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Relationship between oral microbiota and periodontal disease: a systematic review

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Abstract. – OBJECTIVE: In recent years metagenomic analysis has become more accessible for the characterization of biological specimens. There has been an important increase of studies using this technique for subgingival human samples. To date, there are no updated systematic reviews on the relationship between oral microbiota and periodontal disease. The aim of the present systematic review was to update data about studies concerning the influences of changes in oral microbiota composition on the periodontal status in human subjects.

MATERIALS AND METHODS: An electronic search was conducted in four databases (MEDLINE, Scopus, CENTRAL and Web of Science) for articles published in English from January 2014 to April 2018. *In vitro* or animal studies, case reports, case series, retrospective studies, review articles, abstracts and discussions were excluded. Also, studies that evaluated less than 5 microbial species, only viruses or already known periodontal pathogens were excluded. Two independent researches selected the studies and extracted the data. The quality of evidence was assessed as high, moderate or low for each microorganism.

RESULTS: Eight studies and three additional publications recovered from the bibliography search of the selected articles were included in the review. The Bacteria domain was the main detected among the others and it included 53 species. The review confirmed the presence of recognized periodontal pathogens such as the members of the red complex but also identified, with high weight of evidence, the presence of new pathogens.

CONCLUSIONS: The results of this systematic review support high evidence for the association of 3 new species/genera with the etiology of periodontitis. Future investigations on the actual role of these new pathogens in the onset and progression of the disease are needed.

Key Words
Oral microbiota, Periodontal disease, Metagenomic analysis, Pathogens bacteria, Systematic review.

Introduction

Severe periodontitis is the 6th most prevalent disease worldwide, with an overall prevalence of 11.2% and around 743 million people affected. The global burden of periodontal disease increased by 57.3% from 1990 to 2010[1-4]. Periodontal diseases are multifactorial infections induced by a complex of bacterial species that interact with host tissues to determine the destruction of periodontal structures, including the supporting tissues of the teeth, alveolar bone and periodontal ligament. It has recently been shown that some systemic diseases and syndromes are related to an increase on the activity of the cells of the immune system and a worsening of periodontal clinical conditions[5-7]. The importance of bacteria in dental plaque and the key role of plaque in the etiopathogenesis of periodontal disease are already well known[8]. Therefore, the control of oral infection

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has an important clinical relevance. Gram-negative bacteria are the most important bacteria frequently isolated from the periodontal pockets, such as: *Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Fusobacterium nucleatum, Prevotella intermedia, Porphyromonas gingivalis* and *Tannerella forsythia*. However, a recent systematic review has shed light on new bacterial species with a potential pathogen role for periodontal tissues and on the fact that periodontal disease probably is not caused by the presence of specific bacteria, but by changes in the levels of the population of the species in the oral microbiome[9]. The studies dealing with the oral microbiota and its possible connection with the periodontal inflammation are often heterogeneous and do not allow readers to grasp the weight of evidence of the results found. Since the bacteria belonging to the red complex[8] have been found in subgingival samples of patients not suffering from periodontal disease and that recent advances in metagenomic techniques allow to analyze bacteria that were previously not cultivable. In the last five years it has been witnessed a high increase in case-control studies that aimed to assess changes in oral microbiota during periodontitis. The authors of these studies aimed to find new pathogenic microorganisms for periodontal tissues. There is a need for a systematic updating of the literature on this topic because the comprehensive knowledge of the whole dynamic of the oral microbiota and its relationship with periodontal disease is crucial for improving diagnostics and setting effective and rational treatments. The aim of the present systematic review was to analyze studies published in the last five years concerning influences of changes in oral microbiota composition on the periodontal status in human subjects.

Materials and Methods

The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) checklist was used as a guideline for conducting and reporting the present systematic review and meta-analysis[10].

Selection Criteria

Studies were included if they were case-control study published in English, French, German, Spanish or Italian and conducted on human subjects with the aim of comparing microbial composition of supragingival or subgingival samples from adult patients affected and not affected by a form of periodontal disease.

Excluded articles included:

- Publications in languages other than English, French, German, Spanish or Italian;
- *In vitro* or animal studies, case reports, case series, retrospective studies, review articles, abstracts, discussions;
- Studies that evaluated less than 5 microbial species, studies that only evaluated the presence of viruses or studies dealing only with the identification of the already known periodontal pathogens;
- Studies that analyzed the microbial composition of salivary samples;
- Lack of a direct comparison of baseline microbial data between healthy patients and patients affected by periodontitis.

In addition, in case of duplicate publications, the article with the most recent data was preferred.

Information Sources and Search

The following electronic databases were systematically searched from January 2014 to April 2018: MEDLINE-PubMed and all evidence-based medicine reviews via Web of Science, Scopus, and the Cochrane Central Register of Controlled Trials (CENTRAL).

The combination of MeSH terms and free text words used for MEDLINE-Pubmed database are as follows: (((periodontitis OR "periodontal disease" OR "periodontal pocket") AND (identification OR detection OR localization) AND bacteria*) AND invas* OR intracellular OR tissue* OR "epithelial cells")). This search strategy was first designed for MEDLINE and then adapted for the other databases. A supplementary manual search was performed of the following peer-reviewed journals for articles published between January 2014 and April 2018: Journal of clinical periodontology; Journal of periodontology; Journal of periodontal research and Molecular oral microbiology. In addition, the bibliographies of all selected articles were checked and all corresponding authors of included articles were contacted by e-mail in order to recover unpublished articles or raw data and to include as many relevant studies as possible in the analysis.

Study Selection

Screening process was conducted independently and in duplicate, two reviewers (RP and ES) evaluated the titles and abstracts of the retrieved studies from the database searches using the inclusion criteria. Subsequently, the same re-

viewers performed the assessment of the full-text articles. Any disagreements were solved through discussion until consensus.

Data Collection Process

The data were first extracted using specially designed data extraction forms. For studies matching with the inclusion criteria, or for which information in the title and abstract was insufficient to make a clear decision, the reviewers obtained and screened the full report. The studies excluded after full-text evaluation were recorded in the excluded studies table, along with the reasons of the exclusion.

Data Items

The variables extracted from each selected article included: study type, sample size, population details (clinical periodontal parameters, male/female ratio, mean age, smokers' percentage), sample type and intervention type.

Outcome

The outcome was the difference of microbial plaque composition between healthy patients and patients affected by periodontal disease. Summary data were given as levels or prevalence or proportion or abundance for each microorganism.

Risk of Bias in Individual Studies and Quality of Evidence

The reviewers independently extracted the microbial data through a structured form.

Methodological quality scores were given according to predetermined criteria. An additional summary of the certainty of the conclusions and strength of the evidence was developed through the calculation of the number of studies that highlighted the presence of a particular microorganism. The quality of evidence was assessed as high, moderate or low for each microorganism.

Results

Results of the Search

The initial electronic search resulted in 70 titles from the MEDLINE-Pubmed database, 536 titles from the Scopus database, 5 titles from the CENTRAL database, and 128 from the Web of Science database. After the independent elimination of duplicate articles, a total of 635 titles were considered for possible inclusion. A total of 615 articles were removed based on their title and abstract; therefore, 20 full-text articles were selected. Among these studies, eight were included in the review[11-18]. After accurate checking of the bibliographies of all selected articles three additional publications were recovered[19-21].

In conclusion, eleven CCTs were identified as potentially eligible for inclusion in this review[11-21] (Figure 1).

Exclusion of Studies

After full-text evaluation, 2 studies were excluded because of the lack of a direct comparison of baseline microbial data between healthy patients and patients affected by periodontitis. 3 studies were excluded from the review because their design did not fit with the inclusion criteria. 3 studies were excluded because they only evaluated the presence of viruses. One study was excluded because the authors evaluate less than 5 microbial species. 2 studies were excluded because they only considered the presence of periodontal pathogens and another study was not included because it was an *in vitro* study (Table I).

Included studies

Four studies were carried out in Brasil[16-18,20], one in Italy[12], one in USA[15], one in China[11], one in Yemen[19], one in South Korea[21], one in Canada[13] and one in Germany and USA according to a multicenter design[14]. All trials had a parallel group study design[11-21].

All articles investigated the possible variation of oral microbiota in patients affected by periodontal disease through the use of metagenomic analysis and were conducted at University dental clinics[11-18,20,21] except for one in which patients were also enrolled in a private dental center[19]. Characteristics of all the included studies are summarized in Table II. The included studies, overall, were very heterogeneous because they indicated the presence of microorganism using different units of measure and, in some cases, they presented the data graphically, preventing the authors of this article from retrieving them accurately. For these reasons a quantitative analysis of the results was not possible. Only a qualitative analysis was made. The following focused question, formulated in the Patient, Intervention, Comparison and Outcome (PICO) format was developed: "is there any difference in the composition of oral microbiota in adult patients affected by periodontal disease and patients unaffected? Eventual differences could be highlighted by the use of the metagenomic analysis?"

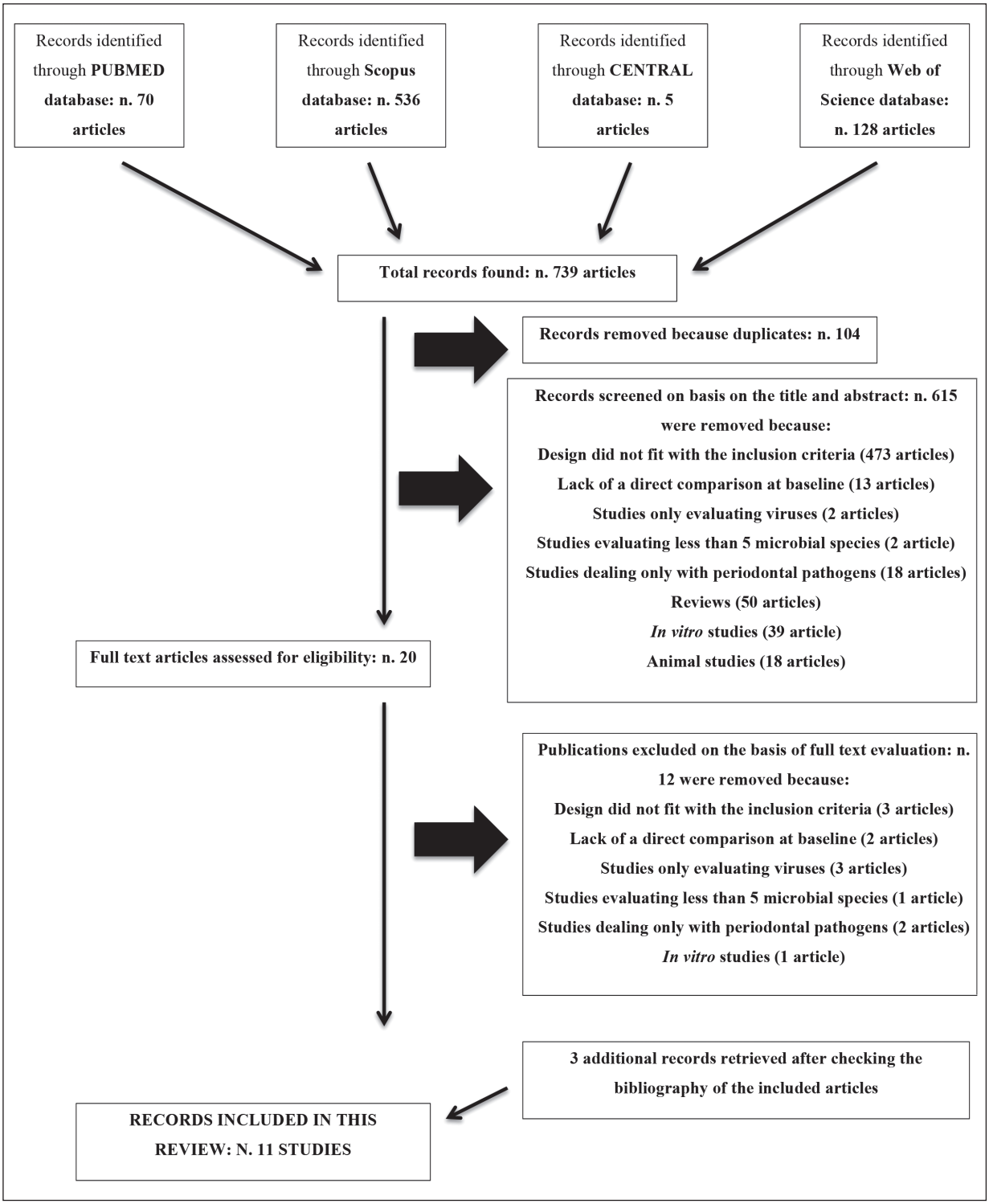

Figure 1. Flow chart of the search strategy.

Table I. Table showing references of excluded studies after full text evaluation with rationale for exclusion.

References	Rationale for exclusion
Baek et al[22]	Design did not fit with the inclusion criteria
Camelo-Castillo et al[23]	Lack of a direct comparison at baseline
Duan et al[24]	Study dealing only with periodontal pathogens
Kazi et al[25]	Study only evaluating viruses
Khorsopanha et al[26]	Study only evaluating viruses
Li CL et al[27]	Study evaluating less than 5 microbial species
Li Y et al[28]	Design did not fit with the inclusion criteria
Loozen et al[29]	Lack of a direct comparison at baseline
Ly et al[30]	Study only evaluating viruses
Moon et al[31]	Design did not fit with the inclusion criteria
Papone et al[32]	Study dealing only with periodontal pathogens
Rodriguez Herrero et al[33]	*In vitro* study

Characteristics of Participants

The selected studies included adult patients (age range: 24.2 – 56 years) among which patients affected by periodontal disease (chronic or aggressive) formed the test group and healthy patients not affected (matched for age and gender) formed the control group. In three cases a third group of patients affected by gingivitis was provided in the study design[18, 20-21]. Among the test group, patients were excluded if they matched at least one of the following exclusion criteria: active caries lesions, orthodontic appliances, previous periodontal treatment in the last 2 years, history of antibiotic, nonsteroidal anti-inflammatory drugs (NSAIDs), contraceptive pills intake and/or continual use of mouthwashes containing antimicrobials in the last 3 months and any condition/disease known to modify subgingival microbial composition such as pregnancy, lactation, diabetes mellitus and immunologic disorders.

Characteristics of Interventions

Data regarding the sample site and metagenomic analysis characteristics are summarized in Table II. The majority of the studies had more cases than controls. A total of 479 individuals affected by periodontal disease and 251 not affected were evaluated. Subgingival biofilm samples were processed individually or pooled even if some studies did not give enough information regarding this aspect. A total of 4,594 and 2,106 subgingival samples were evaluated from subjects affected and not affected by periodontal disease, respectively. All studies used a RNA-based detection method, specifically, the 16S rRNA identification[11-17,19-21]; only one study used a DNA-based metagenomic analysis, the DNA-DNA checkerboard[18].

Characteristics of Outcome Measures

All articles reported the microbial plaque composition measured as levels or prevalence or proportion or abundance for each microorganism in subjects in the test and control groups.

Risk of Bias in Included Studies and Strength of Evidence

The risk of bias is summarized in Table III. Following the evaluation of the risk of bias for each study: one trial was assessed as at high risk[11], nine trials were assessed as at medium risk[12,14-21] and one trial was assessed as at low risk[13]. The evaluation of the certainty of the conclusions and strength of the evidence was developed calculating the number of studies that highlighted the presence of a particular microorganism: the body of evidence reporting the presence of *Desulfobulbus spp., Filifactor alocis*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *TM7 spp. and Treponema denticola* is considered high because of the high number of studies assessing their presence in subgingival samples of patients affected by periodontal disease. Major details and additional data regarding microorganism whose presence was considered with medium or low strength of evidence are given in Table IV.

Effects of Interventions

The microorganisms found in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health were reported in Table V.

Table II. Characteristics of the included studies.

Author	Number of patients	Population details	Male/Female% (age ± SD)	Cigarette Smokers (%)	Sample	Intervention
Ai et al[11]	37 Cases 6 Controls	14 CAL < 2; 16 CAL > 2; 7 NR NR	NR NR	NR NR	Subgingival plaque	16S rRNA sequencing
Al-Hebshi et a[l19]	40 Cases 40 Controls	CPI ≥ 3 CPI < 2	54.1/45.9 (41.5 ± NR) 45.9/54.1 (32 ± NR)	67.6 32.4	Subgingival plaque	16S rRNA sequencing
Coretti et al[12]	12 Cases 8 Controls	CAL > 3 CAL ≤ 3	25/75 (NR) 38/62 (NR)	50 0	Subgingival tissue	16S rRNA sequencing
Galimanas et a[l13]	13 Cases 11 Controls	PPD ≥ 5 and CAL ≥ 3 PPD ≤ 2 and CAL ≤ 1	46/54 (46.8 ± 4.0) 27/73 (38.4 ± 4.1)	46 91	Supragingival plaque Subgingival plaque Plaque from tongue	16S rRNA sequencing
Hunter et a[l14]	4 Cases 4 Controls	PPD > 5 and CAL > 6 PPD ≤ 4 and CAL ≤ 5	NR NR	NR NR	Supragingival plaque Subgingival plaque Biofilm from oral mucosae	16S rRNA sequencing
Kirst et a[l15]	25 Cases 25 Controls	CAL ≥ 5 CAL ≤ 3	NR NR	NR NR	Subgingival plaque	16S rRNA sequencing
Lourenço et al[20]	70 Cases 27 Controls	35 CP: PPD/CAL ≥ 5, BOP+ 24 AgP: PPD/CAL ≥ 5, BOP+, ≤ 39 yr 11 G: > 10% sites PPD/CAL < 3, BOP+ < 10% sites PPD/CAL < 3, BOP+	25.5/74.5 (44.6 ± 11.4) 44.1/55.9 (33.1 ± 3.9) 36.4/63.6 (32.8 ± 15.3) 22.2/77.8 (24.2 ± 6.9)	27.5 2.9 18.2 11.1	Subgingival plaque	16S rRNA sequencing
Oliveira et al[16]	60 Cases 30 Controls	30 CP: PPD/CAL ≥ 4 30 AgP: PPD/CAL ≥ 5 and familiarity PPD/CAL ≤ 3	43/57 (42.0 ± 5.7) 47/53 (26.3 ± 3.5) 40/60 (33.5 ± 11.0)	NR NR NR	Subgingival plaque	16S rRNA sequencing
Park et al[21]	20 Cases 12 Controls	10 CP: PPD > 3; CAL ≥ 4; BOP > 10% 10 G: PPD ≤ 3; BOP ≥ 10% PPD ≤ 3; BOP < 10%	40/60 (56 ± 10.14) 20/80 (42.4 ± 17.32) 25/75 (55.6 ± 13.08)	0 0 0	Subgingival plaque	16S rRNA sequencing
Pérez-Chaparro et al[17]	9 Cases 7 Controls	PPD/CAL ≥ 4 PPD/CAL < 3	NR (46.2 ± 10.6) NR (45.9 ± 9.9)	0 0	Subgingival plaque	16S rRNA sequencing
Vieira Colombo et al[18]	189 Cases 81 Controls	98 CP: PPD/CAL > 4 36 AgP: PPD/CAL > 4 55 G: PPD/CAL < 4, BOP+ PPD/CAL < 4, BOP-	40/60 (35.6 ± 13.6) 41/59 (44.9 ± 11.4) 39/61 (33.0 ± 4.1) 28/72 (25.8 ± 8.6)	27 38 25 7	Subgingival plaque	DNA-DNA checkerboard

CAL = Clinical Attachment Level; PPD = Probing Pocket Depht; CPI = Community Periodontal Index; BOP = Bleeding On Probling; CP = Chronic Periodontitis; AgP = Aggressive Periodontitis; G = Gingivitis; NR = Not Reported

Table III. Review of author judgments on quality assessment for each included study.

Author (year)	Case and Control Definition	Case and Control Selection	Defined exclusion criteria	Comparability of Cases and Controls	Ascertainment of exposure	Blindness	Risk of bias
Ai et al[11]	*	0	0	0	*	*	High
Al-Hebshi et al[19]	**	0	**	*	**	0	Medium
Coretti et al[12]	**	**	**	0	**	0	Medium
Galimanas et al[13]	**	**	**	**	**	0	Low
Hunter et al[14]	**	*	**	0	*	0	Medium
Kirst et al[15]	**	*	**	0	**	0	Medium
Lourenço et al[20]	**	*	**	*	**	0	Medium
Oliveira et al[16]	**	*	**	*	**	0	Medium
Park et al[21]	**	*	*	0	**	0	Medium
Pérez-Chaparro et al[17]	**	*	*	**	*	0	Medium
Vieira Colombo et al[18]	**	**	**	*	**	0	Medium

0 = Not reported, * = not adequately assessed, ** = adequately assessed

Microorganisms presented in Table II belonged to the Bacteria and Eukarya (represented by Fungi) domains. The Eukarya domain was only represented by *Candida albicans* whose presence was detected in patients affected by chronic and aggressive periodontitis by Vieira Colombo et al[18]. The Bacteria domain was the main detected and it included 53 species (in some cases authors of the selected articles did not identify a genus into the family or an order into the genus of a specific bacterium). *Aggregatibacter actinomycetemcomitans*, *Bacteroidales (unidentified family), Candida albicans*, *Enterobacteriaceae (unidentified genus), Filifactor spp., Fretibacterium fastidiosum, Porohyromonas endodontalis, Prevotella intermedia, Pseudomonas aeruginosa, Pseudoramibacter alactolyticus, Selenomonas sputigena, Tannerella forsythia* and *TM7 spp.* were found to have statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in patients affected by aggressive periodontitis than in healthy controls.

Table IV. Number of studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in periodontitis than in periodontal health. 2 (some evidence); 3-4 (moderate evidence); 5-7 (strong evidence)

Taxa/ microorganism (High evidence)	Number of studies	Taxa/ microorganism (Moderate evidence)	Number of studies	Taxa/ microorganism (Low evidence)	Number of studies
Desulfobulbus spp.	5	Bacteroidales (unidentified family)	4	Anaeroglobus geminatus	2
Filifactor alocis	6	Eubacterium spp.	4	Clostridiales (unidentified family)	2
Porphyromonas gingivalis	5	Fretibacterium spp.	4	Filifactor spp.	2
Tannerella forsythia	7	Parvimonas micra	3	Fretibacterium fastidiosum	2
TM7 spp.	5	Peptostreptococcus spp.	3	Fusobacterium spp.	2
Treponema denticola	5	Porphyromonas endodontalis	4	Mogibacteriaceae (unidentified genus)	2
		Selenomonas sputigena	3	Neisseria spp.	2
		Synergistes spp.	4	Olsenella uli	2
		Treponema socranskii	3	Prevotella spp.	2
				Prevotella intermedia	2
				Propionibacterium spp.	2
				Pseudomonas aeruginosa	2
				Spirochaetes spp.	2
				Streptococcus intermedius constellatus	2

Table V. Microorganisms found in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health.

Microorganism	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in CP than in periodontal health	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in AgP than in periodontal health
Actinobaculum spp.	Lourenço et al[20]	
Actinomyces cardiffensis	Galimanas et al[13]	
Actinomyces odontolyticus	Hunter et al[14]	
Aggregatibacter actinomycetemcomitans		Lourenço et al[20]
Alloprevotella tannerae	Lourenço et al[20]	
Anaeroglobus geminatus	Pérez-Chaparro et al[17]; Lourenço et al[20]	
Bacteroidales (unidentified family)	Oliveira et al[16]; Pérez-Chaparro et al[17]; Lourenço et al[20]; Park et al[21]	Oliveira et al[16]
Campylobacter spp. • Campylobacter showae	Lourenço et al[20] Ai et al[11]	
Candida albicans	Vieira Colombo et al[18]	Vieira Colombo et al[18]
Capnocytophaga spp.	Hunter et al[14]	
Catonella morbi	Lourenço et al[20]	
Clostridiales (unidentified family)	Coretti et al[12]; Galimanas et al[13]	
Desulfobulbus spp.	Coretti et al[12]; Hunter et al[14]; Kirst et al[15]; Pérez-Chaparro et al[17]; Lourenço et al[20]	
Dialister spp. • *Dialister invisus*	Lourenço et al[20] Pérez-Chaparro et al[17]	
Enterobacteriaceae (unidentified genus)	Vieira Colombo et al[18]	Vieira Colombo et al[18]
Eubacterium spp. • *Eubacterium brachy* • *Eubacterium saphenum*	Galimanas et al[13]; Kirst et al[15]; Pérez-Chaparro et al[17]; Lourenço et al[20] Pérez-Chaparro et al[17] Pérez-Chaparro et al[17]	
Filifactor spp. • *Filifactor alocis*	Park et al[21] Galimanas et al[13]; Kirst et al[15]; Oliveira et al[16]; Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]; Oliveira et al[16]	
Fretibacterium spp. • *Fretibacterium fastidiosum*	Oliveira et al[16]; Pérez-Chaparro et al[17]; Lourenço et al[20]; Park et al[21] Pérez-Chaparro et al[17]	Oliveira et al[16]
Fusobacterium spp. • *Fusobacterium nucleatum subsp. vincentii* • *Fusobacterium periodonticum*	Pérez-Chaparro et al[17]; Lourenço et al[20] Kirst et al[15] Hunter et al[14]	
Hafnia alvei	Vieira Colombo et al[18]	
Johnsonella spp.	Pérez-Chaparro et al[17]	

Continued

Table V (cont.). Microorganisms found in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health.

Microorganism	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in CP than in periodontal health	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in AgP than in periodontal health
Klebsiella pneumoniae	Hunter et al[14]	
Lachnospiraceae (unidentified genus)	Pérez-Chaparro et al[17]	
Lactobacillus gasseri	Ai et al[11]	
Leptotrichiaceae (unidentified genus)	Pérez-Chaparro et al[17]	
Mogibacteriaceae (unidentified genus)	Coretti et al[12]; Kirst et al[15]	
Mycoplasma spp.	Galimanas et al[13]	
Neisseria spp.	Hunter et al[14]; Vieira Colombo et al[18]	
• *Neisseria flava*	Hunter et al[14]	
• *Neisseria subflava*	Hunter et al[14]	
Olsenella uli	Ai et al[11]; Vieira Colombo et al[18]	
Parvimonas micra	Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]	
Peptococcus spp.	Pérez-Chaparro et al[17]	
Peptostreptococcus spp.	Galimanas et al[13]; Kirst et al[15]; Pérez-Chaparro et al[17]	
• *Peptostreptococcus anaerobius*	Vieira Colombo et al[18]	
• *Peptostreptococcus stomatis*	Lourenço et al[20]	
Phocaeicola spp.	Galimanas et al[13]	
Porphyromonas spp.	Park et al[21]	Oliveira et al[16]
• *Porphyromonas endodontalis*	Galimanas et al[13]; Kirst et al[15]; Pérez-Chaparro et al[17]	
• *Porphyromonas gingivalis*	Kirst et al[15]; Oliveira et al[16]; Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]	
Prevotella spp.	Pérez-Chaparro et al[17]; Lourenço et al[20]	Lourenço et al[20]
• *Prevotella intermedia*	Kirst et al[15]	
• *Prevotella nigrescens*	Hunter et al[14]	
Propionibacterium spp.	Galimanas et al[13]; Hunter et al[14]	
Pseudomonas aeruginosa	Hunter et al[14]; Vieira Colombo et al[18]	Vieira Colombo et al[18]
Pseudoramibacter spp.	Coretti et al[12]	Lourenço et al[20]
• *Pseudoramibacter alactolyticus*		
Rothia spp.	Park et al[21]	
Selenomonas spp.	Lourenço et al[20]	Oliveira et al[16]
• *Selenomonas sputigena*	Hunter et al[14]; Oliveira et al[16]; Pérez-Chaparro et al[17]	
Serratia marcescens	Vieira Colombo et al[18]	

Continued

Table V (cont.). Microorganisms found in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health.

Microorganism	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in CP than in periodontal health	Studies that found statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in AgP than in periodontal health
Spirochaetes spp.	Coretti et al[12]; Park et al[21]	
Staphylococcus capitis	Hunter et al[14]	
Streptococcus gordonii	Hunter et al[14]	
Streptococcus intermedius constellatus	Galimanas et al[13]; Kirst et al[15]	
Streptococcus spp. oral clone BW009; oral strain T1-E5 and T4-E3	Hunter et al[14]	
Synergistes spp.	Galimanas et al[13]; Kirst et al[15]; Al-Hebshi et al[19]; Park et al[21]	
Tannerella forsythia	Galimanas et al[13]; Hunter et al[14]; Kirst et al[15]; Oliveira et al[16]; Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]	Oliveira et al[16]
Tissierellaceae (unidentified genus)	Coretti et al[12]	
TM7 spp.	Galimanas et al[13]; Oliveira et al[16]; Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]	Oliveira et al[16]
Treponema spp.	Pérez-Chaparro et al[17]	
• Treponema denticola	Galimanas et al[13]; Kirst et al[15]; Pérez-Chaparro et al[17]; Al-Hebshi et al[19]; Lourenço et al[20]	
• Treponema maltophilum	Kirst et al[15]	
• Treponema parvum	Kirst et al[15]	
• Treponema pectinovorum 8:A:33768 and OMZ831	Hunter et al[14]	
• Treponema socranskii	Hunter et al[14]; Kirst et al[15]; Pérez-Chaparro et al[17]	
Unidentified Human Oral Bacterium C20	Hunter et al[14]	
Veillonellaceae spp.	Pérez-Chaparro et al[17]	

The same significantly higher levels were recorded in patients affected by chronic periodontitis in all the microorganisms reported in Table II except for *Aggregatibacter actinomycetemcomitans* and *Pseudoramibacter alactolyticus*. With the aim of estimating the current weight of evidence of pathogens associated with periodontitis, the data of Table II were reported in Table III divided according to the following categories: bacteria found in statistically significantly higher levels and/or proportion and/or prevalence and/or abundance in periodontitis than in periodontal health from 5 to 7 studies (high evidence), from 3 to 4 studies (moderate evidence) or in at least 2 studies (low evidence). Six species were included in the high evidence category, nine species in the moderate evidence category and fourteen in the low evidence category. This systematic review confirmed the presence of recognized periodontal pathogens such as the members of the red complex (*Porohyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) but also identified, with high weight of evidence, the presence of new pathogens such as: *Desulfobulbus spp., Filifactor alocis* and *TM7 spp.* Among these newly identified pathogens *Filifactor alocis* was found by 6 studies, more studies than some periodontal pathogens like *Porphyromonas gingivalis* and *Treponema denticola*.

Discussion

The analysis of the results of the studies published in the last five years and included in this systematic review has confirmed some evidence already highlighted in the previous years[9] and detected the presence of some microorganisms not previously found. According to the analysis conducted by the authors regarding the strength of evidence of the results, 3 species/genera can be considered as new periodontal pathogens with strong evidence (*Desulfobulbus spp., Filifactor alocis* and *TM7 spp.*) as the results are confirmed in at least 5 of the studies included in the present review. *Desulfobulbus spp.* and *TM7 spp.* were detected in 5 studies with the same strength of evidence of other already known periodontal pathogens and, surprisingly, *Filifactor alocis* was detected in more studies than *Porphyromonas gingivalis* and *Treponema denticola*. *Desulfobulbus spp.* and *Filifactor alocis* were previously cultivated whereas *TM7 spp.* is a not-yet-cultivable genus; among the cultivable species *Desulfobulbus spp.* is a genus of Gram- anaerobic bacteria and *Filifactor alocis* is a Gram+ anaerobic bacterium. The association of some microorganisms with periodontal disease was evaluated as moderate. Species/genera belonging to this group include: *Eubacterium spp., Fretibacterium spp., Parvimonas micra, Peptostreptococcus spp., Porphyromonas endodontalis, Selenomonas sputigena, Synergistes spp.* and *Treponema socranskii*. An unidentified family of the *Bacteroidales* order was also included in this group. The *Bacteroidales* order and all species/genera belonging to this group were previously cultivated and they variably belong to the Gram+ (*Eubacterium spp., Parvimonas micra* and *Peptostreptococcus spp.*) or Gram- (*Fretibacterium spp., Porphyromonas endodontalis, Selenomonas sputigena, Synergistes spp., Treponema socranskii* and *Bacteroidales* order) category. The association between *Treponema socranskii* and periodontal disease, although with moderate strength of evidence, has been not previously reported in literature. Almost all bacterial species listed as a suspected periodontal pathogen in the present study are mostly found in the oral cavity and rarely involved in extraoral infections. Some exceptions are: *Synergistes spp.* and *Peptostreptococcus spp.*, which are part of the commensal microbiota of the animal gastrointestinal tract but may also act as an opportunistic pathogen when spreading to other mucosa or skin tissues because they have been found in human cysts and abscesses[34]; *Parvimonas micra,* which has been documented to be involved in polymicrobial infections causing pleural empyema[35] and septic arthritis of native joints[36]. Some authors suggested that the ability of *Parvimonas micra* to cause infection in other sites of the human body apart from the oral cavity could be due to the translocation of the bacterium from the oral cavity or from the gastrointestinal tract[37,38]. The presence of microorganisms in subgingival sites that are also associated with extraoral diseases may play an important role in better understanding the connections between oral and systemic infections. This evidence should be considered in further studies. Another aspect emerging from this analysis, that should be considered accurately, is the dramatic increase of studies dealing with metagenomic analysis of samples from human sites: the electronic search of this review regarding publications of the last five years retrieved 635 results. It is very probable that the advent of metagenomics allowed, therefore, to delineate more accurately the composition of the human microbiota and to identify species that were not pre-

viously cultivable, such as *TM7 spp.* On the other hand, the number of plaque samples evaluated by the various studies is also an important point to consider. It has been advocated that the evaluation of a large number of plaque samples per patient is a crucial requirement for obtaining reliable information about the etiology of periodontitis[39,40]. In this regard, there is an important difference between the targeted and open-ended molecular techniques. Unfortunately, the approaches that allow an in-depth characterization of microbial diversity (like the 16S rRNA sequencing) are still relatively costly; therefore, the studies using sequencing have evaluated about the half of the plaque samples of the present review[11-17,19-21]. The other half of the plaque samples were evaluated using the checkerboard DNA-DNA hybridization that allows the evaluation of thousands of plaque samples at a relatively low cost[18]. This systematic review has also allowed the increase of the knowledge about the microbiota connected to aggressive periodontitis. Along with some known periodontal pathogens, the included studies have also detected the presence of new pathogens such as: *Bacteroidales (unidentified family), Candida albicans*, *Enterobacteriaceae (unidentified genus), Filifactor spp., Fretibacterium fastidiosum, Pseudomonas aeruginosa, Pseudoramibacter alactolyticus, Selenomonas sputigena* and *TM7 spp.* The presence of such microorganisms in aggressive periodontitis can be helpful in developing new, more targeted, therapies and should be considered in further studies. The data of this systematic review support the evidence that the subgingival pocket hosts a complex and highly diverse microbiota. It seems evident that other microorganisms besides the already known periodontal pathogens might be involved in the onset and/or progression of periodontitis. Nonetheless, it is important to underline that this review has some limitations. Firstly, the review only confirms or adds evidence to the ones reported by other authors about the topic but it does not give the necessary association of the new pathogens with the etiology of periodontal diseases. Indeed, the etiologic role of these microorganisms would need to be confirmed by risk assessment and interventional (i.e., elimination) studies to evaluate whether their reduction or elimination would be accompanied by clinical improvements and whether their persistence would lead to disease progression[41]. Another important limitation is that a microorganism found in higher levels and proportions in diseased patients rather than in healthy ones might not be sufficient to determine whether it actually initiated the disease process or was merely favored by the inflammatory environment associated with periodontitis[42-45]. Other methodological limitations of this review are the risk of bias of included studies that was overall defined as medium risk and the eventual loss of some reports due to the fact that some authors of the included studies, contacted by e-mail with the aim of retrieving other supplemental data, did not answer.

Conclusions

The results of this systematic review support high evidence for the association of 3 new species/genera with the etiology of periodontitis. These data would be useful to guide future investigations on the actual role of these new pathogens in the onset and progression of this disease as well as on the identification of other pathogens helping to understand the mechanisms that regulate their association and to develop preventive and therapeutic strategies for the control of the disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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