Microbiome and Microbial Biofilm Profiles of Peri-Implantitis: A Systematic Review

Gloria Inés Lafaurie*, María Alejandra Sabogal*, Diana Marcela Castillo*, María Victoria Rincón[†], Luz Amparo Gómez[‡], Yamil Augusto Lesmes[†], and Leandro Chambrone*[‡§]

*Unit of Basic Oral Investigations-UIBO, School of Dentistry, El Bosque University, Bogotá, Colombia.

[†]School of Dentistry, El Bosque University, Bogotá, Colombia.

[‡]School of Dentistry, Ibirapuera University, Sao Paulo, SP, Brazil.

[§]Department of Periodontics, School of Dentistry, The University of Iowa, Iowa City, USA.

Background: This systematic review assesses the microbiological profiles of peri-implantitis, periodontitis and healthy implants based on studies that evaluated microbial biofilms and entire microbiomes to establish their similarities and differences.

Methods: The Medical Literature Analysis and Retrieval System Online, via PubMed, EMBASE (Excerpta Medica Database) and Cochrane Central Register of Controlled Trials (CENTRAL) were searched without language restrictions through July 30, 2016. Observational studies that evaluated the microbial profiles or entire microbiomes of peri-implantitis compared with healthy implants or periodontitis were considered eligible for inclusion. A descriptive summary was created to determine the quantity of data and inter-study variations.

Results: Of the 126 potentially eligible articles, 26 were included in this study; 21 of these articles evaluated the microbiological profile of peri-implantitis vs. healthy implants or periodontitis using conventional microbiological techniques and five articles evaluated the entire microbiome using genomic sequencing. Teeth with periodontitis, healthy implants, or implants with peri-implantitis were colonized by periodontal microorganisms. *Porphyromonas gingivalis* and especially *Prevotella intermedius/nigrescens* were often identified at peri-implantitis sites. Peri-implantitis sites were also colonized by uncultivable asaccharolytic anaerobic gram-positive rods and anaerobic gram-negative rods, which were not frequently identified in teeth with periodontitis or healthy implants. Opportunistic microorganisms were not found very frequently in peri-implantitis sites.

Conclusions: Peri-implantitis represents a heterogeneous mixed infection that includes periodontopathic microorganisms, uncultivable asaccharolytic anaerobic gram-positive rods and other uncultivable gram-negative rods and, rarely, opportunistic microorganisms such as enteric rods and *Staphylococcus aureus.* Sequencing methods that evaluate the entire microbiome improve the identification of microorganisms associated with peri-implantitis.

KEY WORDS (MESH TERMS):

chronic periodontitis; peri-implantitis; dental implants; microbiota; microbial genome; gram-positive rods.

Edentulism remains a public health problem worldwide.[1] Dental implants have improved oral rehabilitation in partial and complete edentulous patients, and their survival is reported with high rates of success.[2] Nevertheless, an increase in peri-implantitis has been reported with frequencies ranging between 14 and 30%.[3] A history of periodontitis,[4] current smoking and diabetes are the most important risk factors associated with peri-implantitis.[5] However, the importance of dental biofilms in the etiology of peri-implantitis has been extensively studied as well.[6]

Peri-implantitis is characterized by an inflammatory process around the implants that includes both soft tissue inflammation and progressive bone loss.[7] However, peri-implantitis is associated with changes in the crestal bone level in conjunction with bleeding on probing with or

without concomitant deepening of peri-implant pockets.[8] Although peri-implant disease has similarities to periodontal disease, they seem to be distinct entities.[8]

Studies[9,10] have investigated whether the dental biofilm profiles of implants are similar to periodontitis or if they represent another type of bacterial colonization, and some studies have shown important differences. Likewise, the bacterial profile differences of healthy and diseased dental implants have also been studied.[11,12] The biofilm profiles of peri-implantitis and healthy implants have shown controversial results. Despite of the presence of periodontopathic microorganisms in peri-implantitis sites, some studies did not find differences in their frequencies between healthy and peri-implant disease.[9,13,14] Observational studies[9,14-17] assessing the colonization of microorganisms around dental implants have suggested that these microorganisms could colonize the peri-implant sulcus of healthy implants in patients with periodontal disease. Other controversial results are related to the methodological design of the studies; studies of the microbiological profiles of peri-implant biofilms at healthy and diseased sites in the same individual differ from studies with parallel design.[16,17]

The microbiological methods used to study the differences between peri-implantitis and periodontitis or healthy implants also seem to influence the results of the microbial profile studies. A previous systematic review (SR)[12] identified that some studies used conventional techniques such as culture-based methods to compare the periodontopathic microorganisms and other unusual microorganisms of clinical importance. On the other hand, other studies have been using molecular methods as well.[18,19] These differences not only appear to be linked to the methods, but to the range of species studied as well. During the last 7 years, data from sequencing methods allowed evaluation of the entire microbiome, which comprises a wide and different distribution of microorganisms, and new non-cultivable species that were identified indicate that periodontitis differs from peri-implant disease.[18,19]

Two recent systematic reviews (SR) on the microbiological profiles of peri-implantitis have been published.[11-12] These reviews evaluated the microbiological profiles of peri-implantitis compared to healthy implants, but not to periodontitis. Recently, data on the entire microbiome of peri-implantitis with comparisons to periodontitis and healthy implants were published,[10] but these were not included in previously published reviews.

Therefore, the aim of this SR is to describe the microbiological profiles of peri-implantitis, periodontitis and healthy implants based on studies that evaluated the microbial biofilms and entire microbiomes to establish similarities and differences. The following focused questions were addressed:

1) **"**What are the differences in the microbial profiles of healthy implants and those affected by peri-implantitis?"; 2) **"**What are the differences in the microbial profiles of periodontitis and peri-implantitis?"; and 3) "Are there differences between the results obtained with conventional subgingival biofilm methods and entire microbiome sequencing methods from healthy vs. peri-implantitis or periodontitis vs. peri-implantitis sites?"

METHODS

This review was registered at the National Institute for Health Research PROSPERO, International Prospective Register of Systematic Reviews (registration number CRD42016038953, available from http://www.crd.york.ac.uk/PROSPERO/ display_record.asp?ID=CRD42016038953)

Types of Studies

Observational studies (i.e., cross sectional, case-control and cohort) were considered eligible for inclusion.

Participants/Population

The participants included adult patients treated with dental implants who were diagnosed with peri-implantitis and those with healthy implants or periodontitis. Studies were considered eligible for inclusion if they assessed microbial biofilms in the peri-implant sulcus/pocket of implants in patients diagnosed with peri-implantitis and in patients with healthy peri-implants or periodontitis and included cultivable or non-cultivable species.

Outcome Measures

1) Differences in the frequency of microorganisms (i.e., the frequency of cultivable species, the frequency of the total count $>10^4$ of each species evaluated)

2) Proportional distribution of bacterial genera

3) Proportional distribution of number of clones by operational taxonomic units (OTUs) at the phylum level

Searches

To streamline the identification of studies potentially eligible for inclusion in this SR, MEDLINE (Medical Literature Analysis and Retrieval System Online, via PubMed), EMBASE (Excerpta Medica Database) and CENTRAL were searched without language restrictions through July 30, 2016, using MeSH (Medical Subject Headings) terms, key words, and other free terms, and Boolean operators (OR, AND) were used to combine searches. Detailed search strategies were developed for each database that were based on the following search strategies presented for MEDLINE:

#1 endosseous dental implantation OR dental implants OR dental implantation OR implants, dental OR dental implants OR endosseous implantation OR implantation, endosseous OR implants OR osseointegrated dental implants OR osseointegration

#2 peri-implantitis OR peri-implant disease OR dental implant, infection OR dental implant, inflammation

#3 #1 OR #2

#4 microbiota OR microbiome OR human microbiome OR microbiome, human OR microbiology OR bacteria OR opportunistic infection

#5 biofilm OR dental biofilm OR oral biofilm OR dental deposits OR dental plaque OR *Porphyromonas gingivalis* OR *Bacteroides gingivalis* OR *Fusobacterium* OR *Sphaerophorus* OR *Prevotella intermedia* OR *Bacteroides intermedius* OR *Aggregatibacter actinomycetemcomitans* OR *Actinobacillus actinomycetemcomitans* OR *Tannerella forsythia* OR *Treponema denticola* OR *Staphylococcus aureus* OR *Candida*

#6 #4 OR #5

#7 PCR OR polymerase chain reaction OR real-time polymerase chain reaction OR real-time PCR OR reverse transcriptase polymerase chain reaction OR reverse transcriptase PCR OR multiplex polymerase chain reaction OR multiplex PCR

#8 sequence OR gene sequence OR molecular sequence OR molecular sequence data OR sequence analysis, protein

#9 #7 OR #8

#10 #3 AND #6 AND #9

Data Extraction (Selection and Coding)

Two independent reviewers screened the titles, abstracts, and full texts of the papers (MAS and LAG) for the PUBMED search, and two independent reviewers screened the EMBASE search (YL and MVR). Disagreement between the reviewers was resolved through discussion. When an agreement could not be reached, a third reviewer was consulted (GIL). When important data for the review were missing, we attempted to contact the authors to resolve ambiguity from the trials. The following data were extracted and recorded in duplicate: citation, publication status, year of publication, location of the trial, study design, characteristics of the participants, outcome measures, methodological quality of the trials and conclusions. One expert (DC) in microbiology reviewed the microbial methods in the studies with molecular methods including DNA sequencing methods (DMC).

Assessment of Validity and Data Extraction

The methodological quality of the observational studies was assessed using an adapted version of the Newcastle-Ottawa scale[20] adapted by Chambrone et al.[21,22]. The following study aspects were evaluated: 1) selection of study groups (sample size calculation, representativeness of the peri-implantitis patients, and selection of patients without peri-implantitis, ascertainment of periodontal/peri-implant conditions, description of the methods used to assess peri-implant conditions, training or calibration of the outcome assessors, prospective data collection/use of clear inclusion/exclusion criteria); 2) comparability (comparability of patients based on study design/analysis and management of confounders); 3) outcome (assessment of microbiological outcomes, ascertainment/criteria applied to evaluate the microbiologic conditions, and adequacy of patient follow-up); and 4) statistical analysis (appropriateness/validity of statistical analysis and unit of analysis reported). Points (stars) were given for each methodological quality criterion, and each included study could receive a maximum of 14 points. Studies with 11 to 14 points ($\geq$ 78.5% of the domains satisfactorily fulfilled) were arbitrarily considered to be of high quality, studies with eight to 10 points were of medium quality, and studies with fewer than eight points ($\leq$50% of domains fulfilled) were of low methodological quality.

Data Synthesis

The data was pooled into evidence tables, and a descriptive summary was created to determine the quantity of data and study variations (characteristics and results).

RESULTS

Search Results and Excluded Trials

Nine hundred seventy-three records potentially relevant to this review were retrieved from the electronic databases in addition to 37 that were identified from hand searching, but 754 were excluded after removing duplicates (see supplementary Figure 1 in online *Journal of Periodontology*). In total, the search strategy identified 256 articles. Of these, 87 were excluded after review of the tittle or abstract, and 39[1,9,10,13,14,16-19,23-30,32-53] full text studies were reviewed; ultimately, 26 studies[9,10,13,14,16-19,36-53] were included (Table 1), and 13[1,23-30,32-35] were excluded for different reasons (12[1,23-30,33-35] for not fulfilling the proposed inclusion criteria and one due to its retrospective[32] design). In total, 1,145 patients with 2,134 implants were assessed in the 26 cross-sectional studies studies[9,10,13,14,16-19,36-53] that were included in this review.

Differences in the Microbial Biofilms of Peri-implantitis and Healthy Implants

A total of 21 studies comparing the microbial profiles of peri-implantitis and healthy implants were evaluated using different microbiological methods: two used methods of cultivation,[37-40] one conventional PCR,[43-38] three quantitative PCR,[16-17,51] one Nested-PCR,[14] eight DNA hybridization[9,13,36,39,41,42,49,50] and five DNA sequencing methods.[18,19,44,47,52] The major periodontopathic microorganisms (Red complex: *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*; Orange complex: *Prevotella intermedia* and *Fusobacterium nucleatum*; and other microorganisms: *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, *Parvimonas micra* and *Campylobacter rectus*) were identified in 11/21 studies (52,3%).[9,14,16-17,37,39,40,43,49,51,53] Three studies also evaluated opportunistic microorganisms.[37,40,53] Five studies using DNA hybridization evaluated between 36 to 40 species including the major periodontopathic microorganisms.[13,36,41,42,50] (Table 2). The reports that used sequencing methods showed vast variability in the number of 16S rRNA gene sequences at the phylum, genus, and species levels, although all studies evaluated periodontal bacteria[18-19,44,47,52] (Table 3).

Comparing the differences between the major periodontopathic microorganisms, eight of 21 studies (38%) found bacteria from the red complex more frequently (i.e., absolute frequency or proportion of the total count based on a concentration of $>10^4$–10^6).[17,37,39,41,42,44,49,50] Orange complex microorganisms (*P. intermedia/nigrescens*, *F. nucleatum* and *E. corrodens*) were more frequent in nine studies (42.8%) in peri-implantitis.[17,36-37,39,40-41,43,44,47] However, seven studies (33.3%) failed to find differences in the frequency of these microorganisms between patients with peri-implantitis and healthy implants.[9,13-14,16,45,51,53] (Tables 2 and 3).

P. gingivalis was the most frequently found red complex organism in peri-implantitis sites in the studies evaluated (7/21, 33.3%),[37,39,41,42,43,44,49,] followed by *T. forsythia* (5/21, 23.8%).[39,41-43,50] *P. intermedia* was more frequently found in peri-implantitis (9/21, 42.8%),[36-37,39-41,43,44,47,52] followed by *F. nucleatum* (4/21, 19%)[36,41,47,52] and *P. micra* (2/21, 9.5%).[17,19,44] *C. rectus* and *E. corrodens* were less frequently found in peri-implantitis (3/21, 14.2%)[17,37,41]. Two[37,40] of the three studies that evaluated opportunistic microorganisms found gram-negative enteric rods and *Staphylococcus aureus* in peri-implantitis sites (Tables 2 and 3).

Figure 1 shows the prevalence of the major periodontopathic microorganisms in patients with healthy implants and peri-implantitis. Both healthy implants and peri-implantitis were colonized by periodontopathic microorganisms. Healthy implants showed colonization by *P. gingivalis* in

the range of 0 to 79% and peri-implantitis in a range of 0 to 65%, and the prevalence of *T. forsythia* ranged from 0 to 80% and from 0 to 80.3% in healthy and peri-implantitis sites, respectively. However, there was a greater difference in *P. intermedia* in healthy implants compared to peri-implantitis sites, with ranges of 6.6-23% vs. 25-66%, respectively, and gram-negative rods ranged from 6 to 13% in healthy implants and from 10 to 65% in peri-implantitis. *S. aureus* was also more frequent in peri-implantitis sites than in healthy implants with a range of 0-43.4% vs. 0 to 19.1%, respectively.

Differences in the Microbial Biofilms of Peri-implantitis and Periodontitis

Only four studies[9,38,43,46] compared the periodontopathic microorganisms in peri-implantitis and periodontitis sites using conventional techniques (Table 2). The results of this investigation confirm the overall similarities of the microbiota compositions associated with periodontitis and peri-implantitis. Although the spectra of the prevalent species are similar, some variation can be detected in the microorganism frequencies. *P. gingivalis* was similar in periodontitis and peri-implantitis in 3/4 studies[9, 43,46]. However, *P. intermedia*, *C. rectus* and *T. forsythia* were more frequent in periodontitis than peri-implantitis[9, 38, 43]. Only 1/4 studies did not find differences in the frequencies of periodontopathic microorganisms between teeth and implants[46]. Enteric rods were recovered more frequently and at higher levels in peri-implantitis compared to periodontitis,[38] and *P. aeruginosa*, *S. aureus* and *C. albicans* were frequent found in peri-implantitis, suggesting they may be associated with implant failure[46].

Comparison of Peri-implantitis With Healthy Implants and Periodontitis Using Sequencing Methods

In studies[19,44,47,52] that used sequencing techniques, the results differed from those that were obtained using other techniques to compare healthy implants and peri-implantitis.[17,39-43,49,50] Most of the bacterial species found in the healthy implants were also detected in peri-implantitis[19]. However, *P. micra*, *P. intermedia* and *F. nucleatum* were in higher proportion in peri-implantitis consortia compared with healthy implants in the same individuals[44,47]. *P. gingivalis* only was observed to be in higher proportion in peri-implantitis consortia in one study[44]. Other non-cultivable microorganisms were associated with peri-implantitis when the entire microbiome was evaluated by sequence techniques, including asaccharolytic anaerobic gram-positive rod (AAGPR) associated species such as *Eubacterium nodatum, Eubacterium brachy, Eubacterium saphenum, Filifactor alocis, Slackia exigua, Parascardovia denticolens*[44]. *Dialister invisus*, *Eubacterium infirmum, Actinomyces cardiffensis, Eubacterium minutum,* and *Gemella sanguinis* as well as anaerobic gram-negative rods (OGNRs) such as *Mitsuokella* sp. HOT 131, *Leptotrichia hofstadii, Kingella denitrificans, Treponema lecithinolyticum*, microorganisms of the orange complex such as *P. intermedia* and gram-positive cocci such as *Streptococcus* sp. human oral taxon (HOT) 064 were also associated with peri-implantitis[47,52], indicating that these microorganisms could be important in the etiology of peri-implantitis (Table 3, Figure 2)

When microorganism sequences were compared between peri-implantitis and periodontitis, ecological differences in the bacterial communities of peri-implantitis and periodontitis were observed; peri-implantitis showed more diversity than periodontitis and was characterized by higher proportions of *Peptococcus, Mycoplasma, Campylobacter, Butyrivibrio, Streptococcus mutans, Eubacterium* spp., *Porphyromonas* sp, *Achromobacter xylosoxidans*, TM7 [G-5] sp.

HOT-437, *Actinomyces massiliensis, Porphyromonas* sp. HOT-395, *P. nigrescens*, and *Prevotella oris* [10,18,50]. Peri-implantitis represented a heterogeneous infection of more complexity predominantly composed of non-cultivable gram-negative species when compared with periodontitis (Table 3, Figure 2). Teeth with periodontitis and implants with ´peri-implantitis were colonized by periodontopathic microorganisms evaluated by culturing methods,[38,46] and these results were corroborated by sequencing techniques.

Risk of Bias (Quality Assessment)

Of the 26 studies included in this SR, one[43] received an 11-point score out of a total of 12 points (high methodological quality); eight received 9-point scores[13,17,37,40,42,47,50] and eight received 8-point scores (medium quality)[10,14,16,19,41,45,49,53]; and four received 7-point scores, four received 6-point scores, and the last one received a 5-point score (low methodological quality)[18,36,38,39,44,46,48,51,52] (see supplementary Figure 2 in online *Journal of Periodontology*). Although none of the studies performed a sample calculation, five studies showed a sample with high representativeness of the peri-implantitis population.[13,17,38,43,50] Descriptions of the inclusion/exclusion criteria and unit of analysis (number of patients per group) were considered adequately addressed for all but 4 studies[38,44,48,52]. All of the studies described standardized, conventional microbiological methods (i.e., culturing, PCR, and DNA hybridization) or sequencing techniques but none of them reported blinding of the examiners. All but 3 studies considered in this review showed adequate assessment of peri-implant conditions using clinical and radiographic evaluation[14,38,46]; however, only 14 studies used pocket depth for peri-implantitis diagnosis: 6 studies used a pocket depth >4 mm[16,17,40,44,45,49] and 8 used a pocket depth >5 mm. [9,10,19,42,43,47,51,53] The comparability of groups according to the use of similar implant therapy was suitable only in 10 studies [7,9,13,14,37,40,41,43,49,52], and the confounders' control (i.e., smoking, diabetes, and periodontal disease activity) by exclusion or statistical analysis was adequate only in 6 studies[9,14,37,42-43,50] (Table 2).

DISCUSSION

Summary of the Main Results

Healthy implants and peri-implantitis are colonized by periodontopathic microorganisms. Although the peri-implantitis microbiota showed a slightly higher frequency of the red complex microorganisms associated with periodontitis, microorganisms of the orange complex such as *Prevotella intermedia/nigrescens* appear to be more associated with peri-implantitis lesions. Non-cultivable microorganisms such as asaccharolytic anaerobic gram-positive rod (AAGPR) associated species and non-culturable gram-negative rods (OGNRs) are more associated with peri-implantitis lesions compared with healthy implants. Periodontitis and peri-implantitis seem to be different entities in terms of microbial composition; peri-implantitis represents a heterogeneous and more complex infection with predominantly non-culturable gram-negative species compared with periodontitis.

Agreements and Disagreements With Previous Studies

Peri-implantitis is recognized as an infection of peri-implant tissues tissues, but currently there is no single definition universally accepted.[57] Although bone loss and these inflammatory signs are the most important factors for diagnosis, a significant crestal bone loss over time should be verified. [58] All the studies included in this SR used bone loss, bleeding on probing and

suppuration as the basis for diagnosis, but only 14/26 (53.8%) of them included pocket depth as well. In a systematic review published in 2016[11] that compared peri-implantitis and healthy implants, only 11 studies that included pocket depth ≥ 5 mm and parallel design were assessed. In this SR, it was possible to include 26 studies, one with high methodological quality and 16 with medium methodological quality, which included parallel and paired/split-mouth designs with intra-individual assessment in peri-implantitis and healthy implants in the same individuals. The paired design could better control biological variability and some confounding factors than the parallel design. The differences in the selection criteria of the studies could explain the differences in the results and the conclusions between the two reviews. The review of Perez-Chaparro et al.[11] supported the association of *P. gingivalis*, *T. denticola*, and *T. forsythia* with peri-implantitis and provided some evidence for the association of *P. intermedia* with the etiology of peri-implantitis. However, in the present SR, the association of the red complex was weaker compared with *P. intermedia* in the etiology of peri-implantitis. Another difference observed between previous reviews and the present SR relates to "the new direction of evidence" generated by microbiome studies and the importance of non-cultivable bacteria in peri-implantitis. In addition, one of those previous SRs[12] concluded that peri-implantitis consisted of gram-negative anaerobic pathogens and opportunistic microorganisms in almost the same ratio. However, the expanded assessment of the available base of evidence performed by this SR does not seem to support these results. The importance of opportunistic microorganisms such as *Staphylococcus aureus* in the etiology of peri-implantitis could not be demonstrated in some studies using conventional methods,[3,49] and the entire microbiome studies[10,18,19,44-48] do not appear to represent the most associated microorganisms in peri-implantitis, although they could be associated in some cases. Recently, another SR including studies published during the last 5 years evaluated the peri-implant diseases microbiota.[59] Based on different objectives and selection criteria, that SR identified a great variability of techniques used for the study of microflora.[58] Thus, these author emphasized on the importance of studying the microbioma profile using metagenomic technics.[58]

Several confounding factors have been reported in the profiles of microorganisms in peri-implantitis. Smokers had significantly lower diversity and shared greater numbers of species than nonsmokers; the genera *Lactobacillus*, *Prevotella*, *Treponema*, *Propionibacterium,* and *Pseudomonas* demonstrated higher abundances in smokers[54]. The presence of periodontitis could influence the colonization of periodontal pathogens such as *P. gingivalis*, *T. forsythia* and *T. denticola* in healthy implants,[15,55] and these pathogens are also low in edentulous patients with implants[37]. The lack of control of these confounders in the revised evidence may have influenced the variability of the studies. Three of four studies[9,16,39,53] with paired design assessment of intra-individual sites with peri-implantitis and healthy implants using conventional microbiological technics found no differences for the red complex microorganisms between peri-implantitis and healthy implants. Another study[39] did not find differences in the same patient except for *A. actinomycetemcomitans* in peri-implantitis, which was evident. In paired design studies using sequence techniques, no differences were observed in the red complex microorganisms between peri-implantitis and healthy implants[19,48]. When comparing studies with paired design between peri-implantitis and periodontitis, two of four[45,47] found no differences between teeth and implants, and two studies found differences for *Porphyromonas* sp. HOT-395[48] and *Porphyromonas* spp[10] but not for *P. gingivalis* in favor of peri-implantitis. These results seem to support that periodontal microorganisms in the same patient can colonize healthy implants. However, *P. intermedia/nigrescens* showed significant differences between peri-implantitis and

healthy implants even in studies with paired design[43] and when compared with periodontitis,[48] which seems to support a greater association of this microorganism with peri-implantitis.

Asaccharolytic anaerobic gram-positive rod (AAGPR) associated species as *Eubacterium nodatum, Eubacterium brachy, Eubacterium saphenum, Eubacterium brachy*[48] *Eubacterium minutum*, and *Eubacterium infirmum* were more abundant in peri-implantitis.[10,18,44,47,52] *Eubacterium minutum* correlated with *P. intermedia*, which suggests an association of *Eubacterium* with peri-implantitis[52]. The importance of these AAGPRs, especially *Eubacterium* species and *Filofactor alosis*, in peri-implantitis as well as other anaerobic gram-negative rods (OGNRs) such as *Mitsuokella* sp. HOT 131, *Leptotrichia hofstadii*, *Kingella denitrificans* and *Treponema lecithinolyticum* that are found in entire microbiome studies comparing peri-implantitis with healthy implants and periodontitis has not yet been evaluated. Due to the loss of representativeness of microbiome studies in peri-implantitis, it is necessary to study these microorganisms in large population samples with implants using prospective cohort studies to determine their role in the initiation of the peri-implantitis. The actual evidence is not conclusive in regards to the initiation of peri-implantitis from peri-implant biofilms and neither is the composition of an established peri-implantitis.

Human oral microbiome has been changing our understanding on the role of bacteria in the pathogenesis of periodontal and peri-implant diseases. A number of species, including some uncultivable ones, that colonize this niche are strongly associated with disease as much or more than some periodontal pathogens commonly isolated by culture techniques[60] Recently, it has been proposed that periodontal disease pathogenesis involves polymicrobial synergy and dysbiosis (i.e., a complex interaction between the commensal microbiota, host susceptibility and environment) and not only the "impact" of a few specific microorganisms.[61-62] Because the bacterial biomass of human microbiome increases with increasing periodontal inflammation.[61-62], the diversity of the peri.implantitis-associated microbiome should direct research towards understanding of the interactions between the inflammatory environment and the development of the microbiome in peri-implantitis

Quality of the Evidence and Potential Biases in the Review Process

Heterogeneity of high methodological quality studies did not allow comparisons between results, thus meta-analyses of such data may be questionable due to potential bias and the lack of control of confounders. Thus, the most transparent approach was a systematic review of observational studies. Different diagnosis criteria for peri-implantitis, clinical and microbiological methods, and study design (parallel or paired-intraindividual assessment) can explain most of the discrepancies between the studies. Another important issue is the sample size calculations, which could underestimate outcomes; due to the low prevalence of peri-implantitis, large samples are required to achieve significant differences. Although evaluation of the entire microbiome using sequencing methods had sufficient sensitivity to find differences in paired samples, these failed to achieve representativeness of the population. Additionally, a clear description of a blind and calibrated assessment of the outcomes should be cited.

CONCLUSIONS

Within the limits of this SR, the following can be concluded:

1) Healthy implants and peri-implantitis are colonized by periodontopathic microorganisms. *P. gingivalis* and especially *P. intermedius/nigrescens* may be more frequent in diseased implants. Peri-implantitis is characterized by the colonization of non-cultivable asaccharolytic anaerobic gram-positive rods and non-cultivable anaerobic gram-negative rods.

2) Peri-implantitis represents a heterogeneous infection of more complexity composed of predominantly non-cultivable gram-negative species compared with periodontitis.

3) There are evident differences between the results obtained with conventional subgingival biofilm methods and entire microbiome sequencing methods in healthy vs. peri-implantitis or periodontitis vs. peri-implantitis. The study of the entire microbiome allows the inclusion of non-cultivable bacteria that colonize implants with peri-implantitis. However, characterizations of the periodontopathic microorganisms in peri-implantitis are similar in conventional and sequencing microbiome studies with paired-intra-individual design.

Implications for Future Research and Practice

Peri-implantitis represent a heterogeneous mixed infection that includes anaerobic gram-negative rods, asaccharolytic anaerobic gram-positive rods and, rarely, opportunistic microorganisms such as enteric rods and *Staphylococcus aureus*. Considering this microbiological profile, it is necessary to evaluate the anti-infectious protocols used in the treatment of this disease.

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CONFLICT OF INTEREST:

The authors report no conflicts of interest related to this study.

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Contact author: Dr Leandro Chambrone, Rua Antonio Pinto Guedes, 626, 08820-430 Mogi das Cruzes, São Paulo, Brazil. Email: leandro_chambrone@hotmail.com

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Figure 1.

Frequency of microorganisms in peri-implantitis and healthy implants identified using conventional methods. A: Bacteria of the red complex and A. actinomycetemcomitans. B: Bacteria of the orange complex. C: Bacteria of the green and yellow complexes and superinfecting microorganisms (fuchsia bars). The colors of the bars for peri-implantitis correspond to the complexes of Socransky[56]. The blue bars correspond to the frequencies of microorganisms in healthy implants.

Figure 2.

Venn diagram of the microbiome distribution in periodontitis (purple set), peri-implantitis (red set) and healthy implants (green set) identified by sequencing. The colors of the bacteria correspond to the complexes of Socransky[56]. The bacteria in blue correspond to microorganisms that are not within these complexes.

Table 1 –

Characteristics of included studies

Autor	Country	Study design	Groups	Participants/implants	Unit of analysis
Salceti et al.[36]	USA	Cross-sectional/parallel	Peri-implantitis	21/28	Implants
			Healthy implants	8/14	
Leonhardt, et al.[37]	Sweden	Cross-sectional/parallel	Peri-implantitis	37/100	Individuals
			Healthy implants	51/93	
Listgarten et al.[38]	USA	Cross-sectional/parallel	Peri-implantitis	41/41	Individuals
			Periodontitis	41/41	
Hultin et al. 2002[39]	Sweden	Cross-sectional/parallel	Peri-implantitis	17/98	Individuals
		Paired	Healthy implants	19/114	
Botero et al.[40]	Colombia	Cross-sectional/parallel	Peri-implantitis	11/16	Implants
			Healthy implants	8/15	
Renvert et al.[13]	Sweden	Cross-sectional/parallel	Peri-implantitis	…/31	Implants
			Healthy implants	…/55	
Shibli et al.[41]	Brasil	Cross-sectional/parallel	Peri-implantitis	22/22	Implant/ individuals
			Healthy implants	22/22	
Maxino et. al. [42]	Brasil	Randomized clinical trial/baseline- Parallel	Peri-implantitis	13/20	Implants
			Healthy implants	10/11	
Koyanagi et al.[19]	Japan	Cross-sectional/paired	Peri-implantitis	….	Individuals
			Healthy implants		
			Periodontitis		
Casado et al.[14]	Brasil	Cross-sectional/parallel	Peri-implantitis	10/10	Individuals
			Healthy implants	10/10	
Cortelli et al.[43]	Brasil	Cross-sectional/parallel	Peri-implantitis	50/100	Implants
			Healthy implants	53/106	
			Periodontitis	50/100	Individuals
Ebadian et al.[9]	Iran	Cross-sectional/paired	Peri-implantitis	….	Individuals
			Healthy implants		
			Periodontitis		

Kumar et al.[18]	USA	Cross-sectional/parallel	Peri-implantitis	10/10	Individuals
			Healthy implants	10/10	
			Periodontitis	10/10	
Tamura et al.[44]	Japan	Cross-sectional/parallel	Peri-implantitis	…	Individuals
			Healthy implants		
Koyanagi et al.[10]	Japan	Cross-sectional/paired	Peri-implantitis	6/6	Individuals
			Periodontitis	6/6	
Schaumann et.al.[45]	Germany	Cross-sectional/paired	Peri-implantitis	7/7	Individuals
			Periodontitis	7/7	
Albertini et al. [46]	Spain	Cross-sectional/paired	Peri-implantitis	….	Individuals
			Periodontitis		
Da Silva et al.[7]	Brasil	Cross-sectional/parallel	Peri-implantitis	….	Individuals
			Healthy implants		
Maruyama et al.[48]	Japan	Cross-sectional/paired	Peri-implantitis	…	Individuals
			Periodontitis		
Ata-Ali et al. [49]	Spain	Cross-sectional/parallel	Peri-implantitis	22/13	individuals
			Healthy implants		
Persson et al.[50]	Sweden	Cross-sectional/parallel	Peri-implantitis	NR/166	Implants
			Healthy implants	NR/47	
Wang et al.[51]	USA	Cross-sectional/parallel	Peri-implantitis	34/34	Implants
			Healthy implants	34/34	
Canullo et al.[16]	Spain	Cross-sectional/pair	Peri-implantitis	….	Implants
			Healthy implants		
Canullo et al.[17]	Spain	Cross-sectional/parallel	Peri-implantitis	53/113	Implants
			Healthy implants	57/122	
Zheng et al.[52]	China	Cross-sectional/parallel	Peri-implantitis	NR/6	Individuals
			Healthy implants	NR/8	
Zhuang et al.[53]	China	Cross-sectional/paired	Peri-implantitis	….	Individuals
			Healthy implants		
			Periodontitis		

NR – not reported

Table 2 –

Descriptive results of studies comparing peri-implantitis with healthy implants by conventional methods.

Author	Case definition Pei-implantitis	Time of function	Confounders control	Outcomes	Microbial technics	Microorganisms evalated	Species with significant differences with healthy implants
Salceti et al.[36]	Peri-implant radiolucency and/or vertical crestal bone loss greater than 2.0 mm after the first year.	≥1 year	Antibiotics, peri-implant therapy and diabetes	Frequency of The total count > 10^4 of each species evaluated	Checkerboard	40 species	*P. nigrescens, P. micros, F. nucleatum* ss vin *F. nucleatum* ss nuc
Leonhardt, et al.[37]	Implant demonstrated a progressive marginal bone loss amounting >3 threads as compared to 1-year intra-oral radiographs.	≥5 years	Periodontal disease history, active periodontal disease and edentulims	Frequency of species cultivate	Culture	Major periodontophatic microorganisms and Opportunistic microorganism	*P. intermedia A. actinomycetemcomitans P. gingivaiis* Gram negative enteric roads
							No microorganisms were isolated in healthy implants in edentulous
Hultin et al. [39]	Implant showing radiographic marginal bone loss of three or more fixture threads (1.8mm) mesially or distally	≥1 year	Antibiotics, periodontal and peri-implant therapy disease history	Frequency of The total count > 10^6 of each species evaluated	Checkerboard	12 species Major periodontophatic microorganisms and *Selenomonas noxia* and *S. intermedia*.	No differences into the same patient except for *A.actinomycetemcomitans*
							Parallel control group *P. gingivalis, P. intermedia T. forsythia A.actinomycetemcomitans T. denticola, C. rectus*, *S.noxia* and *E. corrodens* were not present in edentulous with implants
Botero et al. [40]	Increased probing depth (>4 mm), bleeding on probing, and radiographic loss of bone support.	≥ 1 year	Antibiotics, diabetes and periodontal disease history	Frequency of species cultivate %cultivated (DS)	Culture	Periodontophatic and superinfecting bacteria	*P. intermedia/nigrescens* Gram negative Enteric rods
Renvert et al.[13]	3 threads of bone loss	5 years	Diabetes, periodontal	Proportion of The total	Checkerboard	Microbial complex 38	No differences in any species between

	between the 1-year and the final radio-graphic examinations. Presence of BOP		disease history and edentulims	count > 1 x 10^5 of each species evaluated		species	peri-implantitis and healthy implants
Shibli et al.[41]	Saucer-shaped osseous defects greater than 3mm and an inflamed peri-implant mucosa exhibiting bleeding on probing and/or suppuration	>2 years	Antibiotics, anti-inflammatory, peri-implant therapy, diabetes, active periodontal disease, implant with a coated surface and implants with mobility	mean proportion of each microbial complex and each specie 1 x 10^6±SD	Checkerboard	Checkerboard Microbial complex 36 species	*P gingivalis T. forsythia, T. denticola, Fusobacterium nucleatum ss Nucleatum Fusobacterium nucleatum ss vicentii P. intermedia*
Maxino et. al. [42]	Peri-implantitis: PD % 5mm with BOP and/or SUP and concomitant radiographic bone loss (bone loss % 3 threads until the half of implant length).	≥ 1 year	Antibiotics, peri-implant therapy Diabetes, smoking and periodontal disease activity	Mean Proportion of microbial complex Mean total counts for each species	Checkerboard	Microbial complex 38 species	*P. gingivalis, T. forshytia T. denticola* Species different between healthy implants and peri-implantitis in base line of the randomized clinical trial.
Casado et al.[14]	Clinical signs of inflammation, including implant mobility, suppuration in some cases, and radiographic signs of bone loss.	NR	Antibiotics, diabetes, smoking and periodontal disease activity	Frequency of each species	Nested-PCR	Major periodontopathic microorganisms	No differences in any species between peri-implantitis and healthy implants
Cortelli et al.[43]	Presence of PD > 5 mm with BOP and/or SUP	≥ 1 year	Antibiotics, peri-implant therapy,	Frequency of each specie	Conventional PCR	Major periodontopathic microorganis	*P .gingivalis, P. Intermedia T. Forsythia, C. rectus A.*

	and radiographic bone loss (bone level 3 threads)		diabetes, smoking and periodontal disease			ms	*actinomycetemcomitans T. denticola*
Ebadian et al. [9]	BOP around the implants with or without suppuration, had at least one implant site with PPD>5mm with radiographically crestal bone loss with a minimum of 2 exposed treads	≥ 1 year	Antibiotics, diabetes, periodontal disease GBR therapy All patients had periodontal disease	Frequency of each specie	DNA Hybridization probe	Major periodontopathic microorganisms	No differences in any species between peri-implantitis and healthy implants
Ata-Ali et al. [49]	Probing depth ≥4 mm, loss of supporting bone as estimated on radiographs, bleeding on probing or suppuration	≥2 years	Antibiotics, peri-implant therapy, diabetes, periodontal disease activity	Frequency of each specie	DNA Hybridization probe	Major periodontopathic microorganisms	*P. gingivalis*
Persson et al.[50]	Evidence of a vertical distance of 2 mm from the expected marginal bone level following remodeling post-implant placement.	>2 years	Antibiotics, peri-imlant therapy, smoking, periodontal disease history and periodontal disease activity	Frequency of microorganism by concentration 10^5	Checkerboard	40 species	*T. forsythia S. aureus*
Wang et al.[51]	Site displayed bleeding on probing (BOP) and/or suppuration and PPD >5 mm with radiographic bone loss with the	> 6 month	Antibiotics, smoking and biphosphonates	a) Frequency of detection of microorganims b) total bacterial counts for qPCR or Counts	qPCR	Major periodontopathic microorganisms	No differences in any species between peri-implantitis and healthy implants

	exposure of below the first thread based on radiograph.			with log10-transforme d			
Canullo et al. [16]	radiographic presence of bone loss of >3 mm with Probing pocket depth (PPD) ≥4 mm, Bleeding on probing (BoP) and/or suppuration	≥5 years	Antibiotics and peri-implant therapy	a) Frequency of detection of microorgan ims b) total bacterial counts for qPCR or Counts with log10^6	qPCR	Major periodontoph atic microorganis ms	No differences in any species between peri-implantitis and healthy implants
Canullo et al.[17]	radiographic presence of bone loss of >3 mm with Probing pocket depth (PPD) ≥4 mm, Bleeding on probing (BoP) and/or suppuration	>1year	Antibiotics and peri-imlant therapy	a) Frequency of detection of microorgan ims b) total bacterial counts for qPCR or Counts with log10^6	qPCR	Major periodontoph atic microorganis ms	*T. denticola E. corrodens P. micros*
Zhuang [53]	sites having PPD > 5 mm, presence of BOP confirmed by radiographic bone loss,	NR	Antibiotics, diabetes and periodontal disease history	Frequency of detection of microorgan ims	Conventio nal PCR	Major periodontoph atic microorganis ms *S. aureus*	No differences in any species between peri-implantitis and healthy implants

BOP – bleeding on probing; NR= Not reported; PCR – polymerase chain reaction; qPCR – quantitative polymerase chain reaction

Table 3 –

Descriptive results of studies comparing peri-implantitis with healthy implants or periodontitis by sequency methods.

Authors	Case definition Pei-implantitis	Time of function	Confounders control	Outcomes	Sequencing technic	Microorganims evaluated	Differences between peri-implantitis with healthy implants or periodontitis
Koyanagi et al.[19]	Probing depth (PD) >5 mm with BOP and/or SUP and concomitant radiographic bone loss more than three threads up to half of the implant length).	≥3 year	Antibiotics and smoking	Proportional distribution of number of clones of the phylum by OTU	clones sequenced using 16S rRNA gene clone library	All microbiome 335 sequences of 112 different species of 53 phyla.	The phyla *Chloroflexi, Tenericutes* and *Synergistetes* were observed only in peri-implantitis.
				Proportional distribution of bacterial species		Peri-implantitis vs. healthy implants or periodontitis.	Most of the bacterial species found in the healthy implants were also detected in the peri-implantitis and periodontitis. *Parvimonas micra, Peptostreptococcus stomatis, Pseudoramibacter alactolyticus*, and *Solobacterium moorei* were only detected in peri-implantitis.
Kumar et al. [18]	implant demonstrated clinical signs of inflammation along with radiographic evidence of bone loss after at least 1 year in function	1 year	Antibiotics, diabetes and periodontal status	Proportional distribution of number of clones of the phylum by OTU	16S pyrosequencing	All microbiome 397,286 sequences	Peri-implantitis had higher levels of *Actinomyces, Peptococcus, Campylobacter, non-mutans Streptococcus, Butyrivibrio and S. mutans* than healthy Implants
				Proportional distribution of bacterial species		Peri-implantitis vs. healthy implants or periodontitis	Higher levels of *Peptococcus, Mycoplasma, Eubacterium, Campylobacter, Butyrivibrio, S. mutans* and *Treponema* were observed in peri-implantitis compared with periodontitis.
							Peri-implantitis is a heterogeneous infection with predominantly Gram-negative species.
Tamura et al. [44]	Probing depth ≥ 4 mm, suppuration, BOP and visible three-thread loss of alveolar bone	≥6 month	Antibiotics and diabetes	Proportional distribution of bacterial genera	16S rDNA gene sequences	Sequences of Obligate anaerobic Gram negative rods (OGNRs) and asaccharolytic	The predominant species in the peri-implantitis compared with heathy implants were: *E. nodatum, E brachy, E saphenum, Filifactor alocis, Slackia exigua,*

	clearly extending around the implant as visualized on radiographs.					anaerobic gram-positive rods (AAGPRs) associated species	*Parascardovia denticolens* (AAGPRs)
						Peri-implantitis vs. healthy implants	*P. intermedia, F. nucleatum, P. gingivalis, Centipeda periodontii*, and *P. micra* (OGNRs)
							OGNRs and AAGPRs may also play an important role in peri-implantitis
Koyanagi et al. [10]	PD >5 mm with BOP and/ or SUP and bone loss more than three threads, up to half of the implant length.	5 years	Antibiotics	Proportional distribution of number of clones of the phylum by OTU	clones sequenced using 16S rRNA gene clone library	phylogenetic groups: Firmicutes, Bacteroidetes , Proteobacteria, Fusobacteria, Actinobacteria , TM7, Synergistetes , Spirochetes, Tenericutes, Chloroflexi and Deferribacter.	phyla *Chloroflexi* and *Deferribacteres* were only detected in peri-implantitis.
				Diversity: differences in nucleic acid sequence alignments		Peri-implantitis vs. periodontitis	*Eubacterium* spp. and *Porphyromonas* spp. showed higher prevalence in peri-implantitis than periodontitis.
							The numbers of periodontopathic bacteria were higher in peri-implantitis.
							species belonging to *Firmicutes*, such as *P. micra, Peptostreptococcus stomatis, Pseudoramibacter alactolyticus* and *Solobacterium moorei,* were only observed in peri-implantitis..
							Peri-implantitis had significantly higher 16S rRNA gene diversity than periodontitis.
Schauman et.al. [45]	bleeding on probing (BOP) and	≥ 1 year	Antibiotics, peri-implant therapy	Proportional distribution of	16S rRNA sequencing	43734 sequences 1 phylum, 8 classes, 10	Diversity of biofilms colonizing diseased implants was similar to

	pocket depth ≥ 4 mm and evidence of radiographic bone loss		and diabetes.	number of clones of the phylum by OTU taxonomic assignment		orders, 44 families and 150 genera Peri-implantitis vs. periodontitis	biofilms colonizing teeth affected by periodontitis.
Da Silva et al.[47]	saucer-shaped osseous defects greater than 3mm and an inflamed peri-implant mucosa exhibiting bleeding on probing and/or suppuration and probing depth ≥5 mm and an inflamed peri-implant mucosa		Antibiotics, anti-inflammatory, peri-implant therapy, diabetes, active periodontal disease, implant with a coated surface and edentulims	Proportional distribution of number of clones of the phylum.	16S rRNA sequencing	8 bacterial phyla: *Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, Synergistetes* and TM7. Peri-implantitis vs. healthy implants	Peri-implantitis showed a higher mean of clones of the phylum *Bacteroidetes* than healthy implants.
				Proportional distribution of bacterial genus			*F. nucleatum, C. gracilis, Dialister invisus, Streptococcus* sp.
							Human oral taxon (HOT) 064, *Eubacterium infirmum, Filifactor alocis* and *Mitsuokella* sp. HOT 131 presented a higher mean proportion in peri-implantitis compared with healthy implant in control and into the same individuals. *P. micra* and *P. intermedia* were higher in peri-implantitis in comparison with healthy implants into the same individuals.
Maruyama et al. [48]	Unclear	NR	NR	Overall bacterial community composition at phylum or genus level.	16S rRNA sequencing	436,320 sequences for the 16S rRNA gene 19 phyla, 188 genera, and 235 species were identified	Peri-implantiis microbiome was associated with unusual pathogens, such as *F. alocis*, *D. invisus* and *Mitsuokella* sp. HOT131.
				Diversity: OTU		Peri-implantitis vs. periodontitis	No differences were observed at phylum level between peri-implantitis and periodontitis.
				Core microbiota of peri-implantitis by species-level differences Species associated with			At genus level peri-implantitis had significantly higher levels of the genera *Olsenella,*
							Sphingomonas, Peptostreptococcus, and unclassified *Neisseriaceae* .
							The community diversity for all

				the clinical parameters			samples between peri-implantitis and periodontitis was not significant.
							P. nigrescens was more abundant in peri-implantitis. *Peptostreptococcaceae* SSP, HOT369 and *Desulfomicrobium orale* were more abundant in periodontitis. *Achromobacter xylosoxidans*, TM7 [G-5] sp. HOT-437, *Actinomyces massiliensis, Porphyromonas* sp. HOT-395, *P. nigrescens*, and *Prevotella oris* dominated in peri-implantitis when compared with periodontitis.The severity of the peri-implantitis was associated with an uncultured *Treponema* sp. HOT257 and *Eubacterium nodatum, Peptococcus* sp. HOT168, *Clostridiales* [F-1][G-1] sp. HOT093, and *Catonellamorbi.* Exist ecological differences between peri-implantitis and periodontitis.
Zheng et al. [52]	Unclear	NR	NR	Diversity of OTU	pyrosequencing of the 16S rRNA gene	424,579 sequences for the 16S rRNA gene	Microbial diversity was higher in plaque samples from peri-implant it is compare with healthy implant.
				Relative abundance of microbial taxonomic groups of OUT		Peri-implantitis vs. healthy implants	The phylogenetic diversity was higher In peri-implantitis.
				Analysis at the species level			The dominant phyla at implant sites were: *Firmicutes, Bacteroidetes*, *Fusobacteria, Actinobacteria,* and *Proteobacteria*
				Quantification of			*Leptotrichia hofstadii,*

				bacterial loads of the Eubacterium brachy subgroup			*Eubacterium infirmum, Kingella denitrificans, Actinomyces cardiffensis,*
							Eubacterium minutum, Treponema lecithinolyticum, and *Gemella sanguinis* were higher in Peri-implantitis. *Eubacterium brachy* subgroup was significantly higher in peri-rmplantitis. Analysis of the co-occurrence revealed a positive correlation between
							Eubacterium minutum and Prevotella intermedia Eubacterium minutum was correlated with P. intermedia which suggests the association of Eubacterium with peri-implantitis

BOP - bleeding on probing; OUT - operational taxonomic unit; SUP - suppuration

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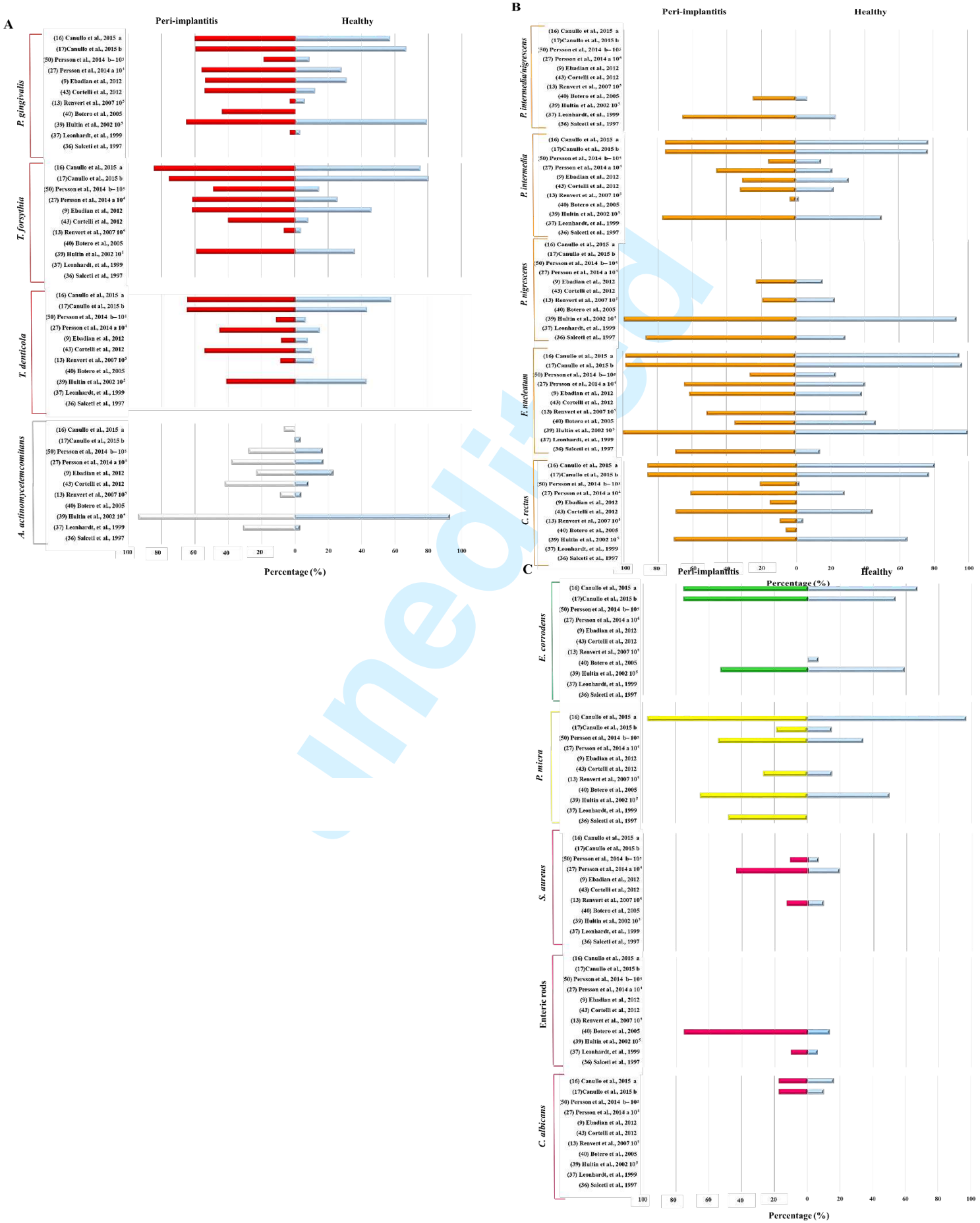

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