Systematic Review
Periodontics

Prevalence of *Enterococcus* species in adults with periodontal health or with periodontitis: a systematic review

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0019

Submitted: November 8, 2021
Accepted for publication: September 19, 2022
Last revision: September 30, 2022

Abstract: The aim of this study was to evaluate the prevalence of *Enterococcus* species in the mouth of adults with periodontal health and periodontitis. A systematic search was made in databases in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The search for articles was conducted in Medline/PubMed, Latin American and Caribbean Health Sciences Literature Database (LILACS), Cochrane Library, Scopus, Embase, Web of Science databases and in the System of Information on Grey Literature in Europe (SINGLE) and included articles published in English up to April 25th, 2021. Observational studies in humans with and without periodontitis were evaluated to identify the prevalence of *Enterococcus* species. Articles that met the inclusion criteria were analyzed and classified to determine the quality rating in good, fair, and poor. A new detailed checklist for quality assessment was developed based on the information required for applicable data extraction in reviews. The study design, sample size, demographic data, periodontal clinical parameters, microbial analysis method, biological sample, prevalence of *Enterococcus* spp., and correlations with periodontal clinical parameters were assessed. After screening and full-text reading, 8 articles met the inclusion criteria. All selected studies showed a significantly higher prevalence of *Enterococcus spp.* in patients with periodontitis compared with periodontally healthy patients. Thus, the present systematic review suggests that the prevalence of *Enterococcus faecalis* in the mouth of periodontitis individuals is higher than that of periodontally healthy individuals.

Keywords: Enterococcus; *Enterococcus faecalis*; Periodontitis.

Introduction

Enterococci are Gram-positive, facultative cocci bacteria that are increasingly associated with nosocomial infections such as septicemia, infective endocarditis, urinary tract infections, burn wounds, and indwelling foreign devices.[1,2] This genus normally inhabits the gastrointestinal tract, oral cavity, and vagina of humans.[1]

Periodontitis is a disease that results in a chronic inflammatory process in the periodontium triggered by an imbalance in the subgingival microbiota of the host. Consequently, this subgingival imbalance and change in

micro-environment may favor colonization and proliferation of Enterococci species. Overall, *E. faecalis* has been found at a low frequency in the healthy oral cavity. Conversely, in individuals with oral diseases, such as caries, endodontic infections, periodontitis, and peri-implantitis, this species has been found in high proportions and frequency. However, data on prevalence vary widely among the different studies.[3-5]

It has been indicated that *E. faecalis* is the species most commonly recovered from teeth with failed endodontic treatment and persistent infection, probably due to its high resistance to endodontic medicaments and the ability to form recalcitrant biofilms both in treated and untreated root canals.[6,7] Some studies have indicated an increase in *E. faecalis* prevalence in individuals with periodontitis, but the correlation between the prevalence of this pathogen and periodontal disease remains unclear.[8,9]

Some enterococci species are categorized by the World Health Organization (WHO) as "in great need of attention for the development of new antimicrobials" for their control. Recently an alarming quantity of *E. faecalis* and *E. faecium* species resistant to vancomycin has appeared worldwide,[10] and therefore several strains, potentially multidrug-resistant, are globally scattered. In addition, standard treatment is ineffective against these strain, hence a great clinical concern has arisen about how best to prevent and treat human enterococcal infections.[11]

In view of the worldwide panorama showing the ever-increasing enterococcus-related infections resistant to antibiotics, it is extremely important to evaluate the prevalence of enterococci in a diseased and healthy mouth, as the oral cavity may constitute a critical reservoir of potently virulent, antibiotic-resistant enterococci species. Therefore, the purpose of this study was to determine the occurrence of enterococci species in samples from healthy patients and from patients with periodontitis through a systematic review of the available literature.

Methodology

Protocol and Registration

This study was submitted to the Prospective Register of Systematic Reviews (CRD42020060942), according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[12]

Literature search

A search strategy was used to identify articles with *Enterococcus spp.* prevalence information in individuals with periodontal health (PH) and with periodontitis (P). Medline/PubMed, Scopus, Embase, Cochrane Library, Web of Science, and Latin American and Caribbean Health Sciences Literature (LILACS) databases were first screened according to the protocol inclusion criteria. Furthermore, the System of Information on Grey Literature in Europe (SINGLE) was used as an additional source to refine the search. The search was performed up to April 25th, 2021.

Keywords and MeSH terms for the search were "Periodontal diseases", "Periodontal disease*", "Periodont*", "Enterococcus", "Enterococcu*", "periodontal", "disease*". These terms were combined using the Boolean operators "AND" and "OR". All the terms were adapted to the different databases.

The articles identified in more than one database were considered duplicates and excluded using a reference manager software (EndNote®, version X7, Thomson Reuters) or manually. Alerts with the search protocol were created for each database. A hand search was performed in the references of the selected articles to complement the previous searches.

Eligibility criteria and selection process

The focus question of this review was "Is there a difference in the prevalence of *Enterococcus* species between periodontally healthy patients and periodontitis patients?". PECO (Population, Exposure, Comparator and Outcomes) question was used as a search strategy framework to identify publications that could answer the main question.

P = adults
E = periodontitis
C = periodontal health
O = prevalence of enterococcus species

The following inclusion criteria were used: observational studies conducted in humans, samples from at least one group of individuals with periodontitis and one with periodontally healthy individuals (control group) and any type of microbiological assessment of at least one Enterococcus species. Periodontitis was considered based on both the 1999 classification, which established "chronic" and "aggressive" periodontitis, and the most recent 2017 classification, which combines the two categories into one, named "periodontitis".

The exclusion criteria were: studies with sample selection focused on systemic diseases or conditions, such as studies of individuals with HIV infection, diabetes, and other similar limitations, studies focused on other subtypes of periodontitis such as apical periodontitis associated with endodontics, necrotizing periodontitis, and periodontitis as a manifestation of a systemic disease. Review articles, case reports, descriptive studies, opinion articles, technical articles, guidelines, animal studies, pilot studies, studies "*in vitro*", studies with exogenous or other manifestations were excluded.

Two researchers (LCPE and AMO) selected the articles by title and abstract from the databases. In case of disagreement, a third research (RMS) was consulted. The null hypothesis of this systematic review was that there is no difference in *Enterococcus* prevalence between patients with periodontal health and patients with periodontitis.

Data extraction, quality assessment and risk of bias

All data extracted from the included articles were tabulated and included: study design, author, year, country in which the study was conducted, number of subjects in case and control groups, statistical analysis, and results.

The articles were read in full and those that met the inclusion criteria were carefully analyzed and their methodological aspects were described in Table 1. The methodological quality and risk of bias of the studies was assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies tool.[13] The possible answers of the tool's categories were: yes, no, cannot determine (ND), not applicable (NA), and not reported (NR).

The assessment for each checklist question was standardized by the examiners. Some criteria were considered in the evaluation of the quality of the studies. The presence of a control group with periodontal health, the sample size calculation and information about the sample origin, and where the study was conducted (private or public institutions) were considered. On the other hand, the lack of exclusion of some known factors related to bias in periodontal research was considered a negative feature in the study. Some of those confounding factors were: tobacco smoking, ongoing orthodontic treatment, diabetics and other systemic diseases that may have periodontal repercussions, use of antibiotics and/or anti-inflammatory drugs in the last 6 or 3 months prior to the study, and periodontal treatment in the last 6 months prior to the study evaluation.

For those studies in which the known confounding factors were considered we evaluated if there was adjustment through statistical analysis. Another relevant information considered was how periodontal status was classified, as the definition of periodontal health, gingivitis and periodontitis is essential for standardized and comparable results that allow reliable assessment of enterococcal species among individuals with different periodontal status.

Calibration of periodontal measurements, details about laboratory protocols of sample preparation and technique used, as well as use of control samples and tests done in duplicate or triplicate were important factors for the evaluation of study quality. After data extraction, confounders and biased results of each study were analyzed to certify that results were not due to chance or biased in one direction.

Once all detailed information was collected and evaluated, the studies were classified as "good", "fair", and "poor". If a study was classified as poor, additional comments were made explaining the reason.

Results

Study selection

A total of 1,461 titles and abstracts were screened from the database search allocated as follows:

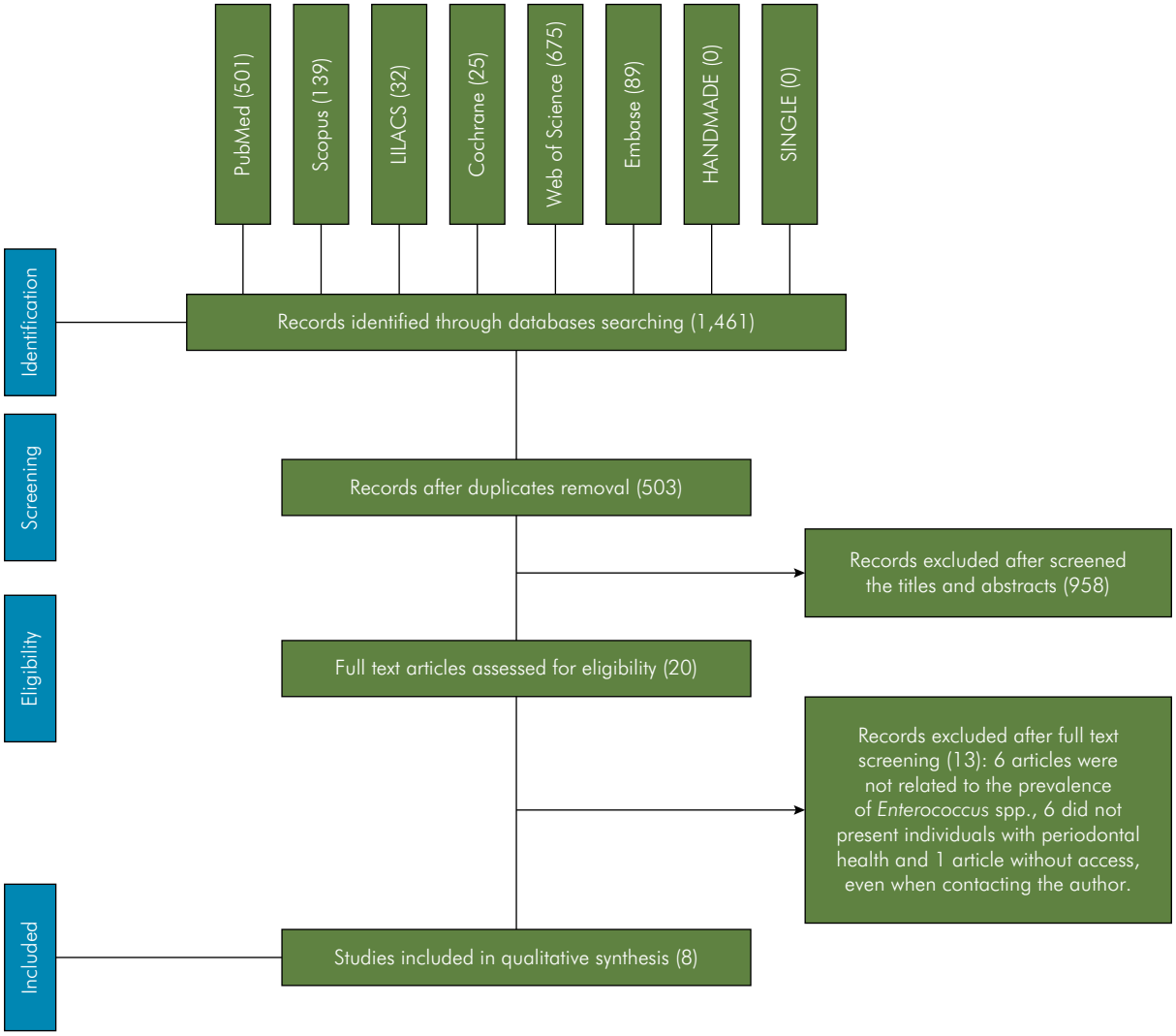
Table 1. Summary of the characteristics of included studies.

Author, year, country	Espíndola et al., 2021, Brazil			Chidambar et al., 2019, India		Colombo et al., 2002, Brazil		Colombo et al., 2013, Brazil		Silva-Boghossian et al., 2011, Brazil			Fritoli et al., 2017, Brazil		Souto et al., 2006, Brazil		Souto & Colombo, 2008, Brazil	
Type of study	Cross-sectional clinical study			Cross-sectional clinical study		Cross-sectional clinical study		Cross-sectional clinical study		Cross-sectional clinical study			Cross-sectional clinical study		Cross-sectional clinical study		Cross-sectional clinical study	
Participants	**PH**	**G**	**P**	**PH**	**PD**	**PH**	**PD**	**PH**	**PD**	**PH**	**CP**	**AP**	**PH**	**PD**	**PH**	**PD**	**PH**	**PD**
(sample size)	**-139**	**-103**	**-305**	**-18**	**-48**	**-14**	**-25**	**-12**	**-46**	**-51**	**-219**	**-90**	**-10**	**-30**	**-3**	**-14**	**-56**	**-169**
Age | mean ± SD	28.3 ± 11.1	29.2 ± 12	43.5 ± 12.9	-	-	34 ± 0.6	41 ± 2	37.3 ± 12.1	47.2 ± 13.7	30.6 ± 1.5*	45.4 ± 0.7*	31.4 ± 0.6*	-	-	-	-	34.3 ± 12	41 ± 14
% Genders		194M 351F		-	-	14 M 86 F	52 M 48 F	25 M 75 F	41 M 59 F	37 M 63 F	40 M 60 F	33 M 67 F	-	-	-	-	35.5 M 64.5 F	34.2 M 65.8 F
% Smokers		-		-	-	15	20	17	22	11.6	44.7	7.1	-	-	-	-	-	-
Missing teeth ± SD	0.9 ± 2.0	1.9 ± 3.1	4.0 ± 4.1	-	-	1.3 ± 0.5*	4.3 ± 0.5*	-	-	-	-	-	-	-	-	-	0.6 ± 1.5	5.2 ± 5.3
Probing depth (mm) ± SD	1.2 ± 0.3	2.0 ± 0.2	3.0 ± 1.07	-	-	1.8 ± 0.2*	3.3 ± 0.3*	2 ± 0.6	6.17 ± 3.2	1.8 ± 0.04*	2.9 ± 0.06*	3.9 ± 0.09*	-	-	1.8 ± 0.04*	4.6 ± 0.1*	2.1 ± 0.5	3.2 ± 1.2
Attachment level (mm) ± SD	1.8 ± 0.5	2.0 ±0.4	3.4 ± 1.3	-	1.6 ± 0.43	1.7 ± 0.1*	3.6 ± 0.2*	1.4 ± 0.5	6.6 ± 2.1	1.7 ± 0.06*	3.5 ± 0.08*	4.3 ± 0.12*	-	-	1.8 ± 0.04*	4.5 ± 0.1*	2.2 ± 0.6	3.9 ± 1.5
% sites with PL ± SD	10.9 ± 12.2	35.7 ± 16.6	51.1 ± 23.2	12.33	68.56	34± 8.5*	70 ± 4*	1	57	13.1 ± 2.7*	63.8 ± 1.8*	70.3 ± 2.5*	-	-	27 ± 4*	82 ± 3*	10.1 ± 10	49 ± 30
% sites with BOP ± SD	3.5 ±3.2	21.2 ± 14.8	41.9 ± 26.8	9.5	64.31	13 ± 7*	55 ± 3*	17	58	4.0 ± 0.5*	40.9 ± 1.6*	64.5 ± 3.1*	-	-	8 ± 1*	56 ± 7*	1.9 ± 4.1	47.2 ± 29
% sites with SUP ± SD	0	0	0.5 ± 2.2	-	-	0*	14 ± 3*	-	-	-	-	-	-	-	-	-	0	3 ± 10
Diagnostic method to detect *Enterococcus spp.*				Culture		Checkerboard DNA-DNA hybridization		PCR		Checkerboard DNA-DNA hybridization			Checkerboard DNA-DNA hybridization		Checkerboard DNA-DNA hybridization		PCR	
Biological sample	Subgingival biofilm			Subgingival biofilm		Subgingival biofilm		Buccal epithelial cells and gingival crevice epithelial cells		Subgingival biofilm			Subgingival biofilm		Subgingival biofilm		Subgingival biofilm and saliva	
Prevalence of *Enterococcus spp.* (% colonized sites)	2.2	7.8	9.8	0	41.7	42	75	24.2 buccal epithelial cells 17.1 gingival crevice epithelial cells	None	35	46	31	18.3 ± 3.4	75.1 ± 5.1	42	83	14.6 saliva 17.1 subgingival biofilm	40.5 saliva 47.8 subgingival biofilm
Correlation between periodontal clinical parameters and presence of *Enterococcus spp.*						Correlation was observed with periodontal clinical parameters, age and sex		No correlations were found with smoke, sex, age, PPD> 6mm, CAL and BOP		Correlation was found in *E. faecalis* and PL			-		No correlations were found with periodontal status		Positive correlations were observed with periodontal clinical parameters, except for SUP and tooth loss	

-: not reported; AP: aggressive periodontitis; BOP: bleeding on probing; CAL: clinical attachment level; CP: chronic periodontitis; PCR: polymerase chain reaction; PPD: Pocket Probing depth; PH: Periodontal Health; PL: supragingival plaque; PD: Periodontitis; SUP: suppuration index; SD: standard deviation; M: male; F: female. * Standard error of mean (SEM) was used in those cases.

PubMed (n = 501), Scopus (n = 139), LILACS (n = 32), Cochrane Library (n =25), Embase (n = 89), and Web of Science (n = 675). No results were found in SINGLE or in the hand search. Five hundred and three duplicate records were removed. All titles and abstracts (n = 957) were methodically examined by three researchers, and 937 were excluded. The full text of 20 articles was analyzed to confirm if they met the inclusion criteria. Eight were included in the systematic review (Figure).

All 8 studies had a cross-sectional design and were performed in university dental schools. Seven studies were carried out in Brazil and 1 in India. None presented sample size calculation, but this was not considered a major qualitative failure due to the limitations of the study design. Other limitations found were the absence of blinding and lack of more than one clnical evaluation over time. Most studies had potential confounding variables, such as inclusion of smokers in the sample. Smoking is known to increase susceptibility to periodontitis, increase its severity, and cause microbiological alterations when compared with non-smokers. Only three studies did not include variables that could confound the results (Table 2), but none of the studies had a high risk of bias in the quality assessment.



LILACS: Latin American and Caribbean Health Sciences Literature Database; HANDMADE: Hand search; SINGLE: System of Information on Grey Literature in Europe.

Figure. PRISMA based flowchart diagram of literature search.

Table 2. NIH assessment for risk of bias.

Author, year, country	Espíndola et al., 2021, Brazil	Chidambar et al., 2019, India	Colombo et al., 2002, Brazil	Colombo et al., 2013, Brazil	Silva-Boghossian et al., 2011, Brazil	Fritoli et al., 2017, Brazil	Souto et al., 2006, Brazil	Souto and Colombo, 2008, Brazil
Clear Objective	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Population clearly specified	Yes	Yes	No	No	Yes	No	No	No
Participation rate of eligible persons of at least 50%	NR	NR	NR	NR	NR	NR	NR	NR
Individuals selected from the same population	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size calculation	Yes	No	No	No	No	No	No	No
Exposure(s) of interest measured prior to the outcome(s) measurement	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sufficient time frame to see an association between exposure and outcome (if present)	No	No	No	No	No	No	No	No
Different levels of the exposure examined	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Exposure measures clearly defined	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Exposure(s) assessed more than once over time	No	No	No	No	No	No	No	No
Outcome measures clearly defined	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Outcome assessors blinded	No	No	No	No	No	No	No	No
Loss to follow-up after baseline - 20% or less	NA	NA	NA	NA	NA	NA	NA	NA
Potential confounding variables measured and statistically adjusted	No	No*	No	No	No	No*	No	No*

ND: cannot determine; NA: not applicable; NR: not reported. * The studies did not include any confounding variables in their samples.

All authors were contacted for the disclosure of additional raw data. Additional data was only provided by the authors Colombo et al.,[14] Silva-Boghossian et al.,[16] Souto et al.,[17] Souto and Colombo,[9] and Espíndola et al.,[20] even though the data did not contribute to the analysis.

Demographic and clinical periodontal parameters

Colombo et al. showed that patients with PD had significantly more signs of disease, including higher means for missing teeth, probing pocket depth (PPD), attachment level (CAL), % of sites with

supragingival plaque (PL), suppuration (SUP), and bleeding on probing (BOP) compared to PH subjects and a significant difference in age and sex between the groups.[14] Those finding were replicated in another study by Colombo et al., in which subjects with PD showed greater mean PPD, CAL, and other parameters like BOP and percentage of sites with supragingival plaque accumulation than healthy patients.[15]

Silva-Boghossian et al.[16] evaluated the difference in clinical periodontal parameters between PH and PD patients and the presence of *E. faecalis* detection correlated positively with PL. Similar clinical results were also found by Souto et al.[9] and Souto and Colombo.[17] Tooth loss and SUP were the only clinical parameters that did not correlate with presence of *E. faecalis* in Souto and Colombo study.[9]

The study by Chidambar et al.[19] corroborated the previous results, in which PD patients had more signs of disease compared to PH subjects and compared even with those with gingivitis (GG). In contrast, Fritoli et al.[18] did not describe the periodontal clinical data. Espíndola et al.[20] found differences in clinical periodontal parameters between PH, GG, and PD patients, following the already expected pattern of higher PPD, CAL, SUP, BOP, and PL in PD than in PH. All the results are in accordance with the literature, as individuals with PD have more pronounced clinical disease parameters, which in turn increase with disease severity.

Enterococcus spp. detection

All eight studies compared the prevalence of *E. faecalis* in PH and PD groups. No other *Enterococcus* species was assessed in the studies. Colombo et al. observed that *E. faecalis* was more frequently detected in PD subjects (75% prevalence in PD and 42% in PH).[14] The difference was statistically significant after adjustment for age and gender. In contrast, Colombo et al. did not find *E. faecalis* in buccal and gingival crevice epithelial cells of PH patients, while in PD individuals the species was found with a prevalence of 24.2% in buccal epithelial cells and 17.1% in gingival crevice epithelial cells. In addition, cells samples containing *E. faecalis* were detected in 57% of subjects with PPD and CAL > 6 mm, but no significant correlations were found.[15]

Silva-Boghossian et al. detected *E. faecalis* in 35% of those with PH and approximately 42% in PD. [16] Souto et al.[17] found a prevalence of 42% in PH and 83% in PD. In a later study conducted by Souto and Colombo,[9] *E. faecalis* was detected in 34.9% of all samples evaluated (34.6% in subgingival biofilm and 35.1% in saliva). No statistical significance was found between sample prevalence. The overall detection of *E. faecalis* was lower than in previous studies of the same group. However, differences in methodology must be considered, as checkerboard and PCR techniques have different sensitivity and specificity levels.

Fritoli et al.,[18] who also used the checkerboard technique, found a similar prevalence to Souto et al.,[17] with 90% of individuals with PD and 50% of subjects with PH having *E. faecalis* in their oral cavity. Chidambar et al.,[19] using the culture method, found a significant difference in the presence of *E. faecalis* in individuals with PH (0%) and P (41.7%) in the Indian population.[19] More recently, Espíndola et al.[20] isolated *Enterococcus* spp. from 7.4% of all samples, with higher prevalence and significant difference in PD (9.8%) and GG (7.8%) than in PH (2.2%), using a selective culture method and further confirmation using MALDI-TOF.

Even though prevalence varies greatly among studies, the overall results were very consistent in describing a higher prevalence of *E. faecalis* in periodontitis subjects compared to healthy controls.

Techniques for detecting Enterococcus spp

Colombo et al.[14] analyzed subgingival plaque samples to determine the prevalence of *E. faecalis* by a modification of the checkerboard DNA-DNA hybridization method described by Socransky et al.,[21,22] and Silva-Boghossian et al.,[16] Fritoli et al.,[17] and Souto et al.[18] used the same method.

Souto & Colombo used conventional PCR method in samples of saliva and subgingival biofilm.[9] Later, Colombo et al.[14] detect *E. faecalis* in buccal and gingival crevice epithelial cells through quantitative real-time PCR using universal and species-specific primer sets. Chidambar et al.[19] and Espíndola et al.[20] did not use a molecular technique, but culture methods to detected *E. faecalis* in subgingival biofilm.

Discussion

Several studies in recent years have focused on the relationship between periodontal diseases and/or oral bacteria and systemic diseases, in particular bacteria of medical importance.[23] Studies focused on prevalence and role of opportunistic species in the oral cavity have been growing, as microorganisms that grown in biofilm, such as dental plaque, tend to be less susceptible to the action of antimicrobials and the immune system, which can lead to serious clinical implications, such as re-infection and therapeutic failure. [24]

Most studies were carried out in convenience samples of the Brazilian population, and one study examined the Indian population. Differences in *Enterococcus spp.* detection described in the literature may result from differences in the studied population, patients' oral and/or systemic health conditions, type and number of clinical samples, and detection methods. Studies have demonstrated that the periodontal microbiota can vary greatly in frequency and proportion in different ethnicities and geographic locations.[14,25] In addition to these factors, the threshold for the classification of health and disease adopted in the various studies can directly influence the results. The role of *Enterococcus spp.* in periodontal disease is still unknown, requiring further research. Nonetheless, *Enterococcus spp.* has various virulence factors that may be related to periodontal inflammation and tissue destruction.[1] Anderson et al. evaluated the virulence of *E. faecalis* isolates from oral cavity, food, and clinical specimens, and reported that oral isolates had the highest percentages of virulence genes, highest levels of extracellular enzymes and the greatest capacity to form biofilms.[26] Several virulence factors in human infections have been studied.[1,27]

Colombo et al.[14] examined the presence and levels of *E. faecalis* in the subgingival microbiota of untreated PD patients and healthy controls using the checkerboard method and observed more higher detection of *E. faecalis* in periodontitis patients. Chidambar et al.,[19] using culture method only, detected *E. faecalis* in PD patients (47.1%), showing the lower sensitivity of this method compared to molecular techniques. Likewise Espíndola et a.l[20] detected *Enterococcus* spp. at a low prevalence in PD (9.8%) and GG (7.8%). In contrast, Rams et al.,[5] using culture methods, detected *E. faecalis* in only 1% of patients with early onset periodontitis and in 5.1% in those with chronic periodontitis.

Investigations have demonstrated that molecular biology methods are more effective than culture in detecting enterococci in different samples.[28,29] There are many reasons for the higher detection of *E. faecalis* by PCR compared to culture, such as the ability of molecular methods to detect DNA from dead cells and the higher sensitivity of molecular biology methods.

The checkerboard DNA-DNA hybridization method used by Colombo et al. detected a similar high prevalence of *E. faecalis* in PD subjects.[14] Molecular biology has emerged as an effective, accurate and reliable form of detection of bacteria that are difficult to grow in culture medium and therefore harder to identify by conventional techniques.[30] Colombo et al.[15] did not detected *E. faecalis* in periodontally healthy patients, but found the species in buccal epithelial cells and gingival crevice epithelial cells of patients with periodontitis in a significantly higher frequency using quantitative real-time PCR.

Souto and Colombo,[17] also using checkerboard method, observed a higher prevalence of *E. faecalis* in patients with periodontitis. However, no correlation could be established between presence of these specie and periodontal clinical parameters. In contrast, Souto and Colombo[9] observed a modest positive correlation between prevalence of *E. faecalis* and PPD, CAL, % sites with PL, and % sites with BOP parameters, but not % sites with SUP and tooth loss.

Silva-Boghossian et al.[16] detected *E. faecalis* in 35% in PH, 46% in CP, and 41% in aggressive periodontitis (AP) using the checkerboard method. This study used a multivariate logistic regression model to differentiate between CP and AP and found that a consortium of microorganism, including *E. faecalis*, were more likely to be found in CP or even PH. It is also possible that this species counterbalances the deleterious effects of A.a, a putative pathogen associated with AP, diminishing the risk of this form of disease. Their study found *E. faecalis* at a lower frequency in patients with AP compared to

CP. However, periodontal classifications can change microbiological associations, such as the most recent classification which does not differentiate between aggressive and chronic periodontitis.[31] It has been shown that *E. faecalis* may be present in different layers of the oral biofilm, aggregating with different oral species.[32] Besides, the ability of *E. faecalis* to form biofilm and adhere and invade soft-tissues allows it to survive in many hostile environments such as the periodontal pocket.[33]

Fritoli et al.[18] showed that the prevalence of *E. faecalis* was higher in periodontitis patients than in periodontally healthy patients. The same group suggested that there was moderate evidence of the role of *E. faecalis* as a periodontal pathogen. Espíndola et al.[20] isolated *Enterococcus* spp. from 7.4% of all samples, 53.7% of which were *Enterococcus faecalis*. Species were more prevalent in periodontitis (9.8%) and gingivitis (7.8%) than in PH (2.2%), but there was no differences among stages of disease severity, per the current periodontal classification.

These clinical studies have shown that the oral cavity is a reservoir for *E. faecalis*, particularly in periodontitis. More attention should be given to periodontitis patients, because the prevalence of opportunistic species in subgingival biofilms poses an increased risk for the development and progression of systemic complications.[34]

Conclusion

Based on the limited data provided by the studies included in this systematic review, it is possible to conclude that the frequency of *Enterococcus faecalis* is higher in individuals with periodontitis in comparison with individuals with periodontal health.

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