sleep apnea in children. Adenoid hypertrophy is characterized by a chronic systemic inflammatory state, which could change the composition of the salivary microbiome in children (41). *Prevotella* species, an obligate gram-negative anaerobe, is frequently linked to human infections and associated with diseases of the oral cavity such as periodontitis and dental caries (49), and, in fact, is increased in all pediatric studies in the obstructive sleep apnea group. Therefore, this increase in these studies could be explained by the inflammatory state in which children with adenoid hypertrophy are subjected, but further studies on it are needed to draw more accurate conclusions.

In the adult population, the results revealed differences in both saliva and swab samples. In swab samples, Yang Wenbo *et al*. (2019)(39) study found a significantly lower bacterial biodiversity in patients diagnosed with OSA, with an increase in the genus *Neisseria* in patients with severe OSA and *Prevotella* in patients with moderate OSA. In Chih-Yuan *et al.* (2019)(45) study, he also separated the increased genera according to the severity of the disease, but the results do not coincide with the previous study, since in the moderate-severe OSA group, the increased bacterial genera were *Treponema, Abiotrophia, Hydrotalea, Schlegelella* and *Gemmiger.* Lastly, in the study by Yinghui Gao *et al.* (2023)(42) which concluded that there were no significant differences in the number of oral microbial species between the groups, and there was no significant clustering of oral microbial communities. In fact, *Prevotella, Alloprevotella, Bacteroides, Veillonella tobetsuensis* and *Candidatus saccharimonas* were found to be increased in the control group, thus showing that the studies with swab samples, presented a great diversity and variety of microorganisms, with no species coinciding between the different studies. This may be due to the different locations of the samples or even the disparity between the ages of participants.

In saliva samples*,* Yanlong Chen *et al.* (2021)(12) found an increase in a specific periodontal pathogen*, Prevotella*, and also, *Rothia,* as well as a lower microbial richness when compared to the control group. In Xuehui Chen *et al.* (2022)(40) *study*, *Rothia, Granulicatella* and *Peptostreptococcus* are the genera increased in OSA group. The increase of *Rothia* coincides with the results of the study by Yanlong Chen *et al*. (2021)(12).

There was no change in alpha and beta diversity in the OSA group compared to the control, only an increase in the genus *Scardovia* in Peinzeng Jia *et al*. (2020)(43) study. In this control group, *Peptostreptococcus* and *Granulicatella* are increased, while, interestingly, in Xuehui Chen *et al.* (2022)(40) study, both these genera are increased in the OSA group. This can probably be explained by the fact that in Peizeng Jia *et al*. (2020)(43) study, people diagnosed with hypertension and with periodontal disease or dental caries were excluded from the study, while Xuehui Chen *et al*. (2022)(40) study, apart from the control group, only included people diagnosed with hypertension.

In Bianchi's systematic review (10), in which culture methods are described, the results showed that using CFU/ml of *Streptococcus mutans* and *lactobacillus*, the differences between the results of control group and OSA were no significant in children, while in another study, also in children, the results effectively showed the opposite, that in OSA group *Streptococcus mutans* and *lactobacilli* were higher. In addition, another study, this time in adults using bacterial culture and qPCR method, showed an increase in gram negative bacteria in the severe OSA with periodontal disease group. This means that, compared to the molecular methods used in our study, there is great heterogeneity at pediatric level, but in the adult age group, many studies have found an increase in various gram-negative bacteria, such as *Veillonella, Capnocytophaga, Aggregatibacter* and others.

As previously stated, patients with OSA are typically mouth breathers and snorers, which is thought to cause changes in the oral microbiome. The reason for this might be due to reduced salivary flow and moisture, and self-cleaning capacity. These changes are also a consequence of intermittent hypoxia (10). However, it is not clear in all studies that snorers were not included in the control groups.

Intermittent hypoxia is responsible for regulating hypoxia and reoxygenation cycles, in both gut and upper airway, which, according to some authors (43) leads to an increase in bacterial diversity in OSA patients. Also, inflammatory biomarkers such as inflammatory cells, IL-8 and IL-6, C-reactive protein, TNF-alpha and proinflammatory cytokine were associated with an altered microbiome, from oral bacteria pathogens such as *Rothia, Haemophilus and Actinomyces* in patients with severe OSA, thus proving that host-microorganism interaction is indeed important in the immune response in OSA patients (40, 43). In the present study, based on the results described above, *Rothia* is in fact increased in some studies (12) (40) as is *Haemophilus* (37) and *Actinomyces* (41).

There are differences in the oral microbiome depending on the severity of OSA, probably due to episodes of hypoxia and increased CO_2 levels (11), and indeed, in the studies where patients were differentiated according to severity, this was the case (41, 45). Nevertheless, since in some studies the severity of OSA was not indicated, this can be a factor which compromises the comparisons between studies.

4.1. Obstructive sleep apnea and periodontal disease

In spite of the fact the only trends can be established, the genera most commonly referred as altered in OSA patients are associated with periodontal disease (*Prevotella, Fusobacterium, Treponema*). Periodontitis just like OSA is in an inflammatory state and both have some risk factors in common like age, stress, diabetes mellitus and obesity. Salivary levels of IL-6 are increased in patients with OSA and are associated with its severity. An increase in salivary concentration of IL-6 can cause or affect periodontal disease in individuals with OSA (50). Also, the fact that many OSA patients are mouth breathers, can lead to an increase in biofilm, thus facilitating the colonization of pathogens caused by xerostomia (51). Species normally associated with periodontitis include *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*, historically defined as the “red complex” (52, 53). This microbiota causes a localized inflammation that is characterized by the infiltration of inflammatory cells such as macrophages, lymphocytes, and plasma cells into the periodontal tissue. There is also release of cytokines, including TNF-alpha, interleukins, prostaglandins and C-reactive protein (54). In addition, other bacteria such as *Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans, Peptostpeptococcus micros* and *Campylobacter rectus* have also been shown to be important in the pathogenesis of the disease (55).

4.2. Obstructive sleep apnea and dental caries

Streptococcus mutans is the bacterial specie most studied for its cariogenic properties. It has even been classified as a specific pathogen of caries. However, new studies showed that other species including *Veillonella, Lactobacillus, Propionibacterium, Actinomycetes,* and *Scardovia* were also detected at high levels in the dental plaque of individuals with history of dental caries (52, 56). In fact, both *Scardovia* (43) and *Veillonella* (38, 42) were increased in some of the studies, but there is not enough data to draw conclusions on this.

Curiously 2 studies (12, 37) refer *Streptococcus* as increased in healthy patients.

4.3. Final Considerations

After analysing the studies included in this systematic review, it can be said that there is great heterogeneity between them, both in terms of conclusions and diversity of bacterial species. It is therefore necessary to carry out more studies on this subject, since with a small sample it is difficult to make comparisons and reach reliable conclusions. There is also a need to standardise which sample is to be used for microbial analysis, as well as studies with a greater diversity of patients, both in terms of age and gender. Moreover, it is assumed that the varying degrees of disease severity are associated with different microorganisms. Therefore, it would be advantageous to conduct studies with populations of the same severity level rather than different ones, as was the case in our studies. The same applies to comorbidities. Only when studies are more homogenous regarding the various aspects mentioned can valid comparisons be made.

CONCLUSION

5. CONCLUSION

There is insufficient data published to support conclusions on the impact of OSA on the oral microbiome. Nevertheless, it seems that periodontopathogens are favoured in the growth conditions established in the mouths of OSA patients, increasing the inflammatory burden and therefore explaining the clinical systemic symptoms associated with OSA.

BIBLIOGRAPHY

6. BIBLIOGRAPHY

1. Yamashita Y, Takeshita T. The oral microbiome and human health. J Oral Sci. 2017;59(2):201-6.
2. Berg G, Rybakova D, Fischer D, Cernava T, Verges MC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):103.
3. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell. 2018;9(5):488-500.
4. Wallace RK. The Microbiome in Health and Disease from the Perspective of Modern Medicine and Ayurveda. Medicina (Kaunas). 2020;56(9).
5. Jia G, Zhi A, Lai PFH, Wang G, Xia Y, Xiong Z, et al. The oral microbiota - a mechanistic role for systemic diseases. Br Dent J. 2018;224(6):447-55.
6. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207-14.
7. Radaic A, Kapila YL. The oralome and its dysbiosis: New insights into oral microbiome-host interactions. Comput Struct Biotechnol J. 2021;19:1335-60.
8. Sharma N, Bhatia S, Sodhi AS, Batra N. Oral microbiome and health. AIMS Microbiol. 2018;4(1):42-66.
9. Gomez A, Nelson KE. The Oral Microbiome of Children: Development, Disease, and Implications Beyond Oral Health. Microb Ecol. 2017;73(2):492-503.
10. Bianchi G, de'Angelis N, Gavriilidis P, Sobhani I, de'Angelis GL, Carra MC. Oral microbiota in obstructive sleep apnea patients: a systematic review. Sleep Breath. 2023;27(4):1203-16.
11. Tellez Corral MA, Herrera Daza E, Cuervo Jimenez HK, Bravo Becerra MDM, Villamil JC, Hidalgo Martinez P, et al. Cryptic Oral Microbiota: What Is Its Role as Obstructive Sleep Apnea-Related Periodontal Pathogens? Int J Environ Res Public Health. 2023;20(3).
12. Chen Y, Chen X, Huang X, Duan Y, Gao H, Gao X. Analysis of Salivary Microbiome and Its Association With Periodontitis in Patients With Obstructive Sleep Apnea. Front Cell Infect Microbiol. 2021;11:752475.

13. Perger E, Mattaliano P, Lombardi C. Menopause and Sleep Apnea. Maturitas. 2019;124:35-8.

14. Yeghiazarians Y, Jneid H, Tietjens JR, Redline S, Brown DL, El-Sherif N, et al. Obstructive Sleep Apnea and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021;144(3):e56-e67.

15. Patil SP, Ayappa IA, Caples SM, Kimoff RJ, Patel SR, Harrod CG. Treatment of Adult Obstructive Sleep Apnea With Positive Airway Pressure: An American Academy of Sleep Medicine Systematic Review, Meta-Analysis, and GRADE Assessment. J Clin Sleep Med. 2019;15(2):301-34.

16. Faber J, Faber C, Faber AP. Obstructive sleep apnea in adults. Dental Press J Orthod. 2019;24(3):99-109.

17. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. Lancet Respir Med. 2019;7(8):687-98.

18. Eckert DJ. Phenotypic approaches to obstructive sleep apnoea - New pathways for targeted therapy. Sleep Med Rev. 2018;37:45-59.

19. Osman AM, Carter SG, Carberry JC, Eckert DJ. Obstructive sleep apnea: current perspectives. Nat Sci Sleep. 2018;10:21-34.

20. Edwards BA, Eckert DJ, Jordan AS. Obstructive sleep apnoea pathogenesis from mild to severe: Is it all the same? Respirology. 2017;22(1):33-42.

21. Levine M, Bennett K, Cantwell M, Postol K, Schwartz D. Dental Sleep Medicine Standards for Screening, Treating, and Managing Adults with Sleep-Related Breathing Disorders. Journal of Dental Sleep Medicine. 2018;5(3):61-8.

22. Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med. 2017;13(3):479-504.

23. Hirani R, Smiley A. A Scoping Review of Sleep Apnea: Where Do We Stand? Life (Basel). 2023;13(2).

24. Laratta CR, Ayas NT, Povitz M, Pendharkar SR. Diagnosis and treatment of obstructive sleep apnea in adults. CMAJ. 2017;189(48):E1481-E8.

25. Chang HP, Chen YF, Du JK. Obstructive sleep apnea treatment in adults. Kaohsiung J Med Sci. 2020;36(1):7-12.

26. Bitners AC, Arens R. Evaluation and Management of Children with Obstructive Sleep Apnea Syndrome. Lung. 2020;198(2):257-70.

27. Solano-Perez E, Coso C, Castillo-Garcia M, Romero-Peralta S, Lopez-Monzoni S, Lavina E, et al. Diagnosis and Treatment of Sleep Apnea in Children: A Future Perspective Is Needed. Biomedicines. 2023;11(6).

28. Lee JJ, Sundar KM. Evaluation and Management of Adults with Obstructive Sleep Apnea Syndrome. Lung. 2021;199(2):87-101.

29. Jordan AS, McSharry DG, Malhotra A. Adult obstructive sleep apnoea. Lancet. 2014;383(9918):736-47.

30. Chang JL, Goldberg AN, Alt JA, Mohammed A, Ashbrook L, Auckley D, et al. International Consensus Statement on Obstructive Sleep Apnea. Int Forum Allergy Rhinol. 2023;13(7):1061-482.

31. Rundo JV. Obstructive sleep apnea basics. Cleve Clin J Med. 2019;86(9 Suppl 1):2-9.

32. Knauert M, Naik S, Gillespie MB, Kryger M. Clinical consequences and economic costs of untreated obstructive sleep apnea syndrome. World J Otorhinolaryngol Head Neck Surg. 2015;1(1):17-27.

33. Milicic Ivanovski D, Milicic Stanic B, Kopitovic I. Comorbidity Profile and Predictors of Obstructive Sleep Apnea Severity and Mortality in Non-Obese Obstructive Sleep Apnea Patients. Medicina (Kaunas). 2023;59(5).

34. Pardak P, Filip R, Wolinski J, Krzaczek M. Associations of Obstructive Sleep Apnea, Obestatin, Leptin, and Ghrelin with Gastroesophageal Reflux. J Clin Med. 2021;10(21).

35. Mahfouz R, Barchuk A, Obeidat AE, Mansour MM, Hernandez D, Darweesh M, et al. The Relationship Between Obstructive Sleep Apnea (OSA) and Gastroesophageal Reflux Disease (GERD) in Inpatient Settings: A Nationwide Study. Cureus. 2022;14(3):e22810.

36. Pinto JA, Ribeiro DK, Cavallini AF, Duarte C, Freitas GS. Comorbidities Associated with Obstructive Sleep Apnea: a Retrospective Study. Int Arch Otorhinolaryngol. 2016;20(2):145-50.

37. Zhang X, Li X, Xu H, Fu Z, Wang F, Huang W, et al. Changes in the oral and nasal microbiota in pediatric obstructive sleep apnea. J Oral Microbiol. 2023;15(1):2182571.

38. Xu H, Li X, Zheng X, Xia Y, Fu Y, Li X, et al. Pediatric Obstructive Sleep Apnea is Associated With Changes in the Oral Microbiome and Urinary Metabolomics Profile: A Pilot Study. J Clin Sleep Med. 2018;14(9):1559-67.

39. Yang W, Shao L, Heizhati M, Wu T, Yao X, Wang Y, et al. Oropharyngeal Microbiome in Obstructive Sleep Apnea: Decreased Diversity and Abundance. J Clin Sleep Med. 2019;15(12):1777-88.

40. Chen X, Chen Y, Feng M, Huang X, Li C, Han F, et al. Altered Salivary Microbiota in Patients with Obstructive Sleep Apnea Comorbid Hypertension. Nat Sci Sleep. 2022;14:593-607.

41. Huang X, Chen X, Gong X, Xu Y, Xu Z, Gao X. Characteristics of salivary microbiota in children with obstructive sleep apnea: A prospective study with polysomnography. Front Cell Infect Microbiol. 2022;12:945284.

42. Gao Y, Wang H, Hu Y, Li J, Xu W, Zhao L, et al. Whole-genome metagenomic analysis of the oral microbiota in patients with obstructive sleep apnea. Sleep Breath. 2023;27(4):1383-98.

43. Jia P, Zou J, Yin S, Chen F, Yi H, Zhang Q. Analysis of the Salivary Microbiome in Obstructive Sleep Apnea Syndrome Patients. Can J Infect Dis Med Microbiol. 2020;2020:6682020.

44. Hong SN, Kim KJ, Baek MG, Yi H, Lee SH, Kim DY, et al. Association of obstructive sleep apnea severity with the composition of the upper airway microbiome. J Clin Sleep Med. 2022;18(2):505-15.

45. Ko CY, Hu AK, Chou D, Huang LM, Su HZ, Yan FR, et al. Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension. Hypertens Res. 2019;42(11):1692-700.

46. Maier T. Oral Microbiome in Health and Disease: Maintaining a Healthy, Balanced Ecosystem and Reversing Dysbiosis. Microorganisms. 2023;11(6).

47. Omori M, Kato-Kogoe N, Sakaguchi S, Fukui N, Yamamoto K, Nakajima Y, et al. Comparative evaluation of microbial profiles of oral samples obtained at different collection time points and using different methods. Clin Oral Investig. 2021;25(5):2779-89.

48. Burcham ZM, Garneau NL, Comstock SS, Tucker RM, Knight R, Metcalf JL, et al. Patterns of Oral Microbiota Diversity in Adults and Children: A Crowdsourced Population Study. Sci Rep. 2020;10(1):2133.

49. Sharma G, Garg N, Hasan S, Shirodkar S. Prevotella: An insight into its characteristics and associated virulence factors. Microb Pathog. 2022;169:105673.

50. Lembo D, Caroccia F, Lopes C, Moscagiuri F, Sinjari B, D'Attilio M. Obstructive Sleep Apnea and Periodontal Disease: A Systematic Review. Medicina (Kaunas). 2021;57(6).

51. Arango Jimenez N, Morales Vera DZ, Latorre Uriza C, Velosa-Porras J, Tellez Corral MA, Escobar Arregoces FM. Relationship of obstructive sleep apnea with periodontal condition and its local and systemic risk factors. Clin Oral Investig. 2023;27(6):2823-32.

52. Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013;69(1):137-43.

53. Zhang Y, Wang X, Li H, Ni C, Du Z, Yan F. Human oral microbiota and its modulation for oral health. Biomed Pharmacother. 2018;99:883-93.

54. Latorre C, Escobar F, Velosa J, Rubiano D, Hidalgo-Martinez P, Otero L. Association between obstructive sleep apnea and comorbidities with periodontal disease in adults. J Indian Soc Periodontol. 2018;22(3):215-20.

55. Visentin D, Gobin I, Maglica Z. Periodontal Pathogens and Their Links to Neuroinflammation and Neurodegeneration. Microorganisms. 2023;11(7).

56. He J, Li Y, Cao Y, Xue J, Zhou X. The oral microbiome diversity and its relation to human diseases. Folia Microbiol (Praha). 2015;60(1):69-80.

Membros do Júri das Provas Públicas

Presidente: Professora Doutora Anna Moura – Professora Associada

Arguente: Professora Doutora Amélia Feliciano – Professora associada convidada da Faculdade de Medicina da UCP de Lisboa

Orientador: Professora Doutora Maria José Correia – Professora Associada

Data das provas públicas: 19 / 07 / 2024

Validação e confirmação pelos serviços escolares:

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