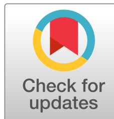

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ORIGINAL ARTICLE

TITLE PAGE

1. Title: Do patients with aggressive and chronic periodontitis exhibit specific differences in the subgingival microbial composition? A systematic review

Authors: Sheyla Christinne Lira Montenegro* (DDS, MSc, DDSc), Belén Retamal-Valdes* (DDS, MSc, DDSc), Bruno Bueno-Silva* (DDS, MSc, DDSc), Poliana Mendes Duarte *† (DDS, MSc, DDSc), Marcelo Faveri* (DDS, MSc, DDSc), Luciene Cristina Figueiredo* (DDS, MSc, DDSc), Magda Feres* (DDS, MSc, DDSc)

* Department of Periodontology, Dental Research Division, Guarulhos University, Guarulhos, Brazil.

† Department of Periodontology, School of Advanced Dental Sciences, College of Dentistry, University of Florida, Gainesville, Florida, The United States of America

Disclaimers: The authors report no conflicts of interest related to this study. The study was self-funded by the authors´ institutions.

Authors responsible for correspondence:

Belén Retamal-Valdes

Centro de Pós-Graduação e Pesquisa-CEPPE

Universidade Guarulhos

Praça Tereza Cristina, 229 Centro

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1902/jper.10547.

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07023-070 Guarulhos, SP, Brazil

e-mail: belenretamalvaldes@gmail.com

Word count and number of figures, tables, and references in the manuscript:

1. Word count: 3,282

2. Number of figures: 1

3. Number of tables: 3

4. Number of references: 86

Running title: Microbiota of periodontitis

Contribution statement: All authors have made considerable contributions to conception and design of this paper and, have given final approval of the version to be published. SCLM, BR-V, BB-S and MFa have been involved in data collection and data analysis. PMD, LCF and MFe have been involved in data interpretation, drafting the manuscript and revising it critically.

One sentence summary: This systematic review showed that no specific species or microbial complexes studied to date were unique to or could differentiate between ChP and AgP, supporting the recently-published Classification Scheme for Periodontal Diseases that introduced the notion that AgP and ChP are not different conditions, but variations of a single disease named Periodontitis.

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ABSTRACT

Background: The 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions grouped the diseases previously recognized as Chronic (ChP) or Aggressive (AgP) periodontitis under a single category named Periodontitis. The rationale for this decision was the lack of specific patterns of immune-inflammatory response or microbial profiles associated with ChP or AgP. However, no previous studies have compiled the results of all studies comparing subgingival microbial data between these clinical conditions. Thus, this systematic review aimed to answer the following focused question: "Do patients with AgP periodontitis present differences in the subgingival microbiota when compared to patients with ChP periodontitis?" **Methods**: A systematic review was conducted according to the PRISMA statement. The MEDLINE, EMBASE and Cochrane databases were searched up to June 2019 for studies of any design (except case reports, case series and reviews) comparing subgingival microbial data from patients with ChP and AgP. **Results**: 467 papers were identified and 56 were included. Thirteen studies found *Aggregatibacter actinomycetemcomitans* elevated in AgP in comparison with ChP, while *Fusobacterium nucleatum, Parvimonas micra* and *Campylobacter rectus* were elevated in AgP in a few studies. None of these species were elevated in ChP. However, the number of studies not showing statistically significant differences between ChP and AgP was always higher than that of studies showing differences. **Conclusion**: These results suggested an association of *A. actinomycetemcomitans* with AgP, but neither this species nor the other species studied to date were unique to or could differentiate between ChP and AgP (PROSPERO #CRD42016039385).

Key-words: Periodontal diseases; Chronic periodontitis; Aggressive periodontitis; Microbiota; Systematic review.

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INTRODUCTION

In 2018, the American Academy of Periodontology and European Federation of Periodontology published the official proceedings from the 2017 World Workshop on the Classification of Periodontal Diseases [1]. According to this new classification scheme, the diseases previously recognized as Chronic (ChP) or Aggressive (AgP) periodontitis were grouped under a single category named Periodontitis[2]. The main rationale for this decision was the lack of specific patterns of immune-inflammatory response or microbial profiles associated with ChP or AgP.

A wide range of studies have been published in the past decades aiming to investigate specific differences between AgP and ChP [3-12]. Regarding host-response, a systematic review has reported insufficient evidence to support the existence of distinct cytokine profiles for patients with AgP and ChP [12]. In terms of microbiology, a previous systematic review [13] assessing five periodontal pathogens suggested that the presence or absence of these microorganisms could not distinguish between patients with ChP and AgP. Nonetheless, no recent review or previous systematic reviews have compiled the results of studies comparing the microbiota of these two clinical conditions, casting doubts on whether ChP and AgP would be associated with specific microbial profiles/periodontal pathogens. Therefore, the aim of this systematic review was to answer the following PECO question: “Do patients with AgP periodontitis present differences in the composition of the subgingival microbiota when compared to patients with ChP periodontitis?”

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METHODS

Protocol and registration

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 [14].

Focused question

"Do untreated patients with AgP periodontitis present differences in the composition of the subgingival microbiota when compared to untreated patients with ChP periodontitis?"

Eligibility criteria

Inclusion criteria (PECOS):

(P)opulation: Untreated patients with periodontitis

(E)xposure: AgP

(C)omparator: ChP

(O)utcome: composition of the subgingival microbiota

(S)tudy design: studies of any design, except case reports, case series and reviews.

Exclusion criteria:

- Lack of a direct comparison of baseline (untreated) microbial data between AgP and ChP, in cases of interventional studies.

- Subgingival biofilm samples were analyzed together with tongue, saliva and/or mucosa samples.

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- No clear definition of AgP and/or ChP.

- No statistical analysis for microbiological data between the two clinical groups.

- Only bacterial morphological data reported.

Search strategy, study selection and data extraction

The MEDLINE, EMBASE and Cochrane databases were searched up to June 2019 by 2 independent reviewers (M.Fa. and L.C.F.), using Mesh terms and other keywords (see Supplemental Table 1 in online Journal of Periodontology). 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.). The inter-reviewer consistency of the full-text analysis was calculated by means of the kappa correlation coefficient.

Risk of bias in individual studies

Two reviewers (S.C.L.M. and B.R.-V.) appraised the risk of bias on the selected studies using the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies[86], since only cross-sectional studies were included. Cases of disagreement were solved by a third reviewer (M.Fe.).

Additional analyses: weight of evidence

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,

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as proposed by Pérez-Chaparro et al.[15]: (i) Strong evidence: difference of > 5 studies, (ii) Moderate evidence: difference 3-5 studies, and (iii) Mild/Some evidence: difference of 2 studies.

Summary measures

The following information was collected from each study and registered in predefined forms: microbiological outcomes (e.g., microorganisms appraised, taxa in higher mean/median levels and/or proportions and/or abundance and/or prevalence in AgP and/or ChP {primary outcome of interest}), study design, characteristics of participants (e.g., age, proportion of men and smokers, inclusion criteria for AgP and ChP, mean probing depth {PD}, clinical attachment level (CAL), proportion of sites with plaque and bleeding and probing {BOP}), as well as number of samples collected, sampling strategy, diagnostic method used and target microorganims. For longitudinal observational/interventional studies, only baseline data were collected.

RESULTS

Studies included

The electronic search strategy provided 488 titles. After title screening and abstract reading, 365 studies were excluded, and 102 full-text publications were comprehensively evaluated. Of these 102 papers, 46 were excluded because they did not match the inclusion/exclusion criteria or had unclear information (see Supplemental Table 2 in online Journal of Periodontology). Therefore, 56 studies [3-6, 16-50, 57, 59, 71-85] were included in this systematic review (Figure 1). Inter-reviewer agreement of the full-text analysis was 90.2% [kappa=0.83; 95% CI (0.67-0.94)].

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 the

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mean age of the populations evaluated. All studies included were cross-sectional. Patients from the majority of studies were selected at Universities, Hospitals or Dental Schools. Two studies reported patients from private practices [21, 22] and six [23-28] did not mention the study site.

In general, the studies evaluated more samples from ChP than from AgP patients. Only ten studies evaluated more samples from AgP [4, 19, 27, 29-35]. A total of 6,376 patients (AgP = 1,978 and ChP = 4,398) and 23,920 subgingival biofilm samples were evaluated (AgP = 8,224 and ChP = 15,696). The samples were analyzed individually in 26 studies [3, 5, 6, 18, 20, 23, 24, 25, 27, 29, 30, 32, 33, 44, 46, 57, 59, 71, 73- 77, 82, 84, 85] and pooled in 28 studies [4, 16, 17, 19, 21, 22, 26, 28, 31, 34, 35, 38-43, 45, 4750, 72, 78, 79, 80, 81, 83]. One study used individual and pooled analysis [36] and one study did not mention the sampling method/analysis used [37] (Table 1).

Ten studies used culture as the main microbial diagnostic test [4, 21, 24, 27, 35, 38-42] and two used an open-ended approach (pyrosequencing) [43, 44]. All 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 [45] to 37.4±10.0 years [33], and of the ChP groups from 30.2±3.9 years [45] to 55.13±7.46 years [46]. 76.7% of the studies showed mean age of above 40 years for the ChP group and 44.6% below 30 years for the AgP group.

Most of the studies evaluated systemically healthy individuals. Four studies did not mention if the participants were systemically healthy [19, 26, 45, 47] and one study included patients with type 2 diabetes in the ChP group [37].

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The inclusion criteria for AgP and ChP and demographic characteristics of the participants from the studies included[3-6, 16-50, 57, 59, 71-85] (% smokers and gender) are presented in Table 3S (see Supplemental Table 3 in online Journal of Periodontology). Of the included studies, 26.7% had smokers in the AgP group [3, 19, 22, 31, 41, 42, 57, 59, 72, 73, 76, 77, 79, 81, 83], and 32.1% had smokers in the ChP group [3, 19, 22, 31, 39, 41, 42, 43, 57, 59, 72, 73, 74, 76, 77, 79, 81, 83]; 53.5% of the studies [3, 6, 5, 17, 21-25, 27, 32, 34-38, 40, 43, 44, 50, 59, 73, 75-79, 81, 83, 84], used age as an inclusion criterion for AgP and ChP.

Clinical data

Mean full-mouth PD, CAL, plaque index and the percentage of sites showing BOP are presented in Table 4S (see Supplemental Table 4 in online Journal of Periodontology). Nine studies did not report clinical data [4, 17, 21, 23, 26, 45, 48-50]. Overall, the mean clinical values presented were compatible with advanced disease. Only eleven [5,20,37,41,44,73,76,77,79,80,83] studies reported full-mouth mean PD and five studies[35, 38, 44, 82, 83] mean CAL below 3.5 mm.

Microbiological data

The microbiological outcomes measured were essentially heterogeneous and could not be assessed by means of statistical tools. This is the main reason why a meta-analysis was not performed.

The microorganisms found in statistically significantly higher counts/abundance/frequency in AgP or ChP are presented in Table 5S (see Supplemental Table 5 in online Journal of Periodontology). Fifty-seven taxa (50 bacterial species, 4 bacterial genera and 3 viruses) differed significantly between AgP and ChP in at least one study. *Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Eubacterium nodatum, Fusobacterium nucleatum, Parvimonas micra, Prevotella intermedia, Eikenella corrodens, Actinomyces odontolyticus, Actinomyces gerencseriae, Actinomyces israelii, Actinomyces naeslundii 1, Eubacterium saburreum, Gemella morbillorum, Propionibacterium acnes, Treponema*

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socranskii, Streptococcus mutans, Treponema lecithinolyticum, Pseudomonas aeruginosa, Neisseria elongata, TM7, Selenomomas sputigena, Filifactor Alocis, Lactobacillus acidophilus, 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 constellatus*, *Capnocytophaga ochracea, Streptococcus gordonii, Streptococcus intermedius*, *Streptococcus mitis*, *Streptococcus oralis, Streptococcus sanguinis, Veillonella parvula, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Dialister pneumosintes, Fusobacterium periodonticum, Prevotella disiens, Fusobacterium nucleatum ssp. Polymorphum,* the genera as *Pseudoramibacter, Klebsiella, Lactococcus* and *Wollinella,* 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) [15]. Thirteen studies[3, 16, 17, 33, 36, 41, 45, 47, 72, 77, 78, 79, 80] found *A. actinomycetemcomitans* elevated in AgP in comparison with ChP, while *F. nucleatum, P. micra* and *C. rectus* were elevated in AgP in 3 [6, 75, 77], 3 [33, 34, 57] and 5 [16, 33, 38, 41, 47] studies, respectively. None of these species were statistically significantly elevated in ChP. *E. nodatum, P. intermedia, E. corrodens, A. gerencseriae, A. israelii, T. socranskii, S. sputigena* and *T. forsythia* showed mild/some association with AgP. No taxa presented strong or moderate evidence for a specific association with ChP; only mild/some evidence was observed for the following bacterial species in ChP: *C. ochracea, S. gordonii, S. oralis, S. aureus* and *Human cytomegalovirus.*

Risk of bias within studies

Table 3 shows the risk of bias analysis of the studies included in this systematic review, according to the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies[86]. All articles explicitly defined a research question (Item #1), the population from which the study participants were selected (Item #2) and had an exposure

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assessed prior to outcome measurement (Item #6). Fifty-five studies defined the outcomes in details (Item #11), 54 studies defined the exposure measures and assessments (Item #9) and elucidated the follow-up rate (Item #13), 53 studies recruited more than 50% of the target population (Item #3), 52 studies showed a sufficient timeframe to see an effect (Item #7), 51 studies used previously determined eligibility criteria (Item #4), and only 16 studies conducted statistical analyses (Item #14). No study complied with the following Items: #10 (Repeated exposure assessment) and #12 (Blinding of outcome assessors) of the NIH Quality Assessment Tool. Only three studies presented sample size justification (Item #5) and showed different levels of the exposure of interest (Item #8). None of the included articles fulfilled all items evaluated by means of the NIH Quality Assessment Tool. However, all articles included in this review accomplished at least 50% of the items assessed.

DISCUSSION

This is the first systematic review to assess the current weight of evidence for the existence of specific differences in the composition of the subgingival microbiota of patients 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) [15]. 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 [3, 16, 17, 33, 36, 41, 45, 47, 72, 77, 78, 79, 80] found *A. actinomycetemcomitans* elevated in AgP, while no study showed this species elevated in ChP. These data supported the notion

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that *A. actinomycetemcomitans* is an important pathogen in the etiology of AgP, which has been suggested by several previous investigations [6, 7, 45, 51-53]. Nonetheless, it is difficult to assure that *A. actinomycetemcomitans* was unique to AgP, since this microorganism was also frequently detected in ChP, and 24 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 patients 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; 60.7% of the studies (34 out of 56) included patients based on the 1999 Classification System [54]. 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 described in Armitage (1999) [54] (see Supplemental Table 3 in online Journal of Periodontology). This lack of standardization probably happened due to some difficulties associated with the use of such classification system, especially when trying to categorize AgP. The three main features of AgP according to the 1999 Classification system were: otherwise clinically healthy patients, familial aggregation and rapid attachment loss. These criteria were not always easy to assess, or not sufficient to discriminate 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,

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professors and researchers used 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 56 studies included in this review used age as an inclusion criterion for AgP and/or ChP, and the majority of them selected AgP patients 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) [33]. 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 [6, 55]. 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 [20]. 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 [20].

While high levels/proportions of red complex species have been previously detected in young patients with AgP [5, 6, 27, 35, 38, 39, 56-59] one would have expected to see higher levels and proportions of these pathogens in older patients with ChP, which was not the case. *Porphyromonas gingivalis*, for example, which was the most extensively studied periodontal pathogen (40 of the 56 included studies evaluated this species), showed no particular association with ChP when compared with AgP. In accordance with these data, a previous systematic review [13] 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)[6] and Feres, Figueiredo, Soares, & Faveri, (2015)[60] evaluated the composition of the subgingival

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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 studies included in this review used targeted diagnostic methods, such as Checkerboard DNA-DNA hybridization, real time PCR, ROQT and immunofluorescence. Only two studies [43, 44] 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 [15, 61-67] or AgP [68, 69] 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 [12.] Taken together, these data support the recently-published Classification Scheme for Periodontal Disease and Conditions that introduced the notion that AgP and ChP are not different diseases, but variations of a single condition [2, 70].

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The main limitation of this systematic review was the lack of standardization of some methodological features of the studies included, such as: the microbiological diagnostic tests used, the way the data were expressed, the number of individuals included per group and the inclusion criteria used to select patients with AgP and ChP. In addition, the severity of periodontitis and the way that clinical data were reported differed considerably among the studies included. In addition, some studies did not report the clinical parameters of the patients inlcuded. Despite these divergences, the studies included in this review compiled data of 6,376 patients and 23,920 subgingival biofilm samples, which allowed for a comprehensive evaluation of the available literature regarding possible microbiological differences between AgP and ChP.

Conclusion

The results of this systematic review suggested an 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.

Acknowledgments

The authors would like to thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES, Brasília, DF, Brazil), the National Council for Scientific and Technological Development (CNPq, Brasília, DF, Brazil) and Latin American Oral Health Association (LAOHA), for supporting researchers involved in this study.

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Figure Legends

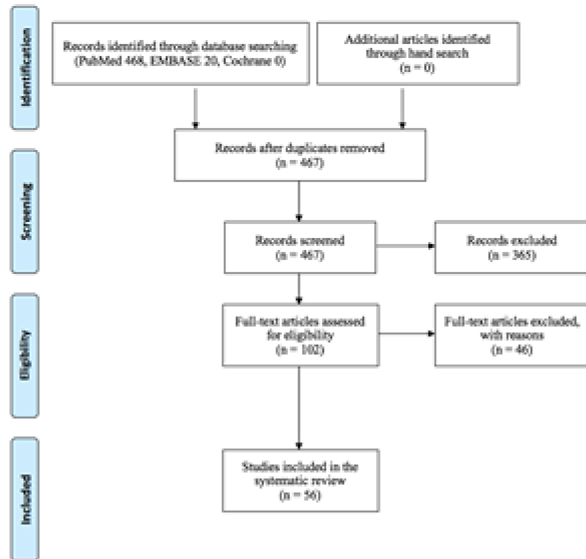

Figure 1. Flow chart of studies selection.

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

Study (Country/Year)	N patients/ N samples (P - I) Mean Age (years)		Method	Target microorganism
	AgP	ChP		
Rams et al. (USA, 1992) [21]	127/127(P) ND	907/907(P) ND	Culture	*P. micra*
Kojima et al. (Japan, 1997) [23]	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) [3]	24/96(I) 33.2 ± 3.4	33/132(I) 46.6 ± 7.1	PCR	*P. gingivalis, P. intermedia, T. forsythia, A. actinomycetemcomitans*‡ and *T. denticola*
Yano-Higuchi et al. (Japan, 2000) [24]	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) [25]	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) [26]	37/37(P) 35.0 ± 8.4	136/136(P) 51.1 ± 12.6	Anti-serum	*E. corrodens*

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Dogan et al. (Turkey, 2003) [4]	17/17(P) 31.0 ± 5.0	14/14(P) 43.9 ± 6.0	Culture and PCR	*P. gingivalis, A. actinomycetemcomitans, P. intermedia, P. nigrescens, T. forsythia, P. micra* and *C. rectus*
Takeuchi, et al. (Japan, 2003) [27]	40/160(I) 28.0 ± 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) [38]	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*‡ and *P. micra*
Yang et al. (Taiwan, 2005) [45]	70/70(P) 17.5 ± 6.8	101/101(P) 30.2 ± 3.9	Indirect immunofluorescence assay	*A. actinomycetemcomitans*‡
Wang et al. (Japan, 2005) [30]	46/184(I) 27.0 ± 5.8	28/112(I) 54.9 ± 6.8	Real-time PCR	*A. actinomycetemcomitans*
Cortelli et al. (Brazil, 2005) [47]	25/25(P) 21.9	178/178(P) 39.1	PCR Prevalence in patients	*A. actinomycetemcomitans*‡, *C. rectus*‡, *P. gingivalis, P. intermedia* and *T. forsythia*
Picolos et al. (USA, 2005) [29]	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) [5]	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,	12/12(P) 22.2 ± 5.7	68/12(P) 42.8 ± 9.3	Culture, biochemical	*S. maltophilia, A. lwoffi, A. baumannii, Pseudomonas spp., P. putida, P. aeruginosa, C.*

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2007)[39]			tests and PCR	*freundii, S. liquefaciens, E. aerogenes, E. gergoviae, E. cloacae, P. gingivalis‡, T. forsythia, P. nigrescens§, P. intermedia, Fusobacterium spp, P. micra, Campylobacter spp., Eubacterium spp., A. actinomycetemcomitans, E. corrodens‡, D. pneumosintes, E. rods* and *K. pneumoniae§*
Botero et al. (Colombia, 2007)[40]	6/6(P) 24.3 ± 6.3	20/20(P) 44.0 ± 12.0	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*
Lafaurie et al. (Colombia, 2007)[41]	158/158(P) 28.0 ± 6.8	325/325(P) 45.6 ± 10.6	PCR and culture	*P. gingivalis, C. rectus‡, T. forsythia, E. corrodens, A. actinomycetemcomitans‡, P. intermedia‡, P. nigrescens* and *E. rods*
Schacher et al. (Germany, 2007) [36]	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‡, P. gingivalis§, T. forsythia§* and *T. denticola§*
Thiha et al. (Japan, 2007) [46]	16/16(I) 35.0 ± 8.2	32/32(I) 55.1 ± 7.4	Real-time PCR	*P. gingivalis, A. actinomycetemcomitans* and *T. forsythia*
Dogan et al. (Turkey, 2008) [42]	8/8(P) 26.5 ± 6.6	8/8(P) 47.4 ± 9.2	Culture	*A. actinomycetemcomitans*
Fritschi et al. (Switzerland	22/88(I) ND	84/336(I) ND	Checkerboard DNA-DNA hybridization	40 bacterial species *(P. micra‡, P. intermedia‡, P. nigrescens‡, A. israelii‡, T. socranskii‡, S. aureus‡ and S.*

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, 2008) [57]				*mutans*‡)
Yamabe et al. (Japan, 2008) [71]	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. (Brazil, 2008) [72]	30/30(P) 27.3 ± 4.8	30/30(P) 42.7 ± 6.7	PCR	*HSV-1*‡, *EBV-1, HCMV, A. actinomycetemcomitans*‡, *P. gingivalis, P. intermedia* and *T. forsythia*
Bilichodmath et al. (India, 2009) [48]	14/14(P) 25.0 ± 3.1	19/19(P) 43.0 ± 7.3	PCR	*HSV-1*§, *HSV-2, EBV*§ and *HCMV*§
Riep et al. (Germany, 2009)[73]	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) [6]	25/225(I) 25.2 ±3.2	30/270(I) 42.0 ± 6.2	Checkerboard DNA-DNA hybridization	38 bacterial species (*P. gingivalis*‡, *F. nucleatum nucleatum*‡, *A naeslundii1*§)
Nibali et al. (England, 2009) [31]	64/64(P) 33.5 ± 5.1	20/20(P) 43.4 ± 11.4	Real-time PCR	*EBV*§ and *HCMV*
Schlafer et al. (Germany, 2010)[32]	72/330(I) 34.8 ± 6.4	30/78(I) 51.0 ± 10.2	PCR	*P. gingivalis, P. intermedia,* *A. actinomycetemcomitans, T. denticola, T. forsythia, F. nucleatum* and *F. alocis*
Rescala et al. (Brazil, 2010) [74]	17/34(I) 29.2 ± 6.6	20/40(I) 48.6 ± 7.5	Checkerboard DNA-DNA hybridization	40 bacterial species
Drescher et al.	62/303(I) 34.2 ± 6.2	82/357(I) 54.4 ±	Dot-blot hybridization, FISH, and	*Selenomonas, P. gingivalis, P. intermedia, T. denticola, T. forsythia, F. nucleatum*

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(Germany, 2010)[75]		12.1	electron microscopy	*nucleatum*‡, *S. sputigena*‡ and *S. noxia*‡
da Silva-Boghossian et al. (Brazil, 2011) [76]	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*§
Das et al. (India, 2012) [50]	25/25(P) ND	25/25(P) ND	PCR	*HSV-1, HSV2, EBV* and *HCMV*
Casarin et al. (Brazil, 2012) [37]	48 27.6 ± 0.9	89(39 diabetic) 43.6 ± 8.3	PCR	*M. timidum*
Heller et al. (Brazil, 2012) [77]	75/525(I) 30.2 ± 4.8	185/1295(I) 45.6 ± 9.3	Checkerboard DNA-DNA hybridization	46 bacterial species *(E. nodatum*‡, *A. gerencseriae*‡, *A. israelii*‡ and *S. aureus*§*)*
Shaker & Ghallab (Egypt, 2012) [78]	25/25(P) 27.5 ± 3.7	25/25(P) 40.2 ± 2.6	PCR	*A. actinomycetemcomitans*‡, *T. forsythia, P. gingivalis*[C], *Treponema denticola*§ and *P. intermedia*§
Benrachadi et al. (Morocco, 2012) [49]	8/8(P) 35.0 ± 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) [59]	20/20(I) 33.3 ± 8.1	20/20(I) 43.6 ± 11.1	Real-time PCR	*A. actinomycetemcomitans, P. gingivalis* and *T. forsythia*§
Silva-Boghossian et al. (Brazil, 2013) [79]	66/66(P) 31.4 ± 0.7	156/156(P) 45.9 ± 0.9	Checkerboard DNA-DNA hybridization	44 bacterial species (*T. forsythia*§, *C. showae*§, *E. nodatum*‡, *P. nigrescens*§, *S. constellatus*§, *A. actinomycetemcomitans*‡, *C.*

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				sputigena§, S. gordonii§, S. oralis§, S. mitis§, S. sanguinIs§, S. intermedius§, V. parvula§, A. naeslundii‡, L. buccalis§, S. aureus§ and D. pneumosintes§)
Silveira et al. (Brazil, 2013) [80]	35/35(P) 33.9 ± 7.1	41/41(P) 44.1 ± 9.4	PCR and Real-time PCR	*A. actinomycetemcomitans‡*
Kowalski & Gorska (Poland, 2014) [16]	17/17(P) 30.3(23–37)	23/23(P) 44.7(37-60)	Real-time PCR	*A. actinomycetemcomitans‡, P. gingivalis, T. forsythia, T. denticola, P. intermedia, P. micra, F. nucleatum, C. rectus‡* and *E. corrodens*
Wang et al. (China, 2014) [18]	10/105(I) 29.7 ± 2.1	10/79(I) 32.5 ± 1.8	Real-time PCR	*A. actinomycetemcomitans*
Haririan et al. (Austria, 2014) [81]	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) [82]	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)[33]	37/329(I) 37.4 ± 10	27/240(I) 50.5 ± 10.8	Checkerboard DNA–DNA hybridization and serum immunoglobulin (Ig) G	*A. actinomycetemcomitans‡, P. gingivalis‡, T. denticola, T. forsythia, P. micra, C. rectus, P. intermedia, F. nucleatum, A. naeslundii, V. parvula* and *E. corrodens*
Schmidt et	15/15(P)	11/11(P)	PCR	51 bacterial species

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al. (Germany, 2014) [34]	32.1 ± 7.1	45.2 ± 8.0		(*P. gingivalis*§ *and P. intermedia*§)
Lourenco et al. (Brazil, 2014) [17]	24/24(P) ND	35/35(P) ND	HOMIM	>250 species/phylotypes (*N. elongata*§, *A. actinomycetemcomitans*‡ *and P. intermedia*‡)
Topcuoglu & Kulekci (Turkey, 2015) [19]	29/29(P) 34.0 ± 8.0	25/25(P) 47.0 ± 9.0	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)[35]	37/37(P) 24.4 ± 5.0	20/20(P) 28.5 ± 4.3	Culture	*A. actinomycetemcomitans*‡, *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) [43]	10/10(P) 22.1 ± 4.9	10/10(P) 46.6 ± 7.4	PCR and pyrosequencing	*36 bacterial species* (*T. forsythia*‡, *P. gingivalis*‡ *and T. denticola*‡)
Vieira-Colombo et al. (Brazil, 2016) [83]	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, 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*

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[28]				
Oliveira et al. (Brazil, 2016) [84]	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‡, F. fastidiosum, Fretibacterium sp., Fretibacterium sp.‡, M. timidum, P. stomatis, P. endodontalis, P. gingivalis, P. denticola, S. sputigena‡, T. forsythia, TM7 sp.‡, T. lecithinolyticum, T. medium* and *T. vincentii*
Lanza et al. (Spain, 2016) [22]	60/60(P) 30.4 ± 7.1	123/123(P) 50.9 ± 9.4	PCR	*P. gingivalis, T. forsythia‡, T. denticola, P. intermedia‡ and A. actinomycetemcomitans*
Kumawat et al. (India, 2016) [85]	30/30(I) 21.2 ± 3.2	30/30(I) 37.5 ± 3.2	PCR	*P. gingivalis* and *T. denticola*
Feres et al. (Brazil, 2018) [20]	74/666 (I) 27.1 ± 3.1	308/2,772 (I) 45.1 ± 5.9	Checkerboard DNA-DNA hybridization and machine learning analyses	40 bacterial species: *A. gerencseriae, A. israelii, A. naeslundii, A. oris, A. odontolyticus, V. parvulla‡, S. gordonii, S. intermedius, S. mitis, S. oralis, S. sanguinis, A. actinomycetencomytans, C. gingivalis‡, C. ochracea‡, C. sputigena, E. corrodens, C. gracilis, C. rectus, C. showae, E. nodatum, F.nucleatum.ssp.nucleatum‡, F.nucleatum.ssp.polymorphum §, F.nucleatum.ssp.vincentii‡, F. periodonticum, P. micra, P. intermedia*[C]*, P. nigrescens, S. constellatus, T. forsythia* §, *P. gingivalis*§, *T. denticola* §, *E. saburreum, G. morbillorum, L. buccalis, P. acnes, P. melaninogenica, N. mucosa, S. anginosus, S. noxia, T. socranskii*

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Shi et al. (China, 2018) [44]	3/9 (I) 27.3	3/9 (I) 37.6	16S rRNA Sequencing (Illumina Hiseq)	The V4 region of the 16S rRNA gene was analyzed to evaluate the diversity of subgingival biofilm samples.

N: Number; **P:** Pooled; **I:** Individually; **AgP:** Aggressive Periodontitis; **ChP:** Chronic Periodontitis; **ND:** Not Described; **PCR:** Polymerase Chain Reaction; **FISH:** Fluorescence In Situ Hybridization; **HOMIM:** Human Oral Microbe Identification Microarray; **‡:** statistically significant difference for AgP; **§:** statistically significant difference for ChP.

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

Type of periodontitis	EVIDENCE	NUMBER OF STUDIES SHOWING BACTERIAL SPECIES		
		ELEVATED IN AgP	ELEVATED IN ChP	NO DIFFERENCE (AgP and ChP)
	Strong			
	Aggregatibacter actinomycetemcomitans	13 [3, 16, 17, 33, 36, 41, 45, 47, 72, 77, 78, 79, 80]	0	24 [4,5,6,18,19,20,22,24,27,29,30,32,38,39,42,46,49,57,59,73,74,76, 81,82]
	Moderate			
	Fusobacterium nucleatum	3 [6,75,77]	0	14 [5,16,17,19,29,33,34,35,38,43,57,73,74,79]
	Parvimonas micra	3 [33,34,57]	0	15 [4,5,6,16,17,19,21,29,35,38,39,74,77,79,81]
	Campylobacter rectus	5 [16,33,38,41,47]	0	15 [5,6,17,19,20,25,29,34,35,57,73,74,77,79,81]
AgP	**Mild/Some**			
	Eubacterium nodatum	2 [77,79]	0	4 [6,29,74,81]
	Prevotella intermedia	5 [17,22,33,41,57]	4 [20,34,39,78]	19 [3,4,5,6,16,19,27,29,35,38,43,47,49,72,73,74,75,79,81]
	Eikenella corrodens	2 [33,39]	0	13 [5,6,16,17,19,29,35,38,41,57,74,77,79,]
	Actinomyces gerencseriae	2 [77,79]	0	3 [6,43,74]
	Actinomyces israelii	3 [57,77,79]	1 [6]	3 [34,43,74]
	Treponema socranskii	2 [25,57]	0	4 [6,17,74,77]
	Selenomomas sputigena	2 [28,84]	0	0
	Tannerella forsythia	6 [3,4,22,33,35,43]	5 [24,27,36,59,79]	22 [5,6,16,17,19,29,35,41,46,47,49,57,72,73,74,75,77,7

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				[8,79,81,82,84]
	Mild/Some			
	Capnocytophaga ochracea	0	2[73,77]	9[5,6,17,29,34,35,38,74,79]
ChP	*Streptococcus gordonii*	0	2[77,79]	5[5,6,43,74]
	Streptococcus oralis	0	2[77,79]	5[5,6,17,43,74]
	Staphylococcus aureus	1[57]	3[76,77,79]	1[83]
	Human cytomegalovirus	0	2[39,48]	2[50,72]

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)[15].

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Table 3. Quality assessment of the included studies according to the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Total
Rams et al. (1992) [21]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Kojima et al. (1997) [23]	Yes	Yes	Yes	No	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	8
Darby et al. (2000) [3]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	No	10
Yano-Higuchi et al. (2000) [24]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Takeuchi et al. (2001) [25]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Suda et al. (2002) [26]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Dogan et al. (2003) [4]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Takeuchi, et al. (2003) [27]	Yes	Yes	Yes	No	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	9
Gajardo, et al. (2005) [38]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Yang et al. (2005)[45]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Wang et al. (2005) [30]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Cortelli et al. (2005) [47]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	Yes	11
Picolos et al. (2005) [29]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Ximenez-Fyvie et al. (2006) [5]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Botero et al. (2007) [39]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	NA	Yes	NA	Yes	NA	9
Botero et al. (2007b) [40]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	NA	Yes	NA	Yes	Yes	10

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Lafaurie et al. (2007) [41]	Yes	Yes	Yes	No	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	8
Schacher et al. (2007) [36]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Thiha et al. (2007) [46]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Dogan et al. (2008) [42]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Fritschi et al. (2008) [57]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Yamabe et al. (2008) [71]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Imbronito et al. (2008) [72]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Bilichodmath et al. (2009)[48]	Yes	Yes	NR	Yes	No	Yes	NA	NA	NA	No	Yes	NA	Yes	Yes	7
Riep et al. (2009)[73]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Faveri et al. (2009) [6]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	10
Nibali et al. (2009) [31]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	No	9
Schlafer et al. (2010) [32]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Rescala et al. (2010) [74]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Drescher et al. (2010) [75]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
da Silva-Boghossian et al. (2011)[76]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	Yes	11
Das et al. (2012) [50]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	No	NA	Yes	No	8
Casarin et al. (2012) [37]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	NA	Yes	NA	Yes	NA	9
Heller et al. (2012) [77]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10

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Shaker & Ghallab (2012) [78]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Benrachadi et al. (2012)[49]	Yes	Yes	NR	Yes	No	Yes	NA	NA	NA	No	Yes	NA	Yes	Yes	7
Tomita et al. (2013) [59]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Silva-Boghossian et al. (2013)[79]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	No	9
Silveira et al. (2013) [80]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	No	9
Kowalski & Gorska (2014) [16]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Wang et al. (2014) [18]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Haririan et al. (2014) [81]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	No	9
Belibasakis et al. (2014)[82]	Yes	Yes	No	Yes	No	Yes	Yes	NA	Yes	No	Yes	NA	Yes	Yes	9
Hwang et al. (2014) [33]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Schimidt et al. (2014) [34]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	8
Lourenco et al. (2014) [17]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Topcuoglu & Kulekci (2015) [19]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Chahboun et al. (2015) [35]	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	NA	Yes	NA	Yes	NA	8
Li et al. (2015) [43]	Yes	Yes	Yes	No	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	No	8
Vieira-Colombo et al. (2015) [83]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	9
Nagpal et al. (2016) [28]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	10

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Oliveira et al. (2016) [84]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	NA	10
Lanza et al., (2016) [22]	Yes	Yes	Yes	Yes	No	Yes	Yes	NA	Yes	NA	Yes	NA	Yes	Yes	10
Kumawat et al. (2016) [85]	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	NA	Yes	NA	Yes	NA	9
Feres et al. (2018) [20]	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	NA	Yes	NA	NA	Yes	8
Shi et al. (2018) [44]	Yes	Yes	Yes	Yes	No	Yes	NA	NA	Yes	NA	Yes	NA	NA	Yes	8
Total	56	56	53	51	3	56	52	3	54	0	55	0	54	16	-

Q1: Question 1. Was the research question or objective in this paper clearly stated?;

Q2: Question 2. Was the study population clearly specified and defined?;

Q3: Question 3. Was the participation rate of eligible persons at least 50%?

Q4: Question 4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)?;

Q5: Question 5. Was a sample size justification, power description, or variance and effect estimates provided?;

Q6: Question 6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?;

Q7: Question 7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?;

Q8: Question 8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?;

Q9: Question 9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?;

Q10: Question 10. Was the exposure(s) assessed more than once over time?;

Q11: Question 11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?;

Q12: Question 12. Were the outcome assessors blinded to the exposure status of participants?;

Q13: Question 13. Was loss to follow-up after baseline 20% or less?;

Q14: Question 14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

*CD, cannot determine; NA, not applicable; NR, not reported

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