DOUTORADO EM ODONTOLOGIA

SHEYLA CHRISTINNE LIRA DA NÓBREGA

PACIENTES COM PERIODONTITE AGRESSIVA E CRÔNICA EXIBEM DIFERENÇAS ESPECÍFICAS NA COMPOSIÇÃO DA MICROBIOTA SUBGENGIVAL? UMA REVISÃO SISTEMÁTICA

Guarulhos

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Guarulhos, 28 de março de 2018.

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(Arthur Schopenhauer)

RESUMO

O sistema de classificação das doenças periodontais proposto em 1999 classificou as periodontites em dois grupos principais: crônica (PCr) e agressiva (PAg). No entanto, nenhum estudo até hoje compilou as informações disponíveis na literatura sobre possíveis diferenças microbiológicas entre essas duas condições clínicas. Portanto, o objetivo desta revisão sistemática foi determinar o peso atual da evidência para a existência de diferenças específicas entre os perfis microbianos/espécies microbianas na PCr e PAg. Esse estudo foi realizado de acordo com as normas do PRISMA. As bases de dados MEDLINE, EMBASE e Cochrane foram pesquisadas até junho de 2017 buscando estudos que comparassem dados microbiológicos de amostras de biofilme subgengival de pacientes com PCr e PAg. Para estimar o peso da evidência, foram definidas as seguintes categorias de nível de evidência, de acordo com a diferença no número de estudos que identificaram algum microrganismo em contagem/abundância/frequência estatisticamente mais altas em PCr ou em PAg: (i) evidência forte, (ii) moderada, e (iii) leve. O estudo foi registrado na base de dados PROSPERO com o número CRD42016039385. Dos 442 estudos identificados, 54 foram incluídos nesta revisão sistemática. Treze estudos encontraram o *Aggregatibacter actinomycetemcomitans* elevado em PAg em comparação com PCr, enquanto *Fusobacterium nucleatum*, *Parvimonas micra* e *Campylobacter rectus* apresentaram evidência moderada para associação com esta condição clínica. Nenhuma espécie mostrou evidência forte ou moderada para associação com PCr. O número de estudos que não observou diferença significativa entre as duas condições clínicas para as espécies bacterianas avaliadas foi sempre superior ao dos estudos que encontraram alguma diferença. Em conclusão, os resultados desta revisão sistemática sugeriram uma forte evidência para a associação de *A. actinomycetemcomitans* com PAg. No entanto, até o momento, nem esta espécie nem as outras espécies bacterianas estudadas podem diferenciar a PCr da PAg.

Palavras-chave: Doenças Periodontais, Periodontite Crônica, Periodontite agressiva, Microbiota, Bactérias.

ABSTRACT

The periodontal disease classification system proposed in 1999 classified the periodontitis into two main groups: chronic (ChP) and aggressive (AgP). However, no study to date has compiled the information available in the literature on possible microbiological differences between these two clinical conditions. Therefore, the objective of this systematic review was to determine the current weight of the evidence for the existence of specific differences between microbial / microbial species profiles in ChP and AgP. This study was performed according to PRISMA standards. The MEDLINE, EMBASE and Cochrane databases were searched until June 2017 for studies comparing microbiological data from subgingival biofilm samples from patients with ChP and AgP. In order to estimate the weight of evidence, the following categories of evidence level were defined according to the difference in the number of studies that identified a statistically significant microorganism at a statistically significantly higher ChP or frequency in ChP and AgP: (i) strong evidence, (ii) moderate, and (iii) mild. The study was registered in the database PROSPERO with the number CRD42016039385. Of the 442 studies identified, 54 were included in this systematic review. Thirteen studies found *Aggregatibacter actinomycetemcomitans* high in AgP compared to ChP while *Fusobacterium nucleatum, Parvimonas micra* and *Campylobacter rectus* presented moderate evidence for association with this clinical condition. No species showed strong or moderate evidence for association with ChP. The number of studies that did not observe a significant difference between the two clinical conditions for the bacterial species evaluated was always higher than in the studies that found some difference. In conclusion, the results of this systematic review suggested strong evidence for the association of *A. actinomycetemcomitans* with AgP. However, to date, neither this species nor the other bacterial species studied can differentiate ChP from AgP.

Keywords: Periodontal diseases, Chronic periodontitis, Aggressive periodontitis; Microbiota, Bacteria.

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LISTA DE ABREVIATURAS E SIGLAS

AAP	Academia Americana de Periodontia
PCr	Periodontite Crônica
PAg	Periodontite Agressiva
PAgL	Periodontite Agressiva Localizada
PAgG	Periodontite Agressiva Generalizada
PCrG	Periodontite Crônica Generalizada
PROSPERO	International Prospective Register Of Systematic Reviews
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-analysis*
AgP	*Aggressive Periodontitis*
ChP	*Chronic Periodontitis*
EOP	*Early Onset Periodontitis*
RPP	*Rapid Progressive Periodontitis*
y	*Years*
PD	*Probing Depth*
CAL	*Clinical Attachment Level*
AL	*Attachment Level*
BOP	*Bleending on Probing*
MEDLINE	*Medical Literature Analysis and Retrieval System Online*
PCR	*Polymerase Chain Reaction*
HOMIM	*Human Oral Microbe Identification Microarray*
ROQT	*RNA-oligonucleotide quantification technique*
mm	*Milimeters*
I	*Individually*
P	*Pool*
PI	*Plaque Index*

LISTA DE SÍMBOLOS

%	Percentual
<	Menor que
>	Maior que
≤	Menor que ou igual a
≥	Maior ou igual a
=	Igual a
±	Mais ou menos
n	Número

SUMÁRIO

1. INTRODUÇÃO

A periodontite é uma doença da cavidade oral iniciada por comunidades polimicrobianas que induzem um processo imunoinflamatório em sítios subgengivais dos elementos dentários, levando à destruição dos tecidos periodontais (LAMONT; HAJISHENGALLIS, 2015; HAJISHENGALLIS et al., 2016).

Ao longo dos anos, as doenças periodontais têm sido classificadas de diferentes formas de acordo com a idade dos pacientes acometidos, fenótipos clínicos, extensão e severidade de destruição tecidual (ARMITAGE, 1999; TONETTI; CLAFFEY, 2005). O primeiro termo usado para definir casos de doenças periodontais destrutivas de início precoce, que levavam a reabsorção óssea e mobilidade dentária, foi "periodontose" (GOTTLIEB, 1928). Em 1999, a Academia Americana de Periodontia (AAP) classificou as periodontites em dois principais tipos: crônica (PCr) e agressiva (PAg). A PCr foi classificada como de progressão lenta, mais comumente encontrada em adultos e associada a acúmulo de grande quantidade de biofilme e/ou cálculo. O termo "Periodontite Agressiva" (PAg) foi introduzido para definir um grupo de doenças periodontais destrutivas com progressão rápida e normalmente associada a pouco acúmulo de biofilme e/ou cálculo (LANG et al., 1999). A PAg foi ainda dividida em (i) localizada (PAgL), que está associada a perda de inserção em primeiros molares e incisivos, e (ii) generalizada (PAgG), não necessariamente derivada da forma localizada, mas sim uma expressão de um processo patogênico diferenciado e mais intenso que acomete pelo menos 3 outros dentes que não os molares e incisivos (TONETTI; MOMBELLI, 1999). O termo PAg abrangeu classificações prévias de periodontite, como: a de início precoce, a juvenil e a de progressão rápida (AAP, 1989). A principal diferença entre a classificação de 1999 e a anterior foi que o termo PAg passou a se basear principalmente na rapidez da progressão da doença e não na idade de início. Além da rápida progressão, a agregação familiar e saúde sistêmica também foram consideradas características importantes da PAg, seguindo conceitos já descritos três décadas antes (BAER, 1971). Ao destruir progressivamente o periodonto, a PCr e a PAg,

se não tratadas, podem promover a perda dos dentes acometidos. Os principais eventos histopatológicos em ambas as doenças são: (i) alterações inflamatórias agudas em resposta à colonização microbiana, (ii) influxo de neutrófilos em relação aos componentes microbianos do biofilme subgengival, (iii) desprendimento do epitélio juncional e a sua conversão para o epitélio da bolsa, (iv) destruição inflamatória do tecido conjuntivo adjacente ao epitélio da bolsa, (v) acúmulo de células inflamatórias crônicas, (vi) migração apical do epitélio para a raiz do dente e, (vii) reabsorção do osso alveolar.

Desde a publicação da Classificação de 1999 (ARMITAGE, 1999), pesquisadores e clínicos se dedicaram a identificar reais diferenças entre PCr e PAg (DOGAN et al., 1999; DARBY et al., 2000; XIMENEZ-FYVIE et al., 2000; FAVERI et al., 2009; ARMITAGE, 2010; ARMITAGE & CULLINAN, 2010; SMITH et al., 2010; STABHOLZ et al., 2010; DUARTE et al., 2015). Alguns estudos propuseram que o tipo da resposta do hospedeiro ao desafio bacteriano ou certas alterações genéticas (SHAPIRA et al., 1994) poderiam contribuir para as diferentes características clínicas ou na velocidade de progressão entre a PAgG, PAgL ou PCr (GARLET et al., 2003; MENG et al., 2007; HWANG et al., 2014; DUARTE et al., 2015). Smith et al. (2010), compararam a natureza do infiltrado inflamatório encontrados nos casos de PCr e PAg e não encontraram diferenças que pudessem explicar distintas velocidades de progressão entre as duas condições clínicas. Bartold et al. (2010) constataram que as lesões periodontais de pacientes com PCr e PAg são bioquimicamente semelhantes em relação aos mediadores moleculares e processos patológicos que levam à perda óssea alveolar. Similarmente, uma revisão sistemática recente sugeriu que o peso atual da evidência não é suficiente para sustentar a existência de perfis distintos de citocinas/quimiocinas em pacientes com PCr e PAg (DUARTE et al., 2015).

Muito também tem sido discutido ao longo dos anos sobre a composição da microbiota subgengival e as possíveis diferenças entre os perfis microbianos da PCr e PAg. Porém, as pesquisas mais precisas nessa área passaram por dificuldades técnicas por muito tempo relacionadas a identificação de uma microbiota mista, na sua maioria anaeróbia estrita, com alta exigência nutricional

e vivendo numa estrutura organizada do biofilme (SOCRANSKY et al., 1998; KAPLAN et al., 2009; ARMITAGE, 2010; FILOCHE et al., 2010). Vale também destacar que apenas 50-60% da microbiota subgengival da PCr e PAg podem ser cultivadas em laboratório usando técnicas de cultura de ponta. O restante da microbiota é classificada como não cultivável (WILSON et al., 1997; FAVERI et al., 2008; PASTER & DEWHIRST et al., 2009), o que dificulta ainda mais o estudo dessas espécies. Apesar de todas dificuldades acima relacionadas, a microbiota periodontal tem sido estudada desde a década de 1970. Ao longo dos anos, vários estudos sugeriram que patógenos e/ou complexos microbianos específicos poderiam estar associados ao início e a progressão de cada condição clínica (BOTERO et al., 2007; SCHACHER et al., 2007; ARMITAGE et al., 2010; HELLER et al., 2012). Proporções elevadas de *Aggregatibacter actinomycetemcomitans* e de *Porphyromonas gingivalis* foram associadas à PAg (LANG et al., 1999) e por muito tempo o *A. actinomycetemcomitans* foi considerado o único patógeno realmente relacionado com a etiologia da PAgL (HAUBEK et al., 2002; 2008; YANG et al., 2004; FINE et al., 2007). Em 2002, uma revisão sistemática (MOMBELLI et al., 2002), questionou se a presença ou ausência de cinco patógenos periodontais, *P. gingivalis, A. actinomycetemcomitans, Prevotella intermedia, T. forsythia* e *C. rectus*. Os autores concluíram que não existia evidência na literatura para afirmar a existência de diferenças específicas entre a microbiota dessas duas condições clínicas. Posteriormente, Faveri et al. (2009) e Feres et al. (2015) avaliaram pela primeira vez as proporções dos complexos bacterianos (SOCRANSKY et al., 1998) no biofilme subgengival de indivíduos com saúde periodontal, PAgL, PAgG e PCr e observaram uma grande semelhança entre os perfis microbianos dessas três condições clínicas. Considerando-se que a literatura referente a possíveis diferenças microbiológicas entre PAg e PCr ainda é controversa, e que nenhum estudo até hoje compilou toda informação científica disponível sobre esse assunto, a realização de uma revisão sistemática nesse tema seria relevante, pois o conhecimento aprofundado sobre a microbiologia das doenças periodontais pode contribuir para um melhor direcionamento das terapias para essas infecções.

2. PROPOSIÇÃO

Determinar o peso atual da evidência para a existência de diferenças específicas entre os perfis microbianos/espécies microbianas encontrados (as) na PCr e PAg.

3. ARTIGO CIENTÍFICO

Artigo científico submetido na revista *Journal of Clinical Periodontology.*

DO SUBJECTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS EXHIBIT SPECIFIC DIFFERENCES IN THE SUBGINGIVAL MICROBIAL COMPOSITION? A SYSTEMATIC REVIEW

Conflict of Interest and Sources of Funding Statement: The authors declare that there are no conflicts of interest in this study.

ABSTRACT

Aim: To determine the current weight of evidence for the existence of microbiological differences between chronic (ChP) and aggressive (AgP) periodontitis. **Methods**: A systematic review was conducted according to the PRISMA statement. The MEDLINE, EMBASE and Cochrane databases were searched up to June 2017 for studies comparing microbial data of subgingival plaque samples collected from patients with ChP and AgP. To estimate the weight of evidence, three categories were defined (strong, moderate and mild/some), according to the difference in number of studies showing microorganisms in statistically higher counts/abundance/frequency in AgP or ChP. **Results**: Of the 442 papers identified, 54 were included. The results showed strong evidence for the association of *Aggregatibacter actinomycetemcomitans* with AgP, while *Fusobacterium nucleatum, Parvimonas micra* and *Campylobacter rectus* showed moderate evidence for their association with AgP. Overall, the number of studies not showing statistically significant microbiological differences between the two clinical conditions was higher than that of studies showing differences. **Conclusion**: The results of this systematic review suggested strong evidence in the literature to support the association of *A. actinomycetemcomitans* with AgP. Nonetheless, neither this species nor the other bacterial species studied to date were unique to, or could differentiate between ChP and AgP. (PROSPERO #CRD42016039385).

Clinical Relevance

Scientific rationale for study: The 1999 classification system for periodontal diseases classified periodontitis into two main groups: chronic (ChP) and aggressive (AgP) periodontitis. However, it is still not clear if these different phenotypes are associated with different microbiological profiles. **Principal findings:** None of the individual species/microbial complexes studied to date is/are unique to, or can differentiate between ChP and AgP. **Practical implications:** The lack of evidence to support specific microbiological differences between ChP and AgP suggest that these two clinical phenotypes may not be sufficiently discriminative to guide the clinical practice, both in terms of diagnosis and treatment.

INTRODUCTION

Periodontitis is an infectious-inflammatory disease triggered by microorganisms of the oral biofilm that colonize the supragingival and subgingival tooth surface. Once tissue destruction is initiated, the disease may follow different patterns of progression, and distinctive clinical phenotypes may be observed (AAP,1989; Armitage, 1999; Flemmig, 1999; Armitage, & Cullinan, 2010).

Based on certain specific clinical traits, especially on the extent and severity of disease and on the rate of tissue destruction, the 1999 World Workshop Classification (WWC) system for periodontal diseases of the American Academy of Periodontology classified periodontitis into two main groups: chronic and aggressive (Armitage, 1999). Chronic periodontitis (ChP) was defined as a slowly progressive disease, commonly found in adults and usually associated with marked accumulation of biofilm and calculus. Conversely, aggressive periodontitis (AgP) was defined as a disease with a rapid periodontal destruction, which is not necessarily correlated with high levels of biofilm and calculus (Armitage, 1999; Armitage, & Cullinan, 2010; Duarte et al., 2015).

Although this classification system has been widely used in the periodontal field, clear differences between AgP and ChP have never been established. The two main biological mechanisms hypothesized by different groups of investigators to explain the occurrence of these distinct phenotypes were (i) different patterns of immune-inflammatory response to the microbial challenge or (ii) different microbial profiles. A wide range of studies have been published in the past

decades aiming to investigate specific differences between AgP and ChP as regards these two mechanisms (Darby, Hodge, Riggio, & Kinane, 2000; Dogan et al., 2003; Ximenez-Fyvie et al., 2006; Faveri et al., 2009; Armitage, 2010; Armitage, & Cullinan, 2010; Ford, Gamonal & Seymour, 2010; Smith, Seymour, & Cullinan, 2010; Stabholz, Soskolne & Shapira, 2010; Duarte et al., 2015).

Regarding host response, a recent systematic review has reported that the current weight of evidence is not sufficient to support the existence of distinct cytokine profiles for patients with AgP and ChP (Duarte et al., 2015). In terms of microbiology, a previous systematic review (Mombelli, Casagni, & Madianos, 2002) assessing five periodontal pathogens suggested that the presence or absence of these microorganisms could not distinguish between subjects with ChP and AgP. Nonetheless, no previous systematic review to date has compiled the results of studies comparing the microbiota of ChP and AgP, and this knowledge is important for decision-making about periodontal treatment and prevention. Thus, the aim of this systematic review was to determine the current weight of evidence for the existence of specific differences between the composition of the subgingival microbiota of subjects diagnosed with AgP and ChP.

METHODS

This systematic review was registered at the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Reviews (registration number: CRD42016039385) and conducted according to

the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement (Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009).

Focused question

What is the weight of evidence for the existence of specific differences in the composition of the subgingival microbiota of ChP and AgP?

Inclusion criteria

The manuscripts meeting the following criteria were included:

- Studies of any design that compared microbial data of subgingival biofilm samples collected from patients with AgP and ChP.

Exclusion criteria

- Studies published in languages other than English, Spanish or Portuguese.
- Lack of a direct comparison of baseline microbial data between AgP and ChP.
- Case reports, case series and reviews.
- Studies evaluating only localized aggressive periodontitis.

Search strategy and data extraction

The MEDLINE, EMBASE and Cochrane databases were searched up to June 2017 by 2 independent reviewers (M.Fa. and L.C.F.), using Mesh terms and other keywords (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 (S.C.L.M. and B.B-S.) and any disagreement was solved through discussion. If disagreement persisted, another

researcher was consulted to achieve consensus (M.Fe.). The studies that fulfilled the inclusion and exclusion criteria were processed for data extraction, conducted by another 2 independent researchers (B.R-V. and P.M.D.).

To estimate the weight of evidence for microorganisms associated with AgP or ChP, the following categories were defined, according to the difference in number of studies showing specific microorganisms in statistically higher counts/abundance/frequency in AgP or ChP: (i) Strong evidence: difference of > 5 studies, (ii) Moderate evidence: difference 3-5 studies, and (iii) Mild/Some evidence: difference of 2 studies (Perez-Chaparro et al., 2014).

RESULTS

Figure 1 presents the flow chart for the selection of the papers. The electronic search strategy provided 442 titles. After title screening and abstract reading, 349 studies were excluded and 93 full-text publications were comprehensively evaluated. Of these 93 papers, 39 studies were excluded because they did not match the inclusion/exclusion criteria or had unclear information (Appendix Table 2). Therefore, 54 studies were included in this systematic review.

Methodological features of the studies included and demographic characteristics of the population evaluated.

Table 1 presents the main methodological characteristics of the studies included, and mean age of the populations evaluated. The majority of studies included were cross-sectional, while four were retrospective (Kowalski, & Gorska, 2014;

Lourenco et al., 2014; Wang et al., 2014; Topcuoglu, & Kulekci, 2015). Patients from the majority of studies were selected at universities, hospitals or dental schools. Two studies reported patients from private practices (Rams, Feik, Listgarten, & Slots, 1992; Lanza, Magan-Fernandez, Bermejo, Marfil-Alvarez, & Mesa, 2016) and six (Kojima, Yano, & Ishikawa, 1997; Yano-Higuchi, Takamatsu, He, Umeda, & Ishikawa, 2000; Takeuchi et al., 2001; Suda, Lai, Yang, & Hasegawa, 2002; Takeuchi, Umeda, Ishizuka, Huang, & Ishikawa, 2003; Nagpal, Prakash, Bhat, & Singh, 2016) did not mention the site of study.

In general, the studies included in this review evaluated more samples from ChP than from AgP subjects. Only ten studies evaluated more samples from AgP (Dogan et al., 2003; Takeuchi, Umeda, Ishizuka, Huang, & Ishikawa, 2003; Picolos, Lerche-Sehm, Abron, Fine, & Papapanou, 2005; Wang et al., 2005; Nibali et al., 2009; Schlafer et al., 2010; Hwang, Stoupel, Celenti, Demmer, & Papapanou, 2014; Schmidt, Jentsch, Stingu, & Sack, 2014; Chahboun, Arnau, Herrera, Sanz, & Ennibi, 2015; Topcuoglu, & Kulekci, 2015). A total of 5,988 subjects (AgP = 1,901 and ChP = 4,087) and 20,476 subgingival biofilm samples were evaluated (AgP = 7,555 and ChP = 12,921). The samples were analyzed individually in 24 studies, and pooled in 28 studies. One study used individual and pooled analysis (Schacher et al., 2007) and one study did not mention the sampling method/analysis used (Casarin et al., 2012) (Table 1).

Ten studies used culture as the main microbial diagnostic test (Rams, Feik, Listgarten, & Slots, 1992; Yano-Higuchi, Takamatsu, He, Umeda, & Ishikawa, 2000; Dogan et al., 2003; Takeuchi, Umeda, Ishizuka, Huang, & Ishikawa, 2003;

Gajardo et al., 2005; Botero et al., 2007a; Botero, Parra, Jaramillo, & Contreras, 2007b; Lafaurie et al., 2007; Dogan, Kipalev, Okte, Sultan, & Asikainen, 2008; Chahboun, Arnau, Herrera, Sanz, & Ennibi, 2015) and one used an open-ended technique (pyrosequencing) (Li et al., 2015). All the other studies used targeted techniques, such as Checkerboard DNA-DNA hybridization, real time PCR, Oligonucleotide DNA-DNA hybridization, Human Oral Microbe Identification Microarray (HOMIM), RNA-oligonucleotide quantification technique (ROQT) (Table 1).

The mean age of the AgP groups analyzed in the various studies ranged from 17.5±6.8 years (Yang, Huang, Chan, & Chou, 2005) to 37.4±10.0 years (Hwang, Stoupel, Celenti, Demmer, & Papapanou, 2014), and of the ChP groups from 30.2±3.9 years (Yang, Huang, Chan, & Chou, 2005) to 55.13±7.46 years (Thiha et al., 2007). 77.7% of the studies showed mean age above 40 years of age for the ChP group and 42.5% below 30 years of age for the AgP group.

Most of the studies evaluated systemically healthy individuals. Four studies did not mention if the participants were systemically healthy (Cortelli, Cortelli, Jordan, Haraszthy, & Zambon, 2005; Yang, Huang, Chan, & Chou, 2005; Suda, Lai, Yang, & Hasegawa, 2002; Topcuoglu, & Kulekci, 2015) and one study included subjects with type 2 diabetes in the ChP group (Casarin et al., 2012) (data not shown).

The inclusion criteria for AgP and ChP and demographic characteristics of the participants from the studies included (% smokers and gender) are presented in

Appendix Table 3. Of the studies, 27.7% had smokers in the AgP group, and 33.3% had smokers in the ChP group; 51.8% of the studies used age as an inclusion criterion for AgP and ChP.

Clinical data

Mean full-mouth Probing Depth (PD), Clinical Attachment Level (CAL), plaque index and the percentage of sites showing bleeding on probing are presented in Appendix Table 4. Nine studies did not report clinical data (Rams, Feik, Listgarten, & Slots, 1992; Kojima, Yano, & Ishikawa, 1997; Suda, Lai, Yang, & Hasegawa, 2002; Dogan et al., 2003; Yang, Huang, Chan, & Chou, 2005; Bilichodmath et al., 2009; Benrachadi, Bouziane, Azziman, Bouziane-Ouartini, & Ennibi, 2012; Das, Krithiga, & Gopalakrishnan, 2012; Lourenco et al., 2014). Overall, the mean clinical values presented were compatible with advanced disease. Only ten studies reported full-mouth mean PD and four studies mean CAL below 3.5 mm.

Microbiological data

The microorganisms found in statistically significantly higher counts/abundance/frequency in AgP or ChP are presented in Appendix Table 5. Fifty-two taxa (49 bacterial species, 2 bacterial genera and 3 viruses) differed significantly between AgP and ChP in at least one study. *A. actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Campylobacter rectus, Eubacterium nodatum, F. nucleatum, Parvimonas micra, Prevotella intermedia, Eikenella corrodens, Actinomyces gerencseriae, Actinomyces israelii, Actinomyces naeslundii 1, Eubacterium saburreum,*

Gemella morbillorum, Treponema socranskii, Streptococcus mutans, Treponema lecithinolyticum, Pseudomonas aeruginosa, Neisseria elongata, TM7, Selenomomas sputigena, Filifactor Alocis, Lactobacillus acidophilus, Prevotella disiens, Prevotella denticola, Anaerococcus prevotii, Prevotella oralis and *Pseudoramibacter alactolyticus* were elevated in a higher number of studies in AgP than in ChP. On the other hand, *Treponema denticola*, *Prevotella nigrescens, Staphylococcus constelattus*, *Capnocytophaga ochracea, Streptococcus gordonii, Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus oralis, Streptococcus sanguinis, Actinomyces odontolyticus*, *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Dialister pneumosintes* and *HCMV* were elevated in a higher number of studies in ChP than in AgP.

Table 2 summarizes the weight of evidence for specific bacterial species associated with either AgP or ChP based on the criteria defined in the study of Perez-Chaparro et al., (2014). Thirteen studies found *A. actinomycetemcomitans* elevated in AgP in comparison with ChP, while *F. nucleatum, P. micra* and *Campylobacter rectus* were elevated in AgP in 3, 3 and 5 studies, respectively. None of these species were elevated to a statistically significant level in ChP. *E. nodatum, P. intermedia, E. corrodens, A. gerencseriae, A. israelii, T. socranskii, Selenomomas sputigena* and *T. forsythia* showed some association with AgP. No taxa presented strong or moderate evidence for a specific association with ChP; only mild evidence was observed for the following bacterial species in ChP: *Prevotella nigrescens*, *Capnocytophaga ochracea, Streptococcus gordonii, Streptococcus oralis, Staphylococcus aureus* and *Human cytomegalovirus.*

DISCUSSION

This is the first systematic review to assess the current weight of evidence for the existence of specific differences in composition of the subgingival microbiota of subjects with AgP and ChP. The results indicated that the only microorganism that showed a specific association with one of the clinical conditions evaluated, with strong evidence, was *A. actinomycetemcomitans* with AgP (Table 2).

The parameter used in this study to categorize strong, moderate or some/mild evidence of the association of a microorganism with one of the clinical conditions was that described by Perez-Chaparro et al., (2014). The categories were defined according to the difference in the number of studies showing a microorganism in higher levels and/or prevalence and/or proportion with statistical significance, in AgP or in ChP as follows: (i) Strong evidence: difference of >5 studies, (ii) Moderate evidence: difference of 3, 4 or 5 studies, and (iii) Mild/Some evidence: difference of 2 studies.

Thirteen studies found *A. actinomycetemcomitans* elevated in AgP, while no study showed this species elevated in ChP. These data supported the notion that *A. actinomycetemcomitans* is an important pathogen in the etiology of AgP, which has been suggested by several previous investigations (Zambon, Christersson, & Slots, 1983; Haraszthy et al., 2000; Haubek, Ennibi, Abdellaoui, Benzarti, & Poulsen, 2002; Yang, Huang, Chan, & Chou, 2005; Faveri et al., 2009; Armitage, 2010). Nonetheless, it was difficult to assure that *A. actinomycetemcomitans* was unique to AgP, since this microorganism was also frequently detected in ChP,

and 23 studies did not show statistically significant differences in the levels and/or prevalence and/or proportion of this microorganism between AgP and ChP.

Three bacterial species, *F. nucleatum, P. micra* and *C. rectus* showed moderate evidence of their association with AgP. Mild evidence was observed for the association of another eight species with AgP, and of four bacterial species and one virus with ChP. Nonetheless, in most cases the number of studies showing no difference was higher than those studies showing a difference. The case of *C. rectus* is illustrative. Although five studies found this species elevated in AgP, another 15 failed to show this association. Thus, the fact that the evidence for the above-mentioned species was rated as mild or, at most, moderate, and that a high number of studies failed to show a difference in their detection between subjects with AgP and ChP, reduced the impact of these associations.

An important piece of information to bear in mind refers to the inclusion criteria used by the different authors; 59.25% of the studies in this review (32 out of 54) included patients based on the WWC criteria (Armitage, 1999). Nonetheless, it is worth noting that the inclusion criteria described in the various studies were overall very heterogeneous and in most cases, did not actually follow the criteria established by the WWC (Appendix Table 3). This lack of standardization probably happened due to some difficulties associated with the use of such classification system, especially when trying to characterize AgP. The three main features of AgP, i.e., otherwise clinically healthy patients, familial aggregation and rapid attachment loss are not always easy to assess, or not sufficiently discriminative between ChP and AgP. Determining the rate of attachment loss,

for example, is not always feasible in private practice, or while selecting patients for cross-sectional studies. Alternatively, clinicians, professors and researchers have been using age as the main diagnostic criterion for AgP, in an attempt to estimate rapid attachment loss by setting the onset of disease in an early age. Actually, 51.8% of the 54 studies included in this review used age as an inclusion criterion for AgP and/or ChP, and the majority of them selected AgP subjects under the age of 35 years, and ChP, over the age of 35 years. In only one study, was the mean age of the AgP group above 35 years (=37.4 years) (Hwang, Stoupel, Celenti, Demmer, & Papapanou, 2014). Thus, one could hypothesize that the slight differences observed between the composition of AgP and ChP in the present study could be due to the difference in age, which may influence the ecology of the oral cavity, more specifically the composition of the subgingival biofilm. In fact, previous studies have suggested that the prevalence/proportion of *A. actinomycetemcomitans* may decrease with increasing age (Rodenburg et al., 1990; Faveri et al., 2009). In a recent study, we were able to distinguish between AgP and ChP by using a mathematical model and a panel of 40 bacterial species (Feres et al., 2017). The mean age was significantly higher in the ChP (45.1±5.9) than in the AgP group (27.1 ± 3.1), and all 40 bacterial species were found in both clinical groups. Thus, we hypothesized that the statistical/microbiological model tested in that study was probably more suitable to differentiate between advanced periodontitis in adults and in young individuals than between ChP and AgP as two different diseases (Feres et al., 2017).

While high levels/proportions of red complex species have been previously detected in young subjects with AgP (Takeuchi, Umeda, Ishizuka, Huang, &

Ishikawa, 2003; Kamma, Nakou, Gmur, & Baehni, 2004; Gajardo et al., 2005; Ximenez-Fyvie et al., 2006; Botero et al., 2007a; Fritschi, Albert-Kiszely, & Persson, 2008; Faveri et al., 2009; Mestnik et al., 2010; Tomita et al., 2013; Chahboun, Arnau, Herrera, Sanz, & Ennibi, 2015) one would have expected to see higher levels and proportions of these pathogens in older patients with ChP, which was not the case. *P. gingivalis*, for example, which was the most extensively studied periodontal pathogen (39 of the 54 included studies evaluated this species), showed no particular association with ChP when compared with AgP. In accordance with these data, a previous systematic review (Mombelli, Casagni, & Madianos, 2002) suggested that the presence or absence of five periodontal pathogens, including *P. gingivalis, A. actinomycetemcomitans, Prevotella intermedia, T. forsythia and C. rectus,* could not distinguish between AgP and ChP. Likewise, Faveri et al., (2009) and Feres, Figueiredo, Soares, & Faveri, (2015) evaluated the composition of the subgingival biofilm of individuals with periodontal health, AgP (localized and generalized) and ChP and observed a great similarity between the microbial profiles of these three clinical conditions.

Although beyond the main scope of this paper, it is worth mentioning that the data here presented supported the notion that the subgingival pocket is a complex environment that harbors a highly diverse microbiota. Independently of the clinical condition being studied, the age of the patients or the microbiological test used, a variety of periodontal pathogens were always detected. The majority of the studies included in this review used targeted diagnostic methods, such as Checkerboard DNA-DNA hybridization, real time PCR, ROQT and

immunofluorescence. Only one study (Li et al., 2015) used high-throughput sequencing technique to compare the subgingival microbiota of AgP and ChP and the results were in agreement with the data obtained with the other techniques. Among all the genera/species detected in the cited study, only *P. gingivalis* and red complex species were in higher abundance in AgP than in ChP. The overall evaluation of the studies that assessed the microbiota of ChP (Griffen et al., 2012; Liu et al., 2012; Abusleme et al., 2013; Galimanas et al., 2014; Perez-Chaparro et al., 2014; Camelo-Castillo et al., 2015; Park et al., 2015, Perez-Chaparro et al., 2017) or AgP (Laksmana et al., 2012; Han, Wang, & Ge, 2017) using sequencing techniques, also suggested a lack of striking differences between these clinical conditions.

Knowledge about the microbiota associated with infectious disease is important to establish effective therapeutic strategies. The lack of specific differences between the subgingival microbiota of AgP and ChP revealed by the data of the present study suggest that the treatment of these clinical conditions may not differ substantially. Similarly, a recent systematic review indicated that the current weight of evidence was not sufficient to support the existence of distinct cytokine/chemokine profiles in patients with these clinical conditions (Duarte et al., 2015). Taken together, these data suggested that AgP and ChP may not be different diseases, but variations of a single disease, as recently proposed (Tonetti, Greenwell, & Kornman, 2018).

The main limitation of this study relates to the lack of standardization of some methodological features of the studies included, such as differences in the microbiological diagnostic tests used, the way the data were expressed, the

number of subjects included per group and the inclusion criteria used to select patients with AgP and ChP. Despite these divergences, the studies included in this review compiled data of 5,988 subjects and 20,476 subgingival biofilm samples, which allowed a comprehensive evaluation of the available literature regarding possible microbiological differences between AgP and ChP.

In conclusion, the results of this systematic review suggested that there was strong evidence in the literature to support the association of *A. actinomycetemcomitans* with AgP. Nonetheless, neither this species nor the other bacterial species studied to date were unique to, or could differentiate between ChP and AgP.

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Figure 1. Flow chart of studies selection.

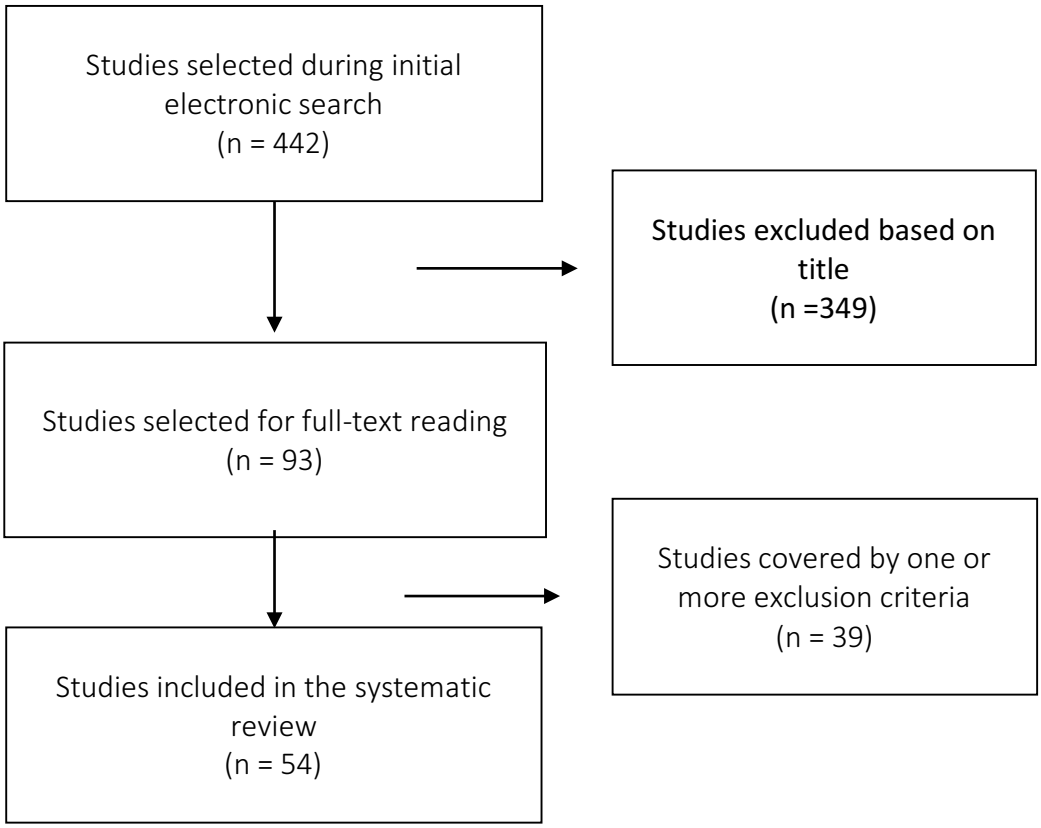

Table 1. Main methodological characteristics of the studies included, and mean age of the populations evaluated.

Study (Country/Year)	N subjects/N samples (P - I) Mean Age		Method	Target microorganism
	AgP	ChP		
Rams et al., (USA, 1992)	127/127(P) ND	907/907(P) ND	Culture	*P. micra*
Kojima et al., (Japan, 1997)	8/109.5 ± 5.2(I) 29.5 ± 5.9	15/97.6 ± 10(I) 53.8 ± 8.0	DNA analysis	*P. gingivalis*
Darby et al. (Scotland, 2000)	24/96(I) 33.2 ± 3.4	33/132(I) 46.6 ± 7.1	PCR	*P. gingivalis, P. intermedia, T. forsythia, A. actinomycetemcomitans*[A] and *T. denticola*
Yano-Higuchi et al. (Japan, 2000)	8/32(I) 31.3/(23-35)	21/84(I) 50.4/(41-62)	Culture, BANA test and oligonucleotide DNA-DNA hybridization	*T. forsythia, P. gingivalis* and *A. actinomycetemcomitans*
Takeuchi et al. (Japan, 2001)	38/152(I) 26.7 ± 6.2	65/260(I) 51.8 ± 8.4	PCR	*P. gingivalis, T. denticola* and *T. socranskii*
Suda et al. (Japan, 2002)	37/37(P) 35 ± 8.4	136/136(P) 51.1 ± 12.6	Anti-serum	*E. corrodens*
Doğan et al. (Turkey 2003)	17/17(P) 31 ± 5	14/14(P) 43.9 ± 6	Culture and PCR	*P. gingivalis, A. actinomycetemcomitans, P. intermedia, P. nigrescens, T. forsythia, P. micra* and *C. rectus*

Takeuchi, et al. (Japan, 2003)	40/160(I) 28 ± 4.4	35/140(I) 51.8 ± 7.2	Culture and PCR	*T. forsythia, A. actinomycetemcomitans, C. rectus, P. gingivalis, P. intermedia, P. nigrescens* and *T. denticola*
Gajardo, et al. (Chile, 2005)	6/6(P) 29.5 ± 6.1	17/17(P) 47.2 ± 7.4	Culture	*A. actinomycetemcomitans, P. gingivalis, P. intermedia, P. nigrescens, E. corrodens, F. nucleatum, Capnocytophaga sp., C. rectus*[A] and *P. micra*
Yang et al. (Taiwan, 2005)	70/70(P) 17.5 ± 6.8	101/101(P) 30.2 ± 3.9	Indirect immunofluorescence assay	*A. actinomycetemcomitans*[A]
Wang et al. (Japan, 2005)	46/184(I) 27 ± 5.8	28/112(I) 54.9 ± 6.8	Real-time PCR	*A. actinomycetemcomitans*
Cortelli et al. (Brazil, 2005)	25/25(P) 21.9	178/178(P) 39.1	PCR Prevalence in subjects	*A. actinomycetemcomitans*[A], *C. rectus*[A], *P. gingivalis, P. intermedia* and *T. forsythia*
Picolos et al. (USA, 2005)	19/57(I) 34.5 ± 12.4	12/36(I) 41.9 ± 10.1	Checkerboard DNA-DNA hybridization	*A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola F. nucleatum, P. intermedia, P. nigrescens, C. rectus, E. nodatum, S. intermedius, P. micra, E. corrodens, C. ochracea, V. parvula* and *A naeslundii*
Ximenez-Fyvie et al. (Mexico, 2006)	19/25.6 media per patient(I) 21.5 ± 1.2	39/25.6 media per patient(I) 48.3 ±1.7	Checkerboard DNA-DNA hybridization	40 bacterial species
Botero et al. (Colombia, 2007a)	12/12(P) 22.2 ± 5.7	68/12(P) 42.8 ± 9.3	Culture, biochemical tests and PCR	*S. maltophilia, A. lwoffi, A. baumannii, Pseudomonas spp., P. putida, P. aeruginosa, C. freundii, S. liquefaciens, E. aerogenes, E.*

				gergoviae, E. cloacae, P. gingivalis[A], *T. forsythia, P. nigrescens*[C], *P. intermedia, Fusobacterium spp, P. micra , Campylobacter spp, Eubacterium spp, A. actinomycetemcomitans, E. corrodens*[A], *D. pneumosintes, E. rods* and *K pneumoniae*[C]
Lafaurie et al. (Colombia, 2007)	158/158(P) 28 ± 6.8	325/325(P) 45.6 ± 10.6	PCR and culture	*P. gingivalis, C. rectus*[A], *T. forsythia, E. corrodens, A. actinomycetemcomitans*[A], *P. intermedia*[A], *P. nigrescens* and *E. rods*
Schacher et al. (Germany, 2007)	30/480(I) and 30(P) 30.3 ± 6.9	30/480(I) and 30(P) 50.1 ± 7.8	Real-time PCR	*A. actinomycetemcomitans*[A], *P. gingivalis*[C], *T. forsythia*[C] and *T. denticola*[C]
Thiha et al. (Japan, 2007)	16/16(I) 35 ± 8.2	32/32(I) 55.1 ± 7.4	Real-time PCR	*P. gingivalis, A. actinomycetemcomitans* and *T. forsythia*
Botero et al. (Colombia, 2007b)	6/6(P) 24.3 ± 6.3	20/20(P) 44 ± 12	PCR and culture	*HCMV, A. actinomycetemcomitans, P. gingivalis, P. intermedia, P. nigrescens, T. forsythia, P. micra, E. corrodens, Campylobacter spp., Eubacterium spp., Fusobacterium spp., Capnocytophaga spp.,* and *D. pneumosintes*
Doğan et al. (Turkey, 2008)	8/8(P) 26.5 ± 6.6	8/8(P) 47.4 ± 9.2	Culture	*A. actinomycetemcomitans*
Fritschi et al. (Switzerland, 2008)	22/88(I) ND	84/336(I) ND	Checkerboard DNA-DNA hybridization	40 bacterial species *(P. micra*[A], *P. intermedia*[A], *P. nigrescens*[A], *A. israelii*[A], *T. socranskii*[A], *S. aureus*[A] *and S. mutans*[A]*)*
Yamabe et al. (Japan, 2008)	17/46(I) ND	32/65(I) ND	PCR and Real-time PCR	*M. oralis DSM 7256* and *M. smithii DSM 861*
Imbronito et al.	30/30(P)	30/30(P)	PCR	*HSV-1*[A], *EBV-1, HCMV, A.*

(Brazil, 2008)	27.3 ± 4.8	42.7 ± 6.7		*actinomycetemcomitans*[A], *P. gingivalis, P. intermedia* and *T. forsythia*
Bilichodmath et al. (India, 2009))	14/14(P) 25 ± 3.1	19/19(P) 43 ± 7.3	PCR	*HSV-1*[C], *HSV-2, EBV*[C] and *HCMV*[C]
Riep et al. (Germany, 2009)	44/220(I) 34.4	46/230(I) 55.2	PCR	*A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, Treponema group III, T. lecithinolyticum, C. rectus, C. ochracea, Fusobacterium spp.,* and *F. nucleatum*
Faveri et al. (Brazil, 2009)	25/225(I) 25.2 ±3.2	30/270(I) 42 ± 6.2	Checkerboard DNA-DNA hybridization	38 bacterial species (*P. gingivalis*[A], *F. nucleatum nucleatum*[A], *A naeslundii1*[C])
Nibali et al. (England, 2009)	64/64(P) 33.5 ± 5.1	20/20(P) 43.4 ± 11.4	Real-time PCR	*EBV*[C] and *HCMV*
Schlafer et al. (Germany, 2010)	72/330(I) 34.8 ± 6.4	30/78(I) 51 ± 10.2	PCR	*P. gingivalis, P. intermedia, A. actinomycetemcomitans, T. denticola, T. forsythia, F. nucleatum* and *F alocis*
Rescala et al. (Brazil, 2010)	17/34(I) 29.2 ± 6.6	20/40(I) 48.6 ± 7.5	Checkerboard DNA-DNA hybridization	40 bacterial species
Drescher et al. (Germay, 2010)	62/303(I) 34.2 ± 6.2	82/357(I) 54.4 ± 12.1	Dot-blot hybridization, FISH, and electron microscopy	*Selenomonas, P. gingivalis, P. intermedia, T. denticola, T. forsythia,* and *F. nucleatum nucleatum*[A], *S. sputigena*[A], *S. noxia*[A]
Da Silva-Boghossian et al. (Brazil, 2011)	90/1260(I) 31.4 ± 0.6	219/3066(I) 45.4 ± 0.7	Checkerboard DNA-DNA hybridization	*P. gingivalis, T. forsythia, T. denticola, A.actinomycetemcomitans, A. baumannii, E. coli, E. faecalis , P. aeruginosa* and *S. aureus*[C]
Das et al. (India, 2012)	25/25(P) ND	25/25(P) ND	PCR	*HSV-1, HSV2, EBV* and *HCMV*
Casarin et al. (Brazil, 2012)	48 27.6 ± 0.9	89(39 diabetic) 43.6 ± 8.3	PCR	*M. timidum*

Heller et al. (Brazil, 2012)	75/525(I) 30.2 ± 4.8	185/1295(I) 45.6 ± 9.3	Checkerboard DNA-DNA hybridization	46 bacterial species *(E. nodatum*[A]*, A. gerencseriae*[A]*, A. israelii*[A]*, S. aureus*[C]*)*
Shaker et al. (Egypt, 2012)	25/25(P) 27.5 ± 3.7	25/25(P) 40.2 ± 2.6	PCR	*A actinomycetemcomitans*[A]*, T. forsythia, P. gingivalis*[C]*, Treponema denticola*[C] and *P. intermedia*[C]
Benrachadi et al. (Morocco, 2012)	8/8(P) 35 ± 10.3	15/15(P) 39.4 ± 7.3	PCR	*P. gingivalis, P. intermedia, T. forsythia, T. denticola,* and *A. Actinomycetemcomitans*
Tomita et al. (Japan, 2013)	20/20(I) 33.3 ± 8.1	20/20(I) 43.6 ± 11.1	Real-time PCR	*A. actinomycetemcomitans, P. gingivalis* and *T. forsythia*[C]
Silva-Boghossian et al. (Brazil, 2013)	66/66(P) 31.4 ± 0.7	156/156(P) 45.9 ± 0.9	Checkerboard DNA-DNA hybridization	44 bacterial species *(T. forsythia*[C]*, C. showae*[C]*, E. nodatum*[A]*, P. nigrescens*[C]*, S. constellatus*[C]*, A. actinomycetemcomitans*[A]*, C. sputigena*[C]*, S. gordonii*[C]*, S. oralis*[C]*, S. mitis*[C]*, S. sanguinIs*[C]*, S. intermedius*[C]*, V parvula*[C]*, A. naeslundii*[A]*, L. buccalis*[C]*, S aureus*[C] *and D. pneumosintes*[C]*)*
Silveira et al. (Brazil, 2013)	35/35(P) 33.9 ± 7.1	41/41(P) 44.1 ± 9.4	PCR and Real-time PCR	*A. actinomycetemcomitans*[A]
Kowalski and Gorska (Poland, 2014)	17/17(P) 30.3(23–37)	23/23(P) 44.7(37-60)	Real-time PCR	*A. actinomycetemcomitans*[A]*, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micra, F. nucleatum, C. rectus*[A]*,* and *E. corrodens*
Wang et al. (China, 2014)	10/105(I) 29.7 ± 2.1	10/79(I) 32.5 ± 1.8	Real-time PCR	*A. actinomycetemcomitans*

Haririan et al. (Austria, 2014)	33/132(P) 34.2 ± 6.2	43/172(P) 48.3 ± 6.5	PCR with microarray technique	*A. actinomycetemcomitans, Capnocytophaga spp., P. gingivalis, T. denticola, T. forsythia, A. viscosus, C. rectus, C showae, E. corrodens, E. nodatum, F. nucleatum, P. intermedia, P. micra, S. mitis, V. parvula, A. odontolyticus, P. nigrescens, S. gordonii group, S. constellatus group, C. concisus* and *C. gracilis*
Belibasakis et al. (Turkey, 2014)	20/20(I) 33.9 ± 5.2	22/22(I) 44.1 ± 7.7	Real-time PCR	*P. gingivalis, T. denticola, T. forsythia,* and *A. actinomycetemcomitans*
Hwang et al. (Colombia, 2014)	37/329(I) 37.4 ± 10	27/240(I) 50.5 ± 10.8	Checkerboard DNA–DNA hybridization and serum immunoglobulin (Ig) G	*A. actinomycetemcomitans*[A], *P. gingivalis*[A], *T. denticola , T. forsythia, P. micra, C. rectus, P. intermedia , F. nucleatum, A. naeslundii, V. parvula,* and *E. corrodens*
Schmidt et al. (Germany, 2014)	15/15(P) 32.1 ± 7.1	11/11(P) 45.2 ± 8.0	PCR	51 bacterial species (*P. gingivalis*[C] *and P. intermedia*[C]*)*
Lourenço et al. (Brazil, 2014)	24/24(P) ND	35/35(P) ND	HOMIM	>250 species/phylotypes *(N. elongata*[C]*, A. actinomycetemcomitans*[A]*, and P. intermedia*[A])
Topcuoglu and Kulekci (Turkey, 2015)	29/29(P) 34 ± 8	25/25(P) 47 ± 9	Microarray	*P. gingivalis, T. forsythia, T. denticola, C. rectus, F. nucleatum, P. micra, P. intermedia; A. actinomycetemcomitans, E. corrodens* and *A. viscosus*
Chahboun et al. (Morocco, 2015)	37/37(P) 24.4 ± 5.0	20/20(P) 28.5 ± 4.3	Culture	*A. actinomycetemcomitans*[A]*, T. forsythia, P. gingivalis, P. intermedia, P. nigrescens, P. micra, C. rectus, E. corrodens. Eubacterium spp., Capnocytophaga spp.,* and *F. nucleatum*
Li et al. (China, 2015)	10/10(P) 22.1 ± 4.9	10/10(P) 46.6 ± 7.4	PCR and pyrosequencing	*36 bacterial species (T. forsythia*[A]*, P. gingivalis*[A] *and T. denticola*[A]*)*

Vieira-Colombo et al. (Brazil, 2016)	36/36(P) 33.0 ± 4.1	98/98(P) 44.9 ± 11.4	Checkerboard DNA–DNA hybridization	*E. faecalis, E. saphenum, F. alocis, G. vaginalis, H. alvei, N. gonorrhoeae F. necrophorum, L. acidophilus, H. influenzae, S. aureus and O. uli, P. aeruginosa, S. marcescens, A baumannii, B fragillis, C albicans, C difficile, D pneumosintes, Enterobacteria, S. pneumoniae, H aprophilus, Neisseria spp, P anaerobius, S enteric ss enterica sorv Typhi* and *S liquefanciens*
Nagpal et al. (India, 2016)	30/30(P) ND	30/30(P) ND	PCR	*S. sputigena*
Oliveira et al. (Brazil, 2016)	30/270(I) 26.3 ± 3.5	30/270(I) 42.0 ± 5.7	RNA-oligonucleotide quantification technique	*A. geminatus, Bacteroidales sp., Desulfobulbus sp., E. faecalis, E. saphenum, F. alocis*[A]*, F. fastidiosum, Fretibacterium sp., Fretibacterium sp.*[C]*, M. timidum, P. stomatis, P. endodontalis, P. gingivalis ,P. denticola, S. sputigena*[A]*, T. forsythia, TM7 sp.*[A]*, T. lecithinolyticum, T. medium* and *T. vincentii*
Lanza et al. (Spain, 2016)	60/60(P) 30.4 ± 7.1	123/123(P) 50.9 ± 9.4	PCR	*P. gingivalis, T. forsythia*[A]*, T. denticola, P. intermedia*[A] *and A. actinomycetemcomitans*
Kumawat et al. (India, 2016)	30/30(I) 21.2 ± 3.2	30/30(I) 37.5 ± 3.2	PCR	*P. gingivalis* and *T. denticola*

I: Individually; P: Pooled; PCR: Polymerase Chain Reaction; FISH: Fluorescence In Situ Hybridization; HOMIM: Human Oral Microbe Identification Microarray; - : Not Described; A: statistically significant difference for AgP; C: statistically significant difference for ChP.

Table 2. Weight of evidence of specific bacterial species associated with AgP or ChP.

	EVIDENCE	NUMBER OF STUDIES SHOWING BACTERIAL SPECIES		
		ELEVATED IN AgP	ELEVATED IN ChP	NO DIFFERENCE (AgP and ChP)
	Strong			
	Aggregatibacter Actinomycetemcomitans	13	0	23
	Moderate			
	Fusobacterium nucleatum	3	0	15
	Parvimonas micra	3	0	15
	Campylobacter rectus	5	0	15
AgP	**Mild/Some**			
	Eubacterium nodatum	2	0	4
	Prevotella intermedia	5	3	19
	Eikenella corrodens	2	0	13
	Actinomyces gerencseriae	2	0	3
	Actinomyces israelii	3	1	3
	Treponema socranskii	2	0	4
	Selenomomas sputigena	2	0	0
	Tannerella forsythia	6	4	23
	Mild/Some			
	Capnocytophaga ochracea	0	2	9
ChP	*Streptococcus gordonii*	0	2	5
	Streptococcus oralis	0	2	5
	Staphylococcus aureus	1	3	1
	Human cytomegalovirus	0	2	2

The following categories were defined, according to the difference in number of studies showing specific microorganisms in statistically higher levels and/or prevalence and/or abundance and/or proportion between AgP and ChP: (i) Strong evidence (difference of > 5 studies), (ii) Moderate evidence (difference of 3, 4 or 5 studies), and (iii) Mild/Some evidence (difference of 2 studies) (Perez-Chaparro et al., 2014).

APPENDIX

Appendix Table 1. Search strategy used in the systematic review.

Search	Add to builder	Query	Items found
#21	Add	Search **((((((((periodontitis OR periodontal disease OR periodontal infection OR periodontal diseases))) AND (adult periodontitis OR Chronic periodontitis)) AND (aggressive periodontitis OR juvenile periodontitis OR localized periodontitis OR early onset OR localized aggressive OR generalized aggressive OR early-onset))) AND (microbiota OR microbiology OR pathogen OR pathogens OR biofilm OR microorganism OR microorganism OR red complex OR orange complex OR bacteria OR checkerboard OR PCR OR gingivalis OR treponema OR forsythia OR rectus OR denticola OR microbiome OR bacteria OR actinomycetemcomitans OR sequencing OR subgingival microbiome OR next generation sequencing OR metagenomic))) AND (plaque sample OR plaque samples OR dental plaque OR subgingival plaque OR subgingival samples OR subgingival biofilm)**	442

Appendix Table 2. Excluded studies and reasons for exclusion.

Exclusion reason	Study
Subgingival biofilm samples were analyzed together with tongue, saliva and/or mucosa samples	Asikainen et al., 1991, Urzua et al., 2008
Lack of direct microbial comparison between AgP and ChP groups	Lai et al., 1986, Savitt and Socransky, 1984, Tempro and Slots, 1986, Rams et al., 1990, Rams et al., 1992, Schenkein et al., 1993, Muller et al., 1995, Sandmeier et al., 1995, Cortelli et al., 2003, De Soete et al., 2001, Picolos et al., 2005, Darby et al., 2005, Collins et al., 2016, Kubar et al., 2005, Mayorga-Fayad et al., 2007, Reichert et al., 2009, Stingu et al., 2012, Belibasakis et al., 2014, Reichert et al., 2015, Savitt et al., 1988, Wohlfeil et al., 2010, Rosalem et al., 2011, Nanaiah et al., 2013, Ramich et al., 2015
Only saliva samples were evaluated	Guentsch et al., 2013
Case Report	Haffajee et al., 1984
Presented data only for Localized Aggressive Periodontitis	Zambon et al., 1983, Chen et al., 1989, Chen, 1996, Jardim Junior et al., 2006
No clear definition of AgP and ChP	Loesche et al., 1985, Kim et al., 2009
No statistical analysis between the two clinical groups	Takeuchi et al., 2006, Slots et al., 1980, Nonnenmacher et al., 2001, Tan et al., 2002
Did not report data at the genera or species levels; only morphological data reported	Liljenberg and Lindhe, 1980

AgP: aggressive periodontitis; ChP: chronic periodontitis.

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Appendix Table 3. Inclusion criteria for AgP and ChP and demographic characteristics of the participants from the studies included

Study/Year	Smoker (%)		Male (%)		Inclusion criteria	
	AgP	ChP	AgP	ChP	AgP	ChP
Rams et al. (USA, 1992)	-	-	-	-	At least 3 sites on separate teeth with PD≥6 mm and ≤35 y	At least 3 sites on separate teeth with PD≥6 mm and >35 y
Kojima et al. (Japan, 1997)	-	-	-	-	Onset age of <35 y	Onset age of over 40 y
Darby et al. (Scotland, 2000)	50	43.5	37.5	65	Systemically healthy, <35 y, CAL ≥5 mm on ≥ 8 teeth, at least 3 of which non-first molars or incisors, and at least 1 of which should be a permanent first molar (Hart et al., 1991)	At least one site with PD ≥6 mm in all sextants
Yano-Higuchi et al. (Japan, 2000)	-	-	12.5	28.5	Advanced destruction of periodontal tissues at an onset age < 35 y (Page et al., 1983)	≥4 sites with CAL ≥4 mm and alveolar bone destruction.
Takeuchi et al. (Japan, 2001)	-	-	-	-	≥4 sites showing ≥4 mm AL and <35 y	≥4 sites showing ≥4 mm AL and >35 y
Suda et al. (Japan, 2002)	-	-	-	-	(AAP, 1989; Caton, 1989; Armitage, 1999)	(AAP, 1989; Caton, 1989; Armitage, 1999)
Doğan et al. (Turkey 2003)	-	-	-	-	(Armitage, 1999)	(Armitage, 1999)
Takeuchi, et al. (Japan, 2003)	0	0	-	-	Age of disease onset <30 y and AL ≥4 mm in ≥ 2 first molars and/or incisors and ≥ 3 cuspids, premolars or second molars	AL ≥4 mm in at least 30% of teeth
Gajardo, et al. (Chile, 2005)	-	-	44	11.7	Age of disease onset <30 y and AL ≥4 mm in ≥ 2 first molars and/or incisors	AL ≥4 mm in at least 30% of teeth

					and ≥ 3 cuspids, premolars or second molars	
Yang et al. (Taiwan, 2005)	-	-	-	-	(AAP, 1999)	
Wang et al. (Japan, 2005)	-	-	-	-	At least five teeth per quadrant. ≥ 3 teeth (other than molars and incisors) with PD ≥5 mm and radiographic evidence of bone loss (Armitage, 1999, Meng et al., 2009).	At least five teeth per quadrant; ≥ 2 teeth with PD ≥5 mm and radiographic evidence of bone loss (Armitage, 1999, Meng et al., 2009).
Cortelli et al. (Brazil, 2005)	-	-	24	36.5	Rapid attachment loss, bone loss and familial aggregation (Lang N, 1999).	≥4 sites with PD ≥4 mm and ≥4 sites with CAL ≥4 mm; radiographic evidence of bone loss.
Picolos et al. (USA, 2005)	-	-	42.1	25	International World Workshop of Periodontology 1999 (Armitage, 1999)	International World Workshop of Periodontology 1999 (Armitage, 1999)
Ximenez-Fyvie et al., (Mexico, 2006)	-	-	15.8	35.9	≥18 sites with CAL ≥5 mm and 12–29 y.	≥18 sites with CAL ≥ 5 mm and >35 y.
Botero et al. (Colombia, 2007a)	0	3.7	14.2	48.1	World Workshop of Periodontology 1999 (Flemmig, 1999, Tonetti and Mombelli, 1999).	World Workshop of Periodontology 1999 (Flemmig, 1999, Tonetti and Mombelli, 1999).
Lafaurie et al. (Colombia, 2007)	14.7	18.7	38	46.5	(Armitage, 1999)	(Armitage, 1999)
Schacher et al. (Germany, 2007)	-	-	43.3	46.7	≤35 y (Kim et al., 2006) and radiographic bone loss ≥50% in at least two different teeth.	>35 y and CAL ≥5 mm in over 30% of sites.
Thiha et al. (Japan, 2007)	-	-	56.2	37.5	(Armitage, 1999)	(Armitage, 1999)
Botero et al, (Colombia, 2007b)	-	-	25	42.8	≥20 teeth (excluding third molars), PD and CAL ≥4 mm+BOP+radiographic bone loss in ≥6 sites, <30 y, severe AL, pockets, bone loss, plaque and	≥20 teeth (excluding third molars), PD and CAL ≥4 mm+BOP+radiographic bone loss in ≥6 sites.

					calculus deposits inconsistent with the severity of the periodontal destruction (Armitage, 1999, Flemmig, 1999, Tonetti and Mombelli, 1999).	
Doğan et al. (Turkey, 2008)	43	50	75	37.5	(Armitage, 1999)	(Armitage, 1999)
Fritschi et al. (Switzerland, 2008)	Yes	yes	-	-	(Armitage, 1999)	(Armitage, 1999)
Yamabe et al. (Japan, 2008)	-	-	-	-	Not described	Not described
Imbronito et al. (Brazil, 2008)	20	13.3	36.6	46.6	AL≥4 mm ≥3 permanent teeth (non-molars or incisors) (Armitage, 1999)	≥20 teeth, PD and CAL ≥4 mm+BOP+radiographic bone loss in ≥6 sites (Armitage, 1999)
Bilichodmath et al. (India, 2009)	-	-	78	63	AL ≥5 mm in > 14 teeth (≥ 3 non-first molars or incisors) with moderate to severe bone loss (Armitage, 1999).	PD≥5 mm and CAL ≥3 mm in >20 teeth with moderate to severe bone loss (Armitage, 1999).
Riep et al. (Germany, 2009)	38.6	32.6	43.2	45.7	Disease onset estimated at <30 y, PD ≥6 mm at >3 permanent teeth (non-first molars or incisors)(Armitage, 1999).	PD ≥4 mm at ≥30% of teeth (Armitage, 1999).
Faveri et al. (Brazil, 2009)	-	-	40	26.6	≤35 y; ≥6 permanent incisors and/or first molars with ≥ 1 site each with PD and CAL ≥5 mm and familial aggregation.	>35 y; ≥6 teeth with ≥ 1 site each with PD and CAL ≥5 mm; ≥30% sites with PD and CAL ≥4mm and BOP.
Nibali et al. (England, 2009)	21.9	25	32.8	50	(Armitage, 1999)	(Armitage, 1999)
Schlafer et al. (Germany, 2010)	0	0	37.5	50	Disease onset estimated at <30 y and PD ≥6 mm at >3 permanent teeth (non-first molars or incisors).	PD≥ 4 mm at ≥30% of teeth.
Rescala et al. (Brazil, 2010)	0	25	30	50	(Armitage, 1999)	(Armitage, 1999)
Drescher et al. (Germay, 2010)	-	-	38.7	43.9	Disease onset was estimated at <30 y and PD ≥6 mm at >3 permanent teeth	PD ≥4 mm at ≥30% of residual teeth (Armitage, 1999).

					other than first molars and incisors (Armitage, 1999).	
Da Silva-Boghossian et al. (Brazil, 2011)	9.1	44.7	37	40	≥30% of teeth with PD and/or CAL ≥5 mm with BOP, including at least one incisor and one first molar, and ≤39 y.	>10% of teeth with PD and/or CAL ≥5 mm and BOP.
Das et al. (India, 2012)	0	0	-	-	≥18 y, 30% of sites involved, PD ≥6 mm, and AL ≥3 mm, with severe bone loss [≥50% of root length]) (Armitage, 1999).	≥35 y, 30% of sites involved, PD ≥6 mm, and AL ≥3 mm, with severe bone loss [≥50% of root length]) (Armitage, 1999).
Casarin et al. (Brazil, 2012)	0	0	27	32	<35 y, good general health, ≥6 permanent incisors and/or first molars with ≥1 site each with PD and CAL >4 mm and BOP; ≥2 sites with PD and CAL ≥7 mm and BOP (Armitage, 1999).	>35 y; ≥30% of the sites with PD and CAL >4 mm and BOP; ≥2 sites with PD and CAL ≥7 mm and BOP (Armitage, 1999)
Heller et al. (Brazil, 2012)	11	45	30	40	≤35 y; systemically healthy; history of familial aggregation; ≥3 sites on 3 teeth (non-first molars or incisors); PD ≥6 mm, CAL ≥5 mm and BOP.	>30% of sites with PD and/or CAL ≥4 mm and BOP
Shaker et al. (Egypt, 2012)	-	-	44	52	≤35 y, ≥6 teeth (non-first molars or incisors); PD and CAL >5 mm and familial aggregation (Armitage, 1999; Faveri et al., 2009).	>35 y, ≥6 teeth with PD and CAL >5 mm (Armitage, 1999) (Faveri et al., 2009).
Benrachadi et al. (Morocco, 2012)	-	-	-	-	Angular bone lysis over more than two-third of radicular height, PD >6 mm, and AL >4 mm. The severity of periodontal lesions is usually not related to local deposits and familial aggregation (Armitage, 1999).	PD >6 mm, AL >4 mm, horizontal bone lysis concerning more than two third of radicular height, and local factors (bacterial plaque and calculus) (Armitage, 1999).
Tomita et al. (Japan, 2013)	25	16	40	25	≤39 y, systemically healthy, rapid attachment loss and bone destruction, familial aggregation, ≥3 sites on 3 teeth (non-first molars or incisors) with	>30% of sites with PD and/or CAL ≥4mm, and BOP (Armitage, 1999).

					PD ≥6 mm, CAL ≥5 mm, and BOP (Armitage, 1999).	
Silva-Boghossian et al. (Brazil, 2013)	12.1	43.6	34.8	40.8	≥30% of teeth with PD and/or CAL ≥5 mm with BOP, including ≥1 incisor and one first molar and ≤39 y (da Silva-Boghossian et al., 2011).	>10% of teeth with PD and/or CAL ≥5 mm and BOP (da Silva-Boghossian et al., 2011)
Silveira et al. (Brazil, 2013)	-	-	-	-	(Tonetti and Mombelli, 1999)	(Tonetti and Mombelli, 1999)
Kowalski et al. (Poland, 2014)	-	-		-	Positive family history of periodontal disease; general health, an inflammatory state not consistent with the amount of dental deposits present on the teeth, PD >5 mm; disease progression intensified in the areas of the central incisors and/or first molars. ≥3 other teeth affected by periodontal inflammation.	Extensive deposits of plaque/calculus, >30% of sites PD >5 mm, and with at least one site of CAL> 4 mm.
Wang et al. (China, 2014)	0	0	5.0	5.0	(Armitage, 1999; Meng, 2009)	(Armitage, 1999; Meng, 2009)
Haririan et al. (Austria, 2014)	27	37	60.6	65.1	Age and pattern of disease as the main criteria for the AgP group. All patients had teeth with PD ≥5 mm, 20 teeth, supporting bone loss ≥30%, ≥6 teeth with PD ≥5 mm (Armitage, 1999).	All patients had teeth with PD ≥5 mm, 20 teeth, supporting bone loss ≥30%, ≥6 teeth with PD ≥5 mm (Armitage, 1999).
Belibasakis et al. (Turkey, 2014)	0	0	40	40.9	Generalized pattern of severe breakdown, CAL ≥5 mm and PD ≥6 mm on ≥8 teeth, ≥3 teeth (non-first molars or incisors), radiographic evidence of bone loss and ≥20 teeth.	≥4 non-adjacent teeth with CAL ≥5 mm, which was commensurate with the amount of plaque accumulation, PD ≥6 mm, full-mouth BOP ≥50%, and radiographic evidence of bone loss
Hwang et al.	0	0	51.4	55.5	(Lindhe, 1999; Lang, 1999)	(Lindhe, 1999; Lang, 1999)

(Colombia, 2014)						
Schimidt et al. (Germany, 2014)	0	0	53.3	45.5	<35 y, rapidly progressive loss of attachment, family aggregation likely (not absolutely necessary), PD and CAL ≥5 mm ≥2 teeth. Radiographic bone loss ≥50%, in ≥2 different teeth (Armitage, 1999).	30 - 60 y with tooth sites exhibiting 3 to 4 mm of CAL and ≥4 mm PD, in ≥3 teeth in two or more different quadrants (Armitage, 1999).
Lourenço et al. (Brazil, 2014)			-	-	≤39 y and ≥30% of teeth with PD and/or CAL ≥5 mm with BOP, including ≥1 incisor and one first molar (Armitage, 1999).	>10% of teeth with PD and/or CAL ≥5 mm and BOP (Armitage, 1999).
Topcuoglu et al. (Turkey, 2015)	14	28	38	20	Not described	Not described
Chahboun et al. (Morocco, 2015)	-	-	21.6	20	At least 20 teeth and ≤35 y; rapid attachment loss and bone breakdown including ≥1 incisor and one first molar; PD ≥4 mm, CAL ≥3 mm and BOP.	At least 20 teeth and ≤35 y; extensive deposits of plaque and calculus, >10% of teeth with PD ≥4 mm and ≥1 site with CAL ≥3 mm.
Li et al. (China, 2015)	0	60	40	70	<35 y, rapid AL and bone destruction; ≥ eight teeth, three of them not being first molars or incisors, had probing depth (PD) >5mm and AL >3 mm.	(Armitage, 1999).
Vieira-Colombo et al. (Brazil, 2015)	25	37.8	40	40.8	≥30% of teeth with PD and/or CAL ≥5 mm with BOP, including at least one incisor and one first molar and ≤39 y (Amitage, 1999; Da Silva-Boghossian, 2011).	>10% of teeth with PD and/or CAL ≥5 mm and BOP. (Amitage, 1999; Da Silva-Boghossian, 2011).
Nagpal et al. (India, 2016)	-	-	-	-	(AAP, 1999)	(AAP, 1999)
Oliveira et al. (Brazil, 2016)	-	-	46.7	43.3	<35 y, ≥6 permanent incisors and/ or first molars with ≥1 site each with PD and CAL ≥5 mm, ≥6 teeth (non-first molars or incisors), with ≥1 site each	≥35 y, ≥6 teeth with ≥1 site each with PD and CAL ≥5 mm, >30% of the sites with PD and CAL ≥4 mm and BOP (Armitage, 1999).

					with PD and CAL ≥5 mm, familial aggregation (≥1 other member of the family presenting or with a history of periodontal disease (Faveri et al., 2009).	
Lanza et al. (Spain, 2016)	45	37.4	28.3	41.4	≤35 y (Kim et al., 2006), non-contributory medical history, self-reported familial aggregation (Lang, 1999), PD ≥4 mm, CAL ≥3 mm, in > 30% of teeth, BOP and bone loss.	PD ≥4 mm, CAL ≥3 mm in > 30% of teeth, BOP and bone loss (Armitage, 1999).
Kumawat et al. (India, 2016)	-	-	43.3	63.3	Rapid attachment loss, ≥4 sites, ≥3 mm CAL, vertical or saucer-shaped bone destruction seen radiographically specially with central incisors and first molars, amount of destruction not consistent with local factors, fair oral hygiene, familial aggregation.	≥4 sites, ≥3 mm CAL, amount of destruction consistent with local factors, horizontal bone loss radiographically, subgingival calculus frequently found, slow to moderate rate of progression with possible periods of rapid progression.

AgP: Aggressive Periodontitis; ChP: Chronic Periodontitis; RP: Rapid Progressive Periodontitis; EOP: Early Onset Periodontitis; CAL: Clinical Attachment Level; BOP: Bleeding on Probing; PD: Probing Depth; Y: Years; AL: Attachment Level and -: not described

Appendix Table A4. Periodontal clinical characteristics of participants within included studies that reported data.

Reference (Country/Year)	Mean PD		Mean CAL		Mean PI		Mean BOP (%)	
	AgP	ChP	AgP	ChP	AgP	ChP	AgP	ChP
Darby et al. (Scotland, 2000)	6.79*	5.92	-	-	1.13	1.47	0.74	0.86
Yano-Higuchi et al. (Japan, 2000)	5.41 ± 1.79	5.68 ± 1.99	4.81 ± 2.26	6.05 ± 2.52	-	-	-	-
Takeuchi et al. (Japan, 2001)	5.84 ± 2.4	5.82 ± 2.21	6.2 ± 2.7	6.66 ± 2.71	-	-	77.6	57.3
Takeuchi, et al. (Japan, 2003)	6.31 ± 2.23	6.39 ± 1.96	6.81 ± 2.66	7.15 ± 2.5	-	-	81.9	64.3
Gajardo, et al. (Chile, 2005)	4.30 ± 0.27*	3.67 ± 0.43	4.0 ± 0.11	3.39 ± 0.44	72.1 ± 14.78	78.77 ± 12.0	57.1 ± 11.54	64.86 ± 17.05
Wang et al. (Japan, 2005)	-	-	-	-	-	-	38.9 ± 27.1	21.7 ± 22.7
Cortelli et al. (Brazil, 2005)	6.49*	5.59	-	-	43	65*	-	-
Picolos et al. (USA, 2005)	5.4 ± 2.4	4.1 ± 0.9	-	-	-	-	-	-
Ximenez-Fyvie et al. (Mexico, 2006)	3.9 ± 0.2	4 ± 0.2	3.9 ± 0.2	4.6 ± 0.2	38.3 ± 8.4	51.7 ± 5.6	44.4 ± 4.8	48.8 ± 3.7
Botero et al. (Colombia, 2007a)	4.42 ± 0.95	4.11 ± 0.55	3.90 ± 0.63	4.54 ± 1.45	43.03 ± 20.15	48.49 ± 3.72	44.91 ± 19.42	54.41 ± 22.13
Lafaurie et al. (Colombia, 2007)	3.8 ± 0.9	3.9 ± 1.1	4.0 ± 1.4	4.2 ± 1.6	-	-	59.3*	53.5
Schacher et al. (Germany, 2007)	7.53 ± 1.38	8.39 ± 1.38*	8.04 ± 1.69	8.98 ± 1.61*	-	-	-	-
Thiha et al. (Japan, 2007)	6.10 ± 1.46	5.74 ± 1.34	6.39 ± 2.06	6.26 ± 1.73	-	-	69	67
Botero et al. (Colombia, 2007b)	4.24 ± 0.42	3.34 ± 0.26	3.97 ± 0.46	3.86 ± 0.22	-	-	-	-
Doğan et al.	3.3 ± 0.76	3.8 ± 1.2	4.4 ± 2.43	4.0 ± 1.73	1.1 ± 0.28	1.5 ± 0.34	-	-

(Turkey, 2008)								
Fritschi et al. (Switzerland, 2008)	-	-	-	-	-	-	100	68.2
Yamabe et al. (Japan, 2008)	6.98 ± 2.86	4.83 ± 2.04	-	-	-	-	-	-
Imbronito et al. (Brazil, 2008)	4.3 ± 0.4*	3.4 ± 0.9	4.8 ± 0.4*	4.4 ± 2.1	-	-	-	-
Riep et al. (Germany, 2009)	7.5 ± 2.9	5.2 ± 2.4	-	-	-	-	-	-
Faveri et al. (Brazil, 2009)	4.8 ± 0.8	3.8 ± 0.7	4.7	4.3	49.2	84.7	68.7	63.8
Nibali et al. (England, 2009)	4.0 ± 0.9	4.0 ± 0.9	4.2 ± 1	4.2 ± 1	-	-	-	-
Schlafer et al. (Germany, 2010)	7.8 ± 2.5	7.1 ± 1.4	-	-	-	-	-	-
Rescala et al. (Brazil, 2010)	3.51 ± 0.51	3.52 ± 0.7	3.65 ± 0.64	4.07 ± 0.99	-	-	0.64 ± 0.16	0.64 ± 0.2
Drescher et al., (Germay, 2010)	7.9 ± 1.3	5.8 ± 1.4	-	-	-	-	-	-
Da Silva-Boghossian et al. (Brazil, 2011)	3.9 ± 0.09*	2.9 ± 0.06	4.3 ± 0.12*	3.5 ± 0.08	70.3 ± 2.5*	63.8 ± 1.8	64.5 ± 3.1	40.9 ± 1.6
Casarin et al. (Brazil, 2012)	3.3 ± 4.6	3.8 ± 4.5	4.1 ± 1*	4.4 ± 1.1	55.4 ± 18.5	69.8 ± 33.7	39.5 ± 29.8	52.3 ± 34.6
Heller et al. (Brazil, 2012)	3.9 ± 0.9*	2.9 ± 0.8	4.2 ± 1.1*	3.6 ± 1.1	70.0 ± 23.5	64.5 ± 25.1	61.5 ± 29.8*	40.4 ± 23.2
Shaker et al. (Egypt, 2012)	6 ± 1.4	5.7 ± 0.9	7.2 ± 1.1*	4.9 ± 0.5	1.8 ± 0.9	2.4 ± 0.5*	1.7 ± 0.8	2.6 ± 0.5
Tomita et al. (Japan, 2013)	7.4 ± 1.4	7.2 ± 1.4	8.1 ± 2.2	7.8 ± 1.4	-	-	55	52.6
Silva-Boghossian et al. (Brazil, 2013)	3.86 ± 0.17*	2.96 ± 0.07	4.28 ± 0.14*	3.67 ± 0.1*	-	-	63.23 ± 4*	46.85 ± 1.83
Silveira et al. (Brazil, 2013)	3.7 ± 0.9*	2.9 ± 0.5	4.3 ± 1.2	3.8 ± 1.1	32.3 ± 19.7	33.5 ± 14	10.7 ± 13.5	20.6 ± 19

Kowalski et al. (Poland, 2014)	5.38 ± 1.29*	4.09 ± 1.80	6.73 ± 1.98	5.28 ± 2.75	56.4 ± 25*	35.0 ± 30.9	74.1 ± 16.9	59.7 ± 30.6
Wang et al. (China, 2014)	4.9 ± 1.9	4.1 ± 2.0	4.5 ± 1.9	4.8 ± 2.0	-	-	30.6 ± 7.2	30.8 ± 5.5
Haririan et al. (Austria, 2014)	3.87 ± 0.91	4.01 ± 0.93	4.39 ± 0.95	4.54 ± 1.21	-	-	46.22 ± 24.82	40.82 ± 23.64
Belibasakis et al. (Turkey, 2014)	6.75 ± 1.11	4.95 ± 0.37	7.60 ± 1.16	7.86 ± 2.12	2.15 ± 0.81	2.54 ± 0.67	64 ± 21	74 ± 41
Schimidt et al. (Germany, 2014)	3.3 ± 1.0	2.7 ± 0.7	3.4 ± 1.0	2.2 ± 0.8	30.7 ± 15.2	35.6 ± 16.4	30.1 ± 20.1	24.8 ± 14.4
Hwang et al. (Colombia, 2014)	4.6 ± 1.1	4.1 ± 0.6	4.6 ± 1.1	4.7 ± 0.7	5.3 ± 1.3	0.9 ± 0.1	0.6 ± 0.2	0.8 ± 0.2
Topcuoglu et al. (Turkey, 2015)	7,1 ± 1,6	5,7 ± 1.8	-	-	-	-	97	76
Chahboun et al. (Morocco, 2015)	6.05 ± 0.95	4.46 ± 0.59	5.18 ± 1.39	3.09 ± 1.20	80.4 (53.9 - 100)	56.2 (45.1 - 76)	76.8 (59.1 – 100)	46.4 (33.7 - 59.9)
Li et al. (China, 2015)	4.84 ± 0.91	4.50 ± 1.24	4.28 ± 1.33	4.40 ± 1.05	-	-	100	100
Vieira-Colombo et al. (Brazil, 2015)	4.2 ± 0.8	2.7 ± 0.7	4.7 ± 1.2	3.2 ± 1.3	66.8 ± 21.6	55.1 ± 25	82.2 ± 17	36.9 ± 22.2
Nagpal et al. (India, 2016)	9.08 ± 0.7	7.81 ± 0.62	-	-	1.06 ± 0.35	2.25 ± 0.48	-	-
Oliveira et al. (Brazil, 2016)	4.1 ± 0.9	4.0 ± 1.1	3.8 ± 1.1	3.7 ± 1.0	78.1 ± 16.7	86.4 ± 18.9	45.5 ± 15.9	42.1 ± 29.9
Lanza et al. (Spain, 2016)	5.98 ± 0.78	5.85 ± 1.35	-	-	38 ± 12.42	46 ± 21.32	-	-
Kumawat et al. (India, 2016)	8.3 ± 2.52	7.93 ± 2.13	8.13 ± 2.41	7.93 ± 2.14	1.24 ± 0.72	2.45 ± 0.43	1.46 ± 0.63	2.55 ± 0.43

PD: Probing Depht; CAL: Clinical Attachment Level; BOP: Bleeding on Probing and - : not described, *statistically significant difference between AgP and ChP.

Appendix Table 5. Microorganisms found in statistically significantly higher counts/abundance/frequency in Aggressive Periodontitis (AgP) or Chronic Periodontitis (ChP), the number of studies showing these differences and the number of subjects and samples included in these studies.

Taxa (N of studies evaluating the species)	**N studies**		**N subjects**	**N samples**
Aggregatibacter actinomycetemcomitans n = 36	Elevated in AgP	13	1602	4170
	Elevated in ChP	0	0	0
	No difference	23	1568	9494
Porphyromonas gingivalis n = 39	Elevated in AgP	5	294	1428
	Elevated in ChP	4	396	2016
	No difference	30	2822	11402
Tannerella forsythia n = 32	Elevated in AgP	5	397	1078
	Elevated in ChP	4	204	576
	No difference	23	2852	11432
Treponema denticola n = 24	Elevated in AgP	1	69	579
	Elevated in ChP	3	370	1990
	No difference	20	1804	9933
Campylobacter rectus n = 20	Elevated in AgP	5	808	1318
	Elevated in ChP	0	0	0
	No difference	15	1237	5893
Campylobacter showae n = 8	Elevated in AgP	1	260	1820
	Elevated in ChP	1	222	222
	No difference	6	391	2840
Eubacterium nodatum n = 6	Elevated in AgP	2	482	2042
	Elevated in ChP	0	0	0
	No difference	4	199	966
Fusobacterium nucleatum n = 18	Elevated in AgP	3	459	2975
	Elevated in ChP	0	0	0
	No difference	15	958	3899
Parvimonas micra n = 18	Elevated in AgP	3	191	1019
	Elevated in ChP	0	0	0
	No difference	15	2117	5870
Prevotella intermedia n = 27	Elevated in AgP	5	890	1718
	Elevated in ChP	3	156	156
	No difference	19	1356	4821
Prevotella nigrescens n = 16	Elevated in AgP	1	106	424
	Elevated in ChP	2	178	1900
	No difference	13	1031	3449
Streptococcus constellatus n = 5	Elevated in AgP	0	0	0
	Elevated in ChP	1	222	222
	No difference	4	428	2693
	Elevated in AgP	0	0	0

Capnocytophaga ochracea n = 11	Elevated in ChP	2	350	2270
	No difference	9	568	2533
Capnocytophaga sputigena n = 10	Elevated in AgP	1	144	660
	Elevated in ChP	1	222	222
	No difference	8	627	4470
Eikenella corrodens n = 15	Elevated in Ag	2	139	649
	Elevated in ChP	0	0	0
	No difference	13	1396	5149
Streptococcus gordonii n = 7	Elevated in AgP	0	0	0
	Elevated in ChP	2	482	2042
	No difference	5	246	2377
Staphylococcus intermedius n = 6	Elevated in AgP	0	0	0
	Elevated in ChP	1	222	222
	No difference	5	430	3893
Streptococcus mitis n = 7	Elevated in AgP	0	0	0
	Elevated in ChP	1	222	222
	No difference	6	506	4197
Streptococcus oralis n = 7	Elevated in AgP	0	0	0
	Elevated in ChP	2	482	2042
	No difference	5	430	3893
Streptococcus sanguinis n = 7	Elevated in AgP	0	0	0
	Elevated in ChP	1	222	222
	No difference	6	536	4317
Actinomyces odontolyticus n = 10	Elevated in AgP	1	260	1820
	Elevated in ChP	0	0	0
	No difference	9	600	3049
Veillonella parvula n = 8	Elevated in AgP	0	0	0
	Elevated in ChP	1	260	1820
	No difference	7	342	3045
Actinomyces gerencseriae n = 5	Elevated in AgP	2	482	2042
	Elevated in ChP	0	0	0
	No difference	3	112	589
Actinomyces israelii n = 7	Elevated in AgP	3	588	2466
	Elevated in ChP	1	55	495
	No difference	3	83	120
Actinomyces naeslundii 1 n = 8	Elevated in AgP	2	281	791
	Elevated in ChP	1	55	495
	No difference	5	401	3424
Eubacterium saburreum n = 4	Elevated in AgP	1	106	424
	Elevated in ChP	0	0	0
	No difference	3	317	1780
Gemella morbillorum n = 6	Elevated in AgP	1	260	1820
	Elevated in ChP	0	0	0
	No difference	5	398	2301
Leptotrichia buccalis n = 6	Elevated in AgP	1	260	1820
	Elevated in ChP	1	222	222

	No difference	4	170	2073
Propionibacterium acnes n = 8	Elevated in AgP	2	366	2244
	Elevated in ChP	1	222	222
	No difference	5	176	2079
Treponema socranskii n = 6	Elevated in AgP	2	209	836
	Elevated in ChP	0	0	0
	No difference	4	411	2448
Enterococcus faecalis n = 5	Elevated in AgP	0	0	0
	Elevated in ChP	1	260	1820
	No difference	4	725	5222
Staphylococcus aureus n = 5	Elevated in AgP	1	106	424
	Elevated in ChP	3	791	6368
	No difference	1	134	134
Streptococcus mutans n = 1	Elevated in AgP	1	106	424
	Elevated in ChP	0	0	0
	No difference	0	0	0
Klebsiella pneumoniae n = 1	Elevated in AgP	0	0	0
	Elevated in ChP	1	80	80
	No difference	0	0	0
Treponema lecithinolyticum n = 3	Elevated in AgP	1	90	450
	Elevated in ChP	0	0	0
	No difference	2	119	599
Dialister pneumosintes n = 3	Elevated in AgP	0	0	0
	Elevated in ChP	1	134	134
	No difference	2	139	139
Neisseria mucosa n = 5	Elevated in AgP	1	106	424
	Elevated in ChP	1	222	222
	No difference	3	355	3378
Pseudomonas aeruginosa n = 4	Elevated in AgP	1	222	222
	Elevated in ChP	0	0	0
	No difference	3	453	2013
Neisseria elongata n = 1	Elevated in AgP	1	59	59
	Elevated in ChP	0	0	0
	No difference	0	0	0
Fretibacterium sp. HOT 360 n = 2	Elevated in AgP	1	59	59
	Elevated in ChP	1	60	540
	No difference	0	0	0
TM7 n = 2	Elevated in AgP	1	60	540
	Elevated in ChP	0	0	0
	No difference	1	59	59
Fusobacterium periodonticum n = 1	Elevated in AgP	0	0	0
	Elevated in ChP	1	260	1820
	No difference	0	0	0
Selenomomas sputigena n = 2	Elevated in AgP	2	120	600
	Elevated in ChP	0	0	0
	No difference	0	0	0
Filifactor alocis	Elevated in AgP	1	60	540

n = 4	Elevated in ChP	0	0	0
	No difference	3	295	601
Prevotella disiens n = 1	Elevated in AgP	0	0	0
	Elevated in ChP	1	26	26
	No difference	0	0	0
Prevotella denticola n = 2	Elevated in AgP	1	26	26
	Elevated in ChP	0	0	0
	No difference	1	59	59
Anaerococcus prevotii n = 1	Elevated in AgP	1	26	26
	Elevated in ChP	0	0	0
	No difference	0	0	0
Prevotella oralis n = 1	Elevated in AgP	1	26	26
	Elevated in ChP	0	0	0
	No difference	0	0	0
Pseudoramibacter alactolyticus n = 1	Elevated in AgP	1	59	59
	Elevated in ChP	0	0	0
	No difference	0	0	0
Human cytomegalovirus n = 4	Elevated in AgP	0	0	0
	Elevated in ChP	2	59	59
	No difference	2	110	110
Epstein Barr Vírus n = 4	Elevated in AgP	1	84	84
	Elevated in ChP	1	33	33
	No difference	2	110	110
Herpes simplex Vírus -1 n = 3	Elevated in AgP	1	60	60
	Elevated in ChP	1	33	33
	No difference	1	50	50

N: Number

4. CONCLUSÃO

Os resultados desta revisão sistemática sugerem uma forte evidência para a associação de *A. actinomycetemcomitans* com PAg, principalmente em indivíduos com menos de 35 anos. No entanto, nem esta espécie ou qualquer outra espécie estudada até o momento é específica da PAg ou da PCr, ou suficiente para diferenciá-las.

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