CLINICAL REVIEW

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Newly Identified Pathogens Associated with Periodontitis: A Systematic Review

ABSTRACT

There is substantial evidence supporting the role of certain oral bacteria species in the onset and progression of periodontitis. Nevertheless, results of independent-culture diagnostic methods introduced about a decade ago have pointed to the existence of new periodontal pathogens. However, the data of these studies have not been evaluated together, which may generate some misunderstanding on the actual role of these microorganisms in the etiology of periodontitis. The aim of this systematic review was to determine the current weight of evidence for newly identified periodontal pathogens based on the results of "association" studies. This review was conducted and reported in accordance with the PRISMA statement. The MEDLINE, EMBASE, and Cochrane databases were searched up to September 2013 for studies (1) comparing microbial data of subgingival plaque samples collected from subjects with periodontitis and periodontal health and (2) evaluating at least 1 microorganism other than the already-known periodontal pathogens. From 1,450 papers identified, 41 studies were eligible. The data were extracted and registered in predefined piloted forms. The results suggested that there is moderate evidence in the literature to support the association of 17 species or phylotypes from the phyla *Bacteroidetes, Candidatus Saccharibacteria, Firmicutes, Proteobacteria, Spirochaetes,* and *Synergistetes.* The phylum *Candidatus Saccharibacteria* and the *Archaea* domain also seem to have an association with disease. These data point out the importance of previously unidentified species in the etiology of periodontitis and might guide future investigations on the actual role of these suspected new pathogens in the onset and progression of this infection.

KEY WORDS: *Archaea*, *Bacteria*, dental plaque, microbiology, periodontal disease, DNA.

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INTRODUCTION

Periodontitis is an infectious disease involving a complex interaction between the oral microorganisms organized in a biofilm structure and the host immune response. Its clinical consequence is the destruction of the tissues that support and protect the tooth. As with any other infection, identification of the microbial pathogens associated with the etiology of periodontitis is the first step toward the development of effective therapeutic approaches. The establishment of a microorganism as a true pathogen should be based on 2 main levels of evidence: (1) the organism should be present in higher prevalence and/or levels in disease than in health ("association" studies), and (2) its suppression or elimination should reduce or stop disease progression ("elimination" studies; Socransky, 1979).

The composition of the oral microbiota—specifically, the subgingival microbiota—has been studied for over a century. Unfortunately, for many decades, research in this field was considerably delayed due to technical difficulties, such as the need to identify microorganisms to the species level using only culture techniques. The use of immunologic and molecular diagnostic tests for the identification of microorganisms independent on cultivation—such as DNA probes, polymerase chain reaction, and immunoassays—began in the 1990s and allowed a great progress in the understanding about the composition of the subgingival microbiota. Using one of these molecular tests—namely, checkerboard DNA-DNA hybridization—Socransky *et al.* (1998) described the role of 5 main microbial complexes in the subgingival biofilm. Some species/complexes were associated with periodontal health, such as the yellow (*Streptococcus species*) and purple (*Veillonela parvula* and *Actinomyces odontolyticus*) complexes, while others were closely associated with disease, such as the red (*Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia*) and orange complexes (*Fusobacterium, Prevotella*, and *Campylobacter species*). Afterward, other association and elimination studies have confirmed the involvement of the 3 members of the red complex and some members of the orange complex, such as *Prevotella intermedia, Parvimonas micra, Fusobacterium nucleatum, Eubacterium nodatum*, and *Aggregatibacter actinomycetemcomitans*, with the etiology of different periodontal conditions (Teles *et al.*, 2013).

In 2001, using cloning and Sanger sequencing, Paster *et al.* suggested a possible role of cultivable and not-yet-cultivable/unrecognized microbial species in the etiology of periodontitis, confirming the idea that the diversity of the oral microbiota was more complex than previously known. Subsequently, a number of other studies using several molecular approaches, including next-generation sequencing techniques, were published in the periodontal literature (Kumar *et al.*, 2005; Matarazzo *et al.*, 2011; Teles *et al.*, 2011; Griffen *et al.*,

2012; Abusleme *et al.*, 2013). The overall data provided by these studies for more than 12 yr suggested the existence of new periodontal pathogens. However, studies are diverse in terms of the diagnostic test used, the taxa assessed, and the number of samples evaluated, which may generate some misunderstanding while trying to draw objective conclusions on the actual role of these microorganisms in the etiology of periodontitis. Thus, a thorough review compiling the results of these studies could be helpful for the accurate interpretation of the present literature on this topic. Therefore, the aim of this systematic review was to determine the current weight of evidence for newly identified periodontal pathogens based on the results of association studies.

MATERIALS & METHODS

This systematic review was conducted in accordance with the recommendations of PRISMA statement (*i.e.*, Preferred Reporting Items for Systematic Reviews and Meta-analysis; Moher *et al*., 2009).

Focused Question

What is the weight of evidence for the existence of newly identified periodontal pathogens based on association studies?

Inclusion Criteria

The manuscripts meeting the following criteria were included:

- Studies of any design that compared microbial data of subgingival plaque samples collected from systemically healthy patients with periodontitis and periodontal health
- Studies evaluating at least 1 new microorganism other than the species already suggested as periodontal pathogens or putative periodontal pathogens (*P. gingivalis, T. denticola, T. forsythia, F. nucleatum, Fusobacterium periodonticum, P. intermedia, Prevotella nigrescens, P. micra, Campylobacter gracilis, Campylobacter rectus, Campylobacter showae, E. nodatum, Streptococcus constellatus and A. actinomycetemcomitans*; "Proceedings of the World Workshop," 1996; Socransky *et al.*, 1998; Teles *et al.*, 2013)

Exclusion Criteria

- Studies published in languages other than English, Spanish, French, or Portuguese
- Lack of baseline data
- Lack of a direct comparison of baseline microbial data between periodontitis and periodontally healthy groups
- Lack of data from subgingival plaque samples in periodontitis and/or periodontally healthy groups
- Lack of data from subgingival plaque samples of systemically healthy subjects
- Studies that evaluated only subjects with localized aggressive periodontitis or refractory periodontitis
- Review studies
- Studies that evaluated only viruses

Search Strategy and Data Extraction

The MEDLINE (via PubMed), EMBASE, and Cochrane Library databases were searched up to September 10, 2013, by 2 independent reviewers (P.J.P.C. and P.D.) using the search strategy described in Appendix Table 1. In addition, a manual search was conducted based on the reference list of the selected manuscripts and review articles. The studies were screened independently by 2 researchers (E.L., M.Fa.), and any disagreement was solved through discussion. When disagreement persisted, another researcher was consulted to achieve consensus (M.Fe.). Those studies that fulfilled the inclusion and exclusion criteria were processed for data extraction, conducted by another 2 independent researchers (P.J.P.C. and C.G.). The following information was collected from each manuscript and registered in predefined piloted forms:

- Study location
- Type of trial
- Characteristics of participants (*e.g.*, systemically health status, number of patients per group, age, periodontal condition)
- Type of microbiological evaluation (*e.g.*, individually or pooled strategy, number of samples evaluated, employed diagnostic method)
- Microbiological outcomes (*e.g.*, microorganisms appraised [*e.g.*, *Bacteria* and/or *Archaea*], taxa in higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health or those reported by the authors as being associated with periodontitis [primary outcome of interest])
- Conflict of interest
- Source of funding

To accurately assign the most updated names to the microorganisms so that we could avoid taxa repetition and to assign a Human Oral Taxon (HOT) number whenever available, the Human Oral Microbiome Database (HOMD, http://www.homd.org/index.php, October 28, 2013) was interrogated for each microorganism cited on the 41 included studies by 3 researchers (P.J.P.C., L.C.F., N.T.). For this step, we used the nomenclature given by each author (*i.e.*, the microorganism/strain/isolate name or the Genbank accession number). When this query did not return any result, the local HOMD blast tool was used to query the available 16S rDNA sequence with length >1,300 nt. In cases in which both queries were unsuccessful, the author's nomenclature was retained. Phyla, class, species, and phylotypes were indexed according to the National Center for Biotechnology Information taxonomy browser (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi, October 29, 2013) when available; otherwise, HOMD classification was retained.

RESULTS

Studies Included

A total of 1,450 titles were found during the electronic search. After title screening, 1,303 studies were excluded, and 147 were

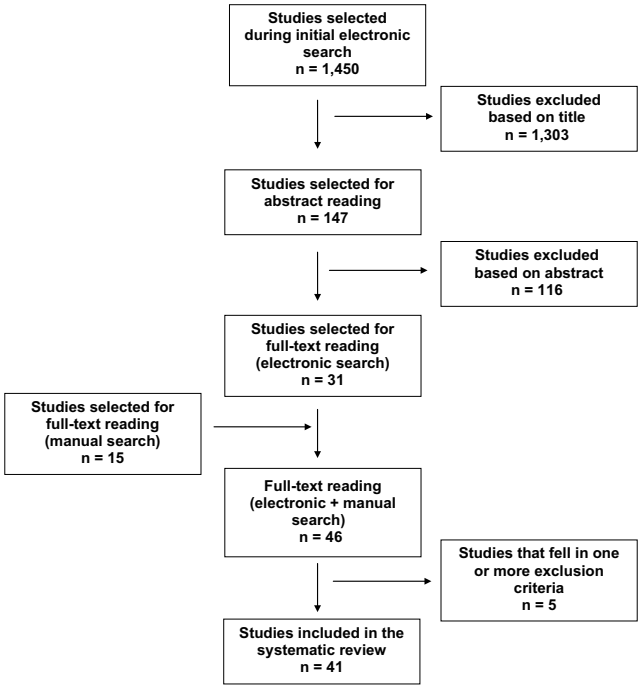

Figure. Flowchart of the search strategy.

selected. After abstract reading, 116 studies were excluded, and 31 full-text publications were comprehensively evaluated. In addition, 15 studies were selected during the manual search. After reading these 46 studies, 5 were excluded for not meeting the inclusion criteria (Appendix Table 2). Therefore, 41 studies were included in this study (Figure).

Study Designs: Periodontal Conditions/Samples Evaluated and Diagnostic Techniques Used

Table 1 presents the studies included and their main methodological features. The majority of the studies had more patients and samples in the periodontitis than in the periodontally healthy group. A total of 912 individuals with periodontal health and 1,918 with periodontitis were evaluated. Subgingival biofilm samples were processed individually in 24 studies and pooled in 13 studies. One study used both sampling methods (Liu *et al.*, 2012); 2 studies did not provide information about the number of samples collected (Dewhirst *et al.*, 2000; Paster *et al.*, 2001); and 1 study (Bringuier *et al.*, 2013) did not clarify whether the samples were analyzed individually or pooled. A total of 3,508 and 10,800 subgingival plaque samples were evaluated from subjects with periodontal health or periodontitis, respectively.

Three studies used culture methods (Macuch and Tanner, 2000; Murdoch *et al.*, 2004; Canabarro *et al.*, 2012), but Macuch and Tanner (2000) also used a protein electrophoresis technique (SDS-PAGE). The other 38 studies used technologies based on nucleic acid detection as follows: 22 used targeted techniques; 10 used open-ended techniques; and 6 used both approaches. Most studies used techniques based on DNA detection; only 2 studies (Teles *et al.*, 2011; Gonçalves *et al.*, 2012) used a RNA-based detection method—specifically, the RNA–oligonucleotide quantification technique.

Microbial Data

The microorganisms found in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health or those reported by the authors as being associated with periodontitis were catalogued, and data are summarized in Appendix Table 3.

Table 2 presents the taxa found in at least 1 study in statistically significantly higher levels and/or proportion and/or abundance and/or prevalence in periodontitis than in periodontal health. Three domain systems were identified: *Bacteria, Archaea*, and *Eukarya* (represented by Fungi). *Bacteria* was the main domain detected, and it included 10 phyla (*Bacteroidetes, Spirochaetes, Firmicutes, Synergistetes, Proteobacteria, Actinobacteria, Fusobacteria, Chloroflexi, Tenericutes* and the *Candidatus Saccharibacteria* [syn. Candidate division TM7]), the Candidate division Sulphur River 1 (SR1, no rank, http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=221235&lvl=3&lin=f&keep=1&srchmode=1&unlock, October 29, 2013), 63 bacterial genera, and 108 species/phylotypes. *Firmicutes*, which harbors mostly Gram-positive bacteria, was the phylum with the highest number of species associated with periodontitis (n = 39), in contrast with *Chloroflexi* (n = 1). One species from the *Archaea* domain (*Methanobrevibacter oralis* HOT 815) and the total levels and proportions of this domain were also associated with periodontitis.

To estimate the current weight of evidence of newly identified pathogens associated with periodontitis, the data of Table 2 were subsetted into the following categories: taxa found in statistically significantly higher levels and/or proportion and/or prevalence and/or abundance in periodontitis than in periodontal health from 3 to 5 studies (moderate evidence) or in 2 studies (some evidence) (Table 3). Seventeen species/phylotypes, the phylum *Candidatus Saccharibacteria*, and the *Archaea* domain were included in the moderate evidence category and other 15 taxa in the some evidence category.

Appendix Table 4 presents the same type of data of Table 2 but for the known pathogens. Recognized periodontal pathogens such as the members of the red complex, *A. actinomycetemcomitans,* and certain members of the orange complex were found in statistically significantly higher levels and/or proportions and/or prevalence in a number of studies using targeted and open-ended techniques. For example, *P. gingivalis, T. forsythia* and *T. denticola* were statistically significantly elevated in periodontitis than in health in 9 studies.

DISCUSSION

This is the first systematic review that assessed the current weight of evidence concerning new candidate periodontal pathogens after 12 yr of what could be considered the "modern era" of oral microbiology. We estimated that at this point no microorganism could be set as a true new periodontal pathogen with strong evidence, since the number of studies that associated each of the taxa with periodontitis is still low—from 1 to 5. Therefore, the highest evidence category specified was moderate.

Table 1. Summary of the Methodological Features of the Included Studies

	Subjects, *n*				Samples, *n*		
	H	GAgP	ChP	RP	H	P	Method/Taxa Evaluated
Willis *et al.*, 1999	10		21		10 (I)	21 (I)	Nested PCR. *7 Treponema* species
Harper-Owen *et al.*, 1999	20		28		40 (I)	56 (I)	PCR/Sanger sequencing. Phylotype PUS3.422, PUS9.170, PUS9.180
Dewhirst *et al.*, 2000	2		1	8	NA	NA	PCR/cloning/Sanger sequencing. *Spirochaetes* phylum
Sawada *et al.*, 2000	20		40		20 (I)	40 (I)	PCR. *Selenomonas sputigena, Centipeda periodontii*
Macuch and Tanner, 2000	18		52		44 (I)	52 (I)	Culture and SDS-Page. *Campylobacter* species
Paster *et al.*, 2001	5		9	11	NA	NA	PCR/cloning/Sanger sequencing. *Bacteria* domain and *Spirochaetes, Bacteroidetes* phyla
Colombo *et al.*, 2002	14		25		1,492 (I)	2,540 (I)	Checkerboard DNA-DNA hybridization. 42 bacterial species
Leys *et al.*, 2002	172		121		172 (P)	121 (P)	Nested PCR/Sanger sequencing. *Bacteroides forsythus* and oral clone BU063
Asai *et al.*, 2002	13		37		13 (P)	37 (P)	PCR and qPCR. Total Treponemes, *T.denticola, T. medium*, and *T. vincentii*
Hutter *et al.*, 2003	6	26			6 (I)	26 (I)	PCR/cloning/Sanger sequencing. *Bacteria* domain
Brinig *et al.*, 2003	4		42		18 (I)	53 (I)	PCR/cloning/Sanger sequencing, qPCR and FISH. Candidate division TM7 (*Phylum Candidatus Saccharibacteria*) and TM7 I025 subgroup
Ouverney *et al.*, 2003	4		12		9 (I)	12 (I)	FISH. Candidate division TM7 (*Phylum Candidatus Saccharibacteria*) and TM7 I025 subgroup
Kumar *et al.*, 2003	66		66		66 (P)	66 (P)	Nested PCR and Sanger sequencing. 39 bacterial species or phylotypes
Zijnge *et al.*, 2003	6		9		6 (P)	9 (P)	PCR/DGGE and DGGE/PCR/Sanger sequencing. *Bacteria* domain
Booth *et al.*, 2004	40		40		40 (P)	80 (P)	Slot-blot hybridization. *Bulleidia extructa, Eubacterium nodatum, Mogibacterium timidum, and Slackia exigua*
Murdoch *et al.*, 2004	28		28		84 (I)	168 (I)	Culture. Oral *staphylococci*
Lepp *et al.*, 2004	8		50		29 (I)	205 (I)	PCR/cloning/Sanger sequencing, FISH and qPCR. *Archaea* and *Bacteria* domains
Mayanagi *et al.*, 2004	12		18		12 (I)	18 (I)	Nested PCR. 25 putative or probable periodontal pathogens
Kumar *et al.*, 2005	15		15		15 (P)	30 (P)	PCR/cloning/Sanger sequencing. *Bacteria* domain
Li *et al.*, 2006	20		35		20 (P)	35 (P)	PCR/Sanger sequencing. Phylotype AU 126 and X 112
Souto *et al.*, 2006	3		14		200 (I)	400 (I)	Checkerboard DNA-DNA hybridization. 11 putative periopathogen bacteria
Ledder *et al.*, 2007	18		29		18 (I)	29 (I)	PCR/DGGE, DGGE/PCR/Sanger sequencing for *Bacteria* and Multiplex PCR for *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythensis*
Souto and Colombo, 2008	56		169		56 (P)	169 (P)	PCR. *Enterococcus faecalis*
Vianna *et al.*, 2008	65		102		65 (P)	102 (P)	qPCR and Sanger sequencing. Hydrogenotrophic *Archaea* and *Bacteria*
Li *et al.*, 2009	15		41		15 (P)	41 (P)	PCR and PCR/cloning/Sanger sequencing. *Archaea* domain
Riep *et al.*, 2009	21	44	46		105 (I)	450 (I)	Dot blot hybridization. 10 Putative periodontal pathogen bacteria
Vartoukian *et al.*, 2009	5		5		5 (P)	10 (P)	PCR/cloning/Sanger sequencing and FISH. *Synergistetes* phylum
Schlafer *et al.*, 2010*	19	72	30		82 (I)	408 (I)	Dot blot hybridization. *Filifactor alocis*, red complex, *A.actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia*
Abiko *et al.*, 2010	12		28		12 (I)	28 (I)	qPCR. Total *Bacteria* and 13 bacterial species

(continued)

Table 1. (continued)

	Subjects, *n*				Samples, *n*		
	H	GAgP	ChP	RP	H	P	Method/Taxa Evaluated
Drescher *et al.*, 2010*	19	62	82		82 (I)	660 (I)	Dot blot hybridization. *Selenomonas* genus, *Centipeda* genus
da Silva-Boghossian *et al.*, 2011	51	90	219		357 (I)	4,326 (I)	Checkerboard DNA-DNA hybridization. Red Complex, *A. actinomycetemcomitans, Acinetobacter baumannii, Escherichia coli, E. faecalis, Pseudomonas aeruginosa, Staphylococcus aureus*
Matarazzo *et al.*, 2011	30	30			60 (I)	103 (I)	qPCR and PCR/cloning/Sanger sequencing. *Bacteria* and *Archaea* domains
Teles *et al.*, 2011	8		11		112 (I)	154 (I)	ROQT. 43 bacterial species
Canabarro *et al.*, 2013	20		40		20 (I)	60 (I)	Culture. *Candida albicans* and other yeast
Griffen *et al.*, 2012	29		29		29 (I)	58 (I)	*16S rDNA* PCR 454 pyrosequencing. *Bacteria* domain
Gonçalves *et al.*, 2012	15	15			135 (I)	135 (I)	ROQT. 10 bacterial species
Liu *et al.*, 2012	3		2		12 (I)	12 (I)	*16S rDNA* PCR 454 pyrosequencing and Illumina Metagenome high-throughput sequencing. *Bacteria* domain
Bringuier *et al.*, 2013	10		22		10 (NA)	22 (NA)	qPCR. *Methanobrevibacter oralis*
Abusleme *et al.*, 2013	10		22		17 (I)	44 (I)	*16SrDNA* PCR 454 pyrosequencing for *Bacteria* domain and qPCR for *Bacteria* domain and *Actinomyces, Streptococcus* and *Veillonella* genera.
You *et al.*, 2013a	10	1	9		10 (P)	10 (P)	PCR/Cloning/Sanger sequencing. *Bacteria* domain
You *et al.*, 2013b	10		10		10 (P)	10 (P)	PCR/Cloning/Sanger sequencing. *Bacteria* domain

*FISH from this study was not taken into account, since no control group was evaluated by this method.

NA, not available; H, periodontal health; GAgP, generalized aggressive periodontitis; ChP, chronic periodontitis; RP, refractory periodontitis; P, periodontitis; (I), samples processed individually; (P), samples processed in pool; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; FISH, fluorescence *in situ* hybridization; DGGE, denaturing gradient gel electrophoresis; ROQT, RNA-oligonucleotide quantification technique.

Four microorganisms of the 17 taxa included in the moderate evidence category are not-yet-cultivable, and 13 have been cultivated before. Five of the cultivable species are Gram positive (*Eubacterium saphenum, Mogibacterium timidum, Peptostreptococcus stomatis, Filifactor alocis and Enterococcus faecalis*), while all the other 8 (Bacteroidales [G-2] *sp. oral taxon 274, Porphyromonas endodontalis, Treponema lecithinolyticum, Treponema medium, Treponema vincentii, Anaeroglobus geminatus*—also known as *Megasphaera* oral clone BB166, *Selenomonas sputigena, Fretibacterium fastidiuosum*) are Gram negative and anaerobic, characteristics of most of the microorganisms involved in polymicrobial infections. Five of these new candidate periodontal pathogens belong to the phyla *Bacteroidetes* and *Spirochaetes*, which include several known periodontal pathogens, such as *P. gingivalis, T. forsythia, T. denticola*, and *T. socranskii* and species from the genera *Prevotella* (Socransky *et al.*, 1998). Seven species were from the *Firmicutes* phylum, and the other 5 species/phylotypes were distributed among the *Proteobacteria, Synergistetes*, and *Candidatus Saccharibacteria* phyla. The phylum *Firmicutes* harbors genera previously associated with periodontal health (*e.g., Streptococcus*) or disease (*e.g., Eubacterium* and *Selenemonas*) (Socransky *et al.*, 1998; Kumar *et al.*, 2003), and several other cultivable or not-yet-cultivable microorganisms from this phylum fell into the moderate (*e.g., F. alocis, E. faecalis*) or some evidence (*Dialister pneumosintes, Lachnospiraceae [G-8] sp. oral taxon 500*) categories.

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. One exception was *E. faecalis*, which is part of the commensal microbiota of the human gastrointestinal tract but may also act as an opportunistic pathogen when spreading to other mucosa or skin tissues (Vu and Carvalho, 2011). With respect to oral diseases, *E. faecalis* has been associated with root canal treatment failure (Wang *et al.*, 2012). It was interesting to note that all the evidence supporting *E. faecalis* as a candidate periodontal pathogen came out of studies that evaluated Brazilian patients (Colombo *et al.*, 2002; Souto *et al.*, 2006; Souto and Colombo 2008; da Silva-Boghossian *et al.*, 2011). This could be an example of a geographic specificity, since it has been suggested that the periodontal microbiota may show specific differences among countries (Haffajee *et al.*, 2004). However, this information would need to be confirmed by future studies evaluating the prevalence and levels of this microorganism in other populations. The other exceptions of microorganisms associated with periodontitis in the present review that may inhabit extraoral environments are *S. sputigena, T. medium*, and species from the

Table 2. Summary of the Data of the Included Studies: Newly Identified Taxa Associated with Periodontitis*

Taxa	Chronic Periodontitis Studies	Generalized Aggressive Periodontitis Studies
Bacteria		
Phylum *Actinobacteria*		
Actinobacteria class		
Actinomyces naeslundii HOT 176	Kumar *et al.*, 2003	
Bifidobacterium dentium HOT 588	Griffen *et al.*, 2012	
Cryptobacterium curtum HOT 579	Kumar *et al.*, 2003	
Corynebacterium diphtheria HOT 591	Souto *et al.*, 2006	
Rothia dentocariosa HOT 587	Kumar *et al.*, 2003	
Slackia exigua HOT 602	Abiko *et al.*, 2010	
Phylum *Bacteroidetes*		
Bacteroidia class		
Bacteroidetes [*G-1*] genus	Abusleme *et al.*, 2013	
Bacteroidaceae [*G-1*] *sp. oral taxon 272* HOT 272 [*Bacteroidetes* [*G-1*] sp. OT 272]	Abusleme *et al.*, 2013	
Bacteroidales [*G-2*] *sp. oral taxon 274* HOT 274 [Bacteroidetes clone AU126 / Phylotype AU126 / Bacteroidales OT 274]	Kumar *et al.*, 2003; Li *et al.*, 2006; Griffen *et al.*, 2012	
Bacteroidetes [*G-3*] genus	Abusleme *et al.*, 2013	
Bacteroidetes [*G-3*] *sp. oral taxon 280* HOT 280	Abusleme *et al.*, 2013	
Bacteroidetes [*G-3*] *sp. oral taxon 365* HOT 365	Abusleme *et al.*, 2013	
Bacteroidetes [*G-6*] genus	Abusleme *et al.*, 2013	
Bacteroidetes [*G-6*] *sp. oral taxon 516* HOT 516	Abusleme *et al.*, 2013	
Porphyromonas endodontalis HOT 273	Kumar *et al.*, 2003; Mayanagi *et al.*, 2004; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Prevotella denticola HOT 291	Kumar *et al.*, 2003; Griffen *et al.*, 2012	
Prevotella sp. oral taxon 526 HOT 526 [*Prevotella* genomo sp. P4]	Griffen *et al.*, 2012	
Prevotella sp. oral taxon 304 HOT 304	Abusleme *et al.*, 2013	
Alloprevotella tannerae HOT 466 [*Prevotella tannerae*]	Mayanagi *et al.*, 2004; Griffen *et al.*, 2012	
Phylum *Chloroflexi*		
Chloroflexi class		
Chloroflexi [*G-1*] genus	Abusleme *et al.*, 2013	
Chloroflexi [*G-1*] *sp. oral taxon 439* HOT 439	Abusleme *et al.*, 2013	
Phylum *Firmicutes*		
Clostridia class	Kumar *et al.*, 2005	
Clostridiales [*F-1*] [*G-1*] *sp. oral taxon 093* HOT 093 [Oral clone MCE_107]	Griffen *et al.*, 2012	
Catonella genus	Liu *et al.*, 2012	
Catonella sp. oral taxon 164 HOT 164 [*Catonella* sp. oral clone BR063]	Kumar *et al.*, 2005	
Shuttleworthia C1	Griffen *et al.*, 2012	
Johnsonella sp. oral taxon 166 HOT 166 [*Johnsonella* CK051]	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Eubacterium [*XI*] [*G-1*] genus	Abusleme *et al.*, 2013	
Eubacterium [*XI*] [*G-3*] *brachy* HOT 557 [*Eubacterium brachy*]	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Eubacterium [*XI*] [*G-5*] *saphenum* HOT 759 [*Eubacterium saphenum*]	Kumar *et al.*, 2003; Mayanagi *et al.*, 2004; Abiko *et al.*, 2010; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Eubacterium [*XI*] [*G-6*] genus	Abusleme *et al.*, 2013	
Eubacterium [*XI*] [*G-6*] *minutum* HOT 673	Abusleme *et al.*, 2013	

(continued)

Table 2. (continued)

Taxa	Chronic Periodontitis Studies	Generalized Aggressive Periodontitis Studies
Mogibacterium genus	Abusleme *et al.*, 2013	
Mogibacterium timidum HOT 042	Mayanagi *et al.*, 2004; Abiko *et al.*, 2010; Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XI*] [*G-2*] genus	Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XI*] [*G-2*] *sp. oral taxon* 091 HOT 091	Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XI*] [*G-4*] genus	Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XI*] [G-4] *sp. oral taxon 103* HOT 103 [*phylotype PUS9.170*]	Harper-Owen *et al.*, 1999	
Peptostreptoccaceae [*XI*] [*G-4*] *sp. oral taxon 369* HOT 369	Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XIII*] [*G-1*] genus	Abusleme *et al.*, 2013	
Peptostreptococcaceae [*XIII*] [*G-1*] *sp. oral taxon* 113 HOT 113 [*Peptoniphilus* oral taxon 113]	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Peptostreptococcus genus	Abusleme *et al.*, 2013	
Peptostreptococcus stomatis HOT 112 [*Peptostreptococcus* sp. oral clone CK035]	Kumar *et al.*, 2005; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Peptococcus sp. oral taxon 167 HOT 167	Abusleme *et al.*, 2013	
Pseudoramibacter genus	Abusleme *et al.*, 2013	
Pseudoramibacter alactolyticus HOT 538	Abusleme *et al.*, 2013	
Filifactor genus	Abusleme *et al.*, 2013	
Filifactor alocis HOT 539	Kumar *et al.*, 2003; Kumar *et al.*, 2005; Schlafer *et al.*, 2010; Griffen *et al.*, 2012, Abusleme *et al.*, 2013	Schlafer *et al.*, 2010
Lachnospiraceae [*G-8*] genus	Abusleme *et al.*, 2013	
Lachnospiraceae [*G-8*] *sp. oral taxon 500* HOT 500 [*Lachnospiraceae* JM048]	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Lachnospiraceae [*G-4*] genus	Abusleme *et al.*, 2013	
Stomatobaculum sp. oral taxon 373 HOT 373 [*Lachnospiraceae* [*G-4*] sp. OT 373]	Abusleme *et al.*, 2013	
Unclassified clostridiales ord	Abusleme *et al.*, 2013	
Negativicutes class		
Anaeroglobus geminatus HOT 121 [*Megasphaerao* oral clone BB166]	Kumar *et al.*, 2003; Kumar *et al.*, 2005; Griffen *et al.*, 2012	
Centipeda genus	Drescher *et al.*, 2010	Drescher *et al.*, 2010
Dialister invisus HOT 118 [*Dialister* sp. oral strain GBA27]	Kumar *et al.*, 2003	
Dialister sp. oral taxon 119 HOT 119 [*Dialister* sp. oral clone MCE7_134]	Kumar *et al.*, 2005	
Dialister pneumosintes HOT 736	Mayanagi *et al.*, 2004; Kumar *et al.*, 2005	
Megasphaera sp. oral clone MCE3_141	Kumar *et al.*, 2005	
Megasphaera sp. oral taxon 123 HOT 123 [*Megasphaera* sp. oral clone BS073]	Kumar *et al.*, 2005	
Mitsuokella sp. HOT 131 [*Selenomonas* CS002]		Gonçalves *et al.*, 2012
Selenomonas genus	Liu *et al.*, 2012; Drescher *et al.*, 2010	Drescher *et al.*, 2010
Selenomonas sputigena HOT 151	Kumar *et al.*, 2003; Mayanagi *et al.*, 2004; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	Gonçalves *et al.*, 2012
Selenomonas sp. oral clone D0042	Kumar *et al.*, 2005	
Selenomonas sp. oral clone 126 HOT 126 [*Selenomonas* EY047]	Griffen *et al.*, 2012	
Selenomonas dianae HOT 139	Griffen *et al.*, 2012	
Veillonellaceae [*G-1*] genus	Abusleme *et al.*, 2013	

(continued)

Table 2. (continued)

Taxa	Chronic Periodontitis Studies	Generalized Aggressive Periodontitis Studies
Veillonellaceae [*G-1*] *sp. oral taxon 129* HOT 129	Griffen *et al.*, 2012	
Veillonellaceae [*G-1*] *sp. oral taxon 132* HOT 132	Abusleme *et al.*, 2013	
Veillonellaceae [*G-1*] *sp. oral taxon 155* HOT 155	Abusleme *et al.*, 2013	
Bacilli class		
Enterococcus faecalis HOT 604	Colombo *et al.*, 2002; Souto *et al.*, 2006; Souto and Colombo, 2008; da Silva-Boghossian *et al.*, 2011	
Streptococcus sp. *oral strain 9F*	Kumar *et al.*, 2005	
Streptococcus sp. oral taxon 061 HOT 061 [*Streptococcus* sp. oral clone DP009]	Kumar *et al.*, 2005	
Streptococcus constellatus HOT 576	Abusleme *et al.*, 2013	
Streptococcus anginosus HOT 543	Abusleme *et al.*, 2013	
Streptococcus sp. oral taxon 071 HOT 071	Abusleme *et al.*, 2013	
Staphylococcus aureus HOT 550	Souto *et al.*, 2006	
Phylum *Fusobacteria*		
Fusobacteriia class		
Fusobacterium oral taxon A71	Griffen *et al.*, 2012	
Fusobacterium nucleatum subsp. animalis HOT 420 [*Fusobacterium animalis*]	Abusleme *et al.*, 2013	
Leptotrichiaceae [*G-1*] *sp. oral taxon 210* HOT 210	Griffen *et al.*, 2012	
Leptotrichia sp. oral taxon 498 HOT 498 [*Leptotrichia* IK040]	Griffen *et al.*, 2012	
Leptotrichia EX103	Griffen *et al.*, 2012	
Sneathia sanguinegens HOT 837	Abusleme *et al.*, 2013	
Phylum *Proteobacteria*		
Alphaproteobacteria class		
Bartonella sp.	Colombo *et al.*, 2002	
Gammaproteobacteria class		
Acinetobacter baumannii HOT 554	da Silva-Boghossian *et al.*, 2011; Souto *et al.*, 2006	da Silva-Boghossian *et al.*, 2011
Aggregatibacter sp. oral taxon 458 HOT 458 [*Aggregatibacter* AY349380]	Griffen *et al.*, 2012	
Escherichia coli HOT 574	Colombo *et al.*, 2002; Souto *et al.*, 2006	
Klebsiella pneumoniae HOT 731	Souto *et al.*, 2006	
Pseudomonas sp.	Ledder *et al.*, 2007	
Pseudomonas aeruginosa HOT 536	Souto *et al.*, 2006	
Deltaproteobacteria class		
Desulfobulbus genus	Abusleme *et al.*, 2013	
Desulfobulbos sp. oral taxon 041 HOT 041 [Clone *Desulfobulbus sp.* R004 / *Desulfobulbus* sp. oral clone R004 / *Desulfobulbos* sp. OT 041 / *Desulfobulbus* R004]	Kumar *et al.*, 2005; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Desulfobulbus oral clone CH031	Kumar *et al.*, 2005	
Epsilonproteobacteria class		
Campylobacter sputorum HOT 776	Kumar *et al.*, 2005	
Campylobacter sp. oral taxon 044 HOT 044 [*Campylobac*ter sp. oral clone BB120]	Kumar *et al.*, 2005	
Phylum *Spirochaetes*		
Spirochaetia class		
Treponema genus	Abusleme *et al.* 2013	
Treponema phylogroup II	You *et al.*, 2013a	Riep *et al.*, 2009; You *et al.*, 2013a
Treponema phylogroup III	You *et al.*, 2013a	You *et al.*, 2013a

(continued)

Table 2. (continued)

Taxa	Chronic Periodontitis Studies	Generalized Aggressive Periodontitis Studies
Treponema phylogroup V	You *et al.*, 2013a	You *et al.*, 2013a
Treponema phylogroup I:OTU 8P68	You *et al.*, 2013a	You *et al.*, 2013a
Treponema sp. oral taxon 246 HOT 246 [*Treponema II CT1*]	Griffen *et al.*, 2012	
Treponema phylogroup II:OTU 1P26	You *et al.*, 2013a	You *et al.*, 2013a
Treponema amylovorum HOT 541	Griffen *et al.*, 2012	
Treponema lecithinolyticum HOT 653	Kumar *et al.*, 2003; Griffen *et al.*, 2012; Abusleme *et al.*, 2013;	Riep *et al.*, 2009
Treponema medium HOT 667	Asai *et al.*, 2002; Kumar *et al.*, 2003; Mayanagi *et al.*, 2004; Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
Treponema vincentii HOT 029	Willis *et al.*, 1999; Asai *et al.*, 2002; Griffen *et al.*, 2012	
Treponema sp. oral taxon 230 HOT 230	Griffen *et al.*, 2012	
Treponema sp. oral taxon 490 HOT 490 [*Treponema E25-8*]	Griffen *et al.*, 2012	
Treponema E_D_05_72	Griffen *et al.*, 2012	
Treponema sp. oral taxon 237 HOT 237	Abusleme *et al.*, 2013	
Treponema maltophilum HOT 664	Abusleme *et al.*, 2013	
Treponema sp. oral taxon 257 HOT 257 [*Treponema* D36ER-1]	Abusleme *et al.*, 2013	
Treponema sp. oral taxon 249 HOT 249	Abusleme *et al.*, 2013	
Treponema sp. parvum HOT 274	Abusleme *et al.*, 2013	
Treponema sp. oral taxon 253 HOT 253	Abusleme *et al.*, 2013	
Treponema sp. oral taxon 258 HOT 258	Abusleme *et al.*, 2013	
Phylum *Synergistetes*	Vartoukian *et al.*, 2009	
Unclassified class		
Synergistetes Oral Clone A2F_22 ["*Synergistetes*" OTU 4.2 A2F_22-OTU 4.2 FJ490414]	Vartoukian *et al.*, 2009	
Synergistes oral taxon G36	Griffen *et al.*, 2012	
Fretibacterium sp. oral taxon 359 HOT 359 [*Deferribacteres* sp. oral clone BH007 / *Synergistetes* OTU 7P1]	Kumar *et al.*, 2005; You *et al.*, 2013b	
Fretibacterium sp. oral taxon 360 HOT 360 [*Deferribacteres* clone BH017 / Synergistes oral taxon 360 / *Synergistetes* OTU 7P22 / *Synergistes* [*G-3*] sp. OT 360]	Kumar *et al.*, 2003; Griffen *et al.*, 2012; You *et al.*, 2013b; Abusleme *et al.*, 2013	
Fretibacterium sp. oral taxon 361 HOT 361 [*Synergistes* [*G-3*] *sp. OT* 361]	Abusleme *et al.*, 2013	
Fretibacterium sp. oral taxon 362 HOT 362 [*Deferribacteres* clone D084 / *Synergistetes* [*G-3*] sp. OT 362 / *Synergistetes* OTU 2P9 / *Synergistetes* OTU 6P18]	Kumar *et al.*, 2003; You *et al.*, 2013b; Abusleme *et al.*, 2013	
Fretibacterium fastidiosum HOT 363 [*Deferribacteres* sp. oral clone W090 / *Synergistetes* [*G-3*] sp. OT 363 / *Synergistetes* OT 4P12]	Kumar *et al.*, 2005; You *et al.*, 2013b; Abusleme *et al.*, 2013	
Fretibacterium sp.oral taxon 453 HOT 453 [*Synergistes* OT 453]	Griffen *et al.*, 2012	
Phylum *Tenericutes*		
Mollicutes class		
Mycoplasma genus	Abusleme *et al.*, 2013	
Mycoplasma facium HOT 606	Abusleme *et al.*, 2013	
Phylum *Candidatus Saccharibacteria* (Syn. Candidate division TM7)	Brinig *et al.*, 2003; Ouverney *et al.*, 2003; Liu *et al.*, 2012	

(continued)

Table 2. (continued)

Taxa	Chronic Periodontitis Studies	Generalized Aggressive Periodontitis Studies
TM7 [*G-1*] *sp. oral taxon 346* HOT 346 [*TM7* 401H12]	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
TM7 [*G-1*] *sp. oral taxon 347* HOT 347	Griffen *et al.*, 2012	
TM7 [*G-1*] *sp. oral taxon 349* HOT 349	Griffen *et al.*, 2012; Abusleme *et al.*, 2013	
TM7 [*G-5*] genus	Abusleme *et al.*, 2013	
TM7 [*G-5*] *sp. oral taxon 356* HOT 356 [TM7 Clone I025]	Kumar *et al.*, 2003; Brinig *et al.*, 2003; Abusleme *et al.*, 2013	
Candidate division Sulphur River 1 (Candidate division SR1)		
SR1 [*G-1*] *sp. oral taxon 345* HOT 345 [OP11 clone X112 / phylotype X112]	Kumar *et al.*, 2003; Li *et al.*, 2006	
Archaea	Lepp *et al.*, 2004; Li *et al.*, 2009	Matarazzo *et al.*, 2011
Phylum *Euryarchaeota*		
Methanobacteria class		
Methanobrevibacter oralis HOT 815 [Uncultured *Methanobrevibacter* isolate mcrA-II]	Bringuier *et al.*, 2013	
Eukarya		
Fungi Kingdom	Canabarro *et al.*, 2012	

*As found in statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in periodontitis than in periodontal health.
[Brackets] indicate other nomenclatures for the species/phylotype used on the different studies.
HOT, Human Oral Taxon (designations provided in accordance with the Human Oral Microbiome Database).

Synergistetes and *Candidatus Saccharibacteria* phyla. *S. sputigena* is a normal resident of the upper respiratory tract and has been associated with a case of septicemia (McCarthy and Carlson, 1981), while *T. medium* has been detected in the human brain cortex of subjects with Alzheimer but not in healthy controls (Riviere *et al.*, 2002). Species from the *Synergistetes* phylum, such as *Synergistetes jonesii* and *Peritoneal fluid isolate RMA 16088*, have been isolated from the peritoneal fluid (Horz *et al.*, 2006). Species from the *Candidatus Saccharibacteria* phylum have been detected in vaginosis and bowel disease (Fredricks *et al.*, 2005; Kuehbacher *et al.*, 2008). The presence of microorganisms in the subgingival biofilm that are also associated with extraoral diseases may be an important link between oral and systemic infections and should be considered in further studies.

Another finding that deserves attention in the present review concerns the *Archaea* domain, which also fell into the moderate evidence category. Among the 41 studies included in this review, only 5 searched for *Archaea*, and 4 of them showed an association between this domain and periodontitis (Lepp *et al.*, 2004; Li *et al.*, 2009; Matarazzo *et al.*, 2011; Bringuier *et al.*, 2013). Although the fifth study (Vianna *et al.*, 2008) did not find statistically significant higher prevalence or counts of metanogenic *Archaea* in subjects with periodontitis in comparison with periodontally healthy subjects, this taxa was not detected in any of the healthy subjects evaluated. Hence, while the number of studies that examined *Archaea* is still modest, all of them suggested some type of association between this domain and periodontitis, and it would be important to conduct future investigations to elucidate this evidence more clearly. To date, *Archaea* has not been associated with other infections in the body.

Some of the microorganisms showing moderate evidence of being periodontal pathogens have not yet been cultivated. It was possible to detect these species due to molecular diagnostic approaches, such as polymerase chain reaction and DNA probes introduced in the late 1990s and, more recently, the open-ended polymerase chain reaction/sequencing techniques. The results of studies using these techniques have broadened our knowledge about oral cavity ecology, including the possible role of some not-yet-cultivable taxa in the etiology of periodontitis. The *Candidatus Saccharibacteria* and *Synergistetes* phyla, for example, comprise mainly uncultivated species, and many of them fell into the moderate or some evidence categories. Some of the studies using independent-culture techniques have also contributed to showing that the diversity of certain genera already associated with periodontitis, such as *Treponema*, might be greater than previously reported. It is interesting to observe that 21 species from the *Treponema* genus, other than those already recognized as periodontal pathogens, have been found in statistically significant higher levels and/or proportions and/or abundance in subjects with periodontitis in 9 studies (Table 2).

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 large number of plaque samples per patient is a crucial requirement for obtaining reliable information about the etiology of periodontitis (Haffajee and Socransky, 2006). In this regard, there is an important difference between the targeted and open-ended molecular techniques. For instance, while the open-ended *16S rDNA* pyrosequencing approaches allow an in-depth characterization of microbial diversity, these techniques are still relatively costly; therefore, the studies using pyrosequencing have

Table 3. Weight of Evidence for Newly Identified Periodontal Pathogens in the Etiology of Periodontitis

Taxa	Studies, *n*
Evidence: Moderate	
Phylum *Bacteroidetes*	
Bacteroidales [*G-2*] *sp. oral taxon 274* HOT 274 (–)[a] [Bacteroidetes clone AU126 / Phylotype AU126 / Bacteroidales OT 274]	3
Porphyromonas endodontalis HOT 273 (–)[a]	4
Phylum *Firmicutes*	
Eubacterium [*XI*] [*G-5*] *saphenum* HOT 759 (+)[a] [*Eubacterium saphenum*]	5
Mogibacterium timidum HOT 042 (+)[a]	3
Peptostreptococcus stomatis HOT 112 (+)[a] [*Peptostreptococcus* sp. oral clone CK035]	3
Filifactor alocis HOT 539 (+)[a]	5
Anaeroglobus geminatus HOT 121 (–)[a] [*Megasphaera* oral clone BB166]	3
Selenomonas sputigena HOT 151 (–)[a]	5
Enterococcus faecalis HOT 604 (+)[b]	4
Phylum *Proteobacteria*	
Desulfobulbus sp. oral taxon 041 HOT 041 [*Desulfobulbus* sp. oral clone R004 / *Desulfobulbos* sp. OT 041 / *Desulfobulbus* R004][c]	3
Phylum *Spirochaetes*	
Treponema lecithinolyticum HOT 653 (–)[a]	4
Treponema medium HOT 667 (–)[a]	5
Treponema vincentii HOT 029 (–)[a]	3
Phylum *Synergistetes*	
Fretibacterium sp. oral taxon 360 HOT 360 [*Deferribacteres* clone BH017 / Synergistes oral taxon 360 / *Synergistetes* OTU 7P22 / *Synergistes* [*G-3*] *sp. OT 360*][c]	4
Fretibacterium sp. oral taxon 362 HOT 362 [*Deferribacteres* clone D084 / *Synergistetes* [*G-3*] *sp.* OT 362 / *Synergistetes* OTU 2P9 / *Synergistetes* OTU 6P18][c]	3
Fretibacterium fastidiuosum HOT 363 (–)[a] [*Deferribacteres* sp. oral clone W090 / *Synergistetes* [*G-3*] sp. OT 363 / *Synergistetes* OT 4P12]	3
Phylum *Candidatus saccharibacteria* (Syn. Candidate division TM7)	3
TM7 [*G-5*] *sp. oral taxon 356* HOT 356 [TM7 clone I025][c]	3
Archaea domain	3
Evidence: Some	
Phylum *Bacteroidetes*	
Prevotella denticola HOT 291 (–)[a]	2
Alloprevotella tannerae HOT 466 (–)[a] [*Prevotella tannerae*]	2
Phylum *Firmicutes*	
Selenomonas genus (–)[a]	2
Johnsonella sp. oral taxon 166 HOT 166 [*Johnsonella* CK051][c]	2
Eubacterium [*X1*] [*G-3*] *brachy* HOT 557 (+)[a] [*Eubacterium brachy*]	2
Peptostreptococcaceae [*XIII*] [*G-1*] *sp. oral taxon 113* HOT 113 [*Peptoniphilus* oral taxon 113][c]	2
Lachnospiraceae [*G-8*] *sp. oral taxon 500* HOT 500 [*Lachnospiraceae* JM048][c]	2
Dialister pneumosintes HOT 736 (–)[a]	2
Phylum *Proteobacteria*	
Acinetobacter baumannii HOT 554 (–)[a]	2
Escherichia coli HOT 574 (–)[b]	2
Phylum *Spirochaetes*	
Treponema phylogroup II (–)[a]	2
Phylum *Synergistetes*	
Fretibacterium sp. oral taxon 359 HOT 359 [*Deferribacteres sp.* Oral Clone BH007 / *Synergistetes* OTU 7P1][c]	2
Phylum *Candidatus saccharibacteria* (Syn. Candidate division TM7)	
TM7 [*G-1*] *sp. oral taxon 346* HOT 346 [TM7 401H12][c]	2
TM7 [*G-1*] *sp. oral taxon 349* HOT 349[c]	2
Candidate division Sulphur River 1 (Candidate division SR1)	
SR1 [*G-1*] *sp. oral taxon 345* HOT 345 [OP11 clone X112 / Phylotype X112][c]	2

Species, phylothype, phylum, or domain found in statistically significantly higher levels and/or prevalence and/or proportion and/or abundance in periodontitis than in periodontal health in 3, 4, or 5 studies (moderate evidence) or in 2 studies (some evidence). [Brackets] indicate other nomenclatures for the species or phylotype used among the different studies.

+, Gram positive; –, Gram negative.

[a]Anaerobic.

[b]Facultative anaerobic.

[c]Species not-yet-cultivable.

evaluated a limited number of plaque samples. However, some of the target techniques, such as checkerboard DNA-DNA hybridization and RNA–oligonucleotide quantification technique, allow the evaluation of thousands of plaque samples at a relatively low cost. Specifically, one-third of the studies included in this review used open-ended diagnostic tests and evaluated approximately 230 and 630 subgingival plaque samples from periodontally healthy or periodontitis subjects, respectively, in contrast to 3,220 and 10,160 analyzed by the two-thirds of the studies using targeted approaches. Thus, the combination of open-ended and targeted methods seems to be our best option toward full understanding of the etiology and, consequently, the treatment of periodontitis. Probes or primers for the suspected new pathogens detected by the *16S rDNA* pyrosequencing studies might be developed and used on a large scale by target techniques. In an even more optimistic future perspective, the cost associated with this next-generation sequencing technology will be reduced and the processing of the data would be simplified, allowing for the sequencing of large numbers of samples.

Overall, the data of this systematic review support the notion that the subgingival pocket is a complex environment that harbors a 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 essential to emphasize that this review provides only the first evidence necessary to associate a microorganism with the etiopathogenesis of periodontitis—that is, higher levels and/or proportions of the species in cases than in controls (association studies). 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 (Socransky, 1979). In addition, further investigation into their mechanisms of pathogenicity and their ability to promote or evade host immune response would be required.

Another important idea to keep in mind while interpreting the results of association studies is the "causal versus casual" concept. The fact that a microorganism is found in higher levels and proportions in disease than in health might not be sufficient to determine whether it actually initiated the disease process or was merely favored by the inflammatory environment associated with periodontitis. In recent years, this discussion around causality/casualty has gained new momentum with the introduction of novel theories about the ecological events associated with periodontal destruction (Marsh, 2003; Socransky and Haffajee, 2005; Darveau, 2010; Hajishengallis *et al.*, 2011; Hajishengallis and Lamont, 2012). Although they differ in several aspects, a common principle of these theories is that there is a reciprocal interaction between the environment and the microbiota; specifically, environmental factors may lead to the selection or overgrowth of certain pathogens. An interesting hypothesis has suggested that certain known periodontal pathogens—termed "keystone pathogens"—that have the capacity to evade host response would be able to mediate the microbial community's conversion into dysbiosis, and a wide perturbation of this community would cause and/or sustain the process of periodontal breakdown (Hajishengallis *et al.*, 2011). Apparently, these keystone pathogens might elevate the virulence of the entire biofilm through specific interactions with accessory pathogens (Hajishengallis and Lamont, 2012). The results of the present review might serve as the initial step for the identification of new keystone or accessory pathogens, contributing to future preventive and therapeutic strategies for periodontitis.

In summary, the results of this systematic review support moderate evidence for the association of 17 species/phylotypes from the *Bacteria* domain, the *Candidatus Saccharibacteria* phylum, and the *Archaea* domain with the etiology of periodontitis. These findings would be useful to guide future investigations on the actual role of these suspected new pathogens in the onset and progression of this disease.

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