

Periodontal pathogens and clinical parameters in chronic periodontitis

Emile Boyer, Bénédicte Martin, Sandrine Le Gall-David, Shao Bing Fong,

Yves Deugnier, Martine Bonnaure-Mallet, Vincent Meuric

► To cite this version:

Emile Boyer, Bénédicte Martin, Sandrine Le Gall-David, Shao Bing Fong, Yves Deugnier, et al.. Periodontal pathogens and clinical parameters in chronic periodontitis. Molecular Oral Microbiology, 2020, 35 (1), pp.19-28. 10.1111/omi.12274 . hal-02394270

HAL Id: hal-02394270 https://univ-rennes.hal.science/hal-02394270

Submitted on 19 Dec 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Periodontal pathogens and clinical parameters in chronic periodontitis

Journal:	Molecular Oral Microbiology
Manuscript ID	MOM-07-19-1075.R3
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Boyer, Emile; INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer Martin, Bénédicte; Univeristé de Rennes 1, Microbiology - EA1254 Le Gall-David, Sandrine; INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer Fong, Shao Bing; INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer Deugnier, Yves; INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer Bonnaure-Mallet, Martine; Université de Rennes 1, Equipe de Microbiologie UPRES-EA 1254 Meuric, Vincent; INSERM, INRA, Univ Rennes 1, Univ Bretagne Loire, Nutrition Metabolisms and Cancer, ;
Keywords:	Genetics < Oral Microbiology, Periodontal Disease, Ecology < Oral Microbiology, Dysbiosis
Summary	<u>Background</u> : The use of Next Generation Sequencing and bioinformatics have revealed the complexity and richness of the human oral microbiota. While some species are well known for their periodontal pathogenicity, the molecular-based approaches for bacterial identification have raised awareness about new putative periodontal pathogens. Although they are found increased in case of periodontitis, there is currently a lack of data on their interrelationship with the periodontal measures. <u>Methods</u> : We processed the sequencing data of the subgingival microbiota of 75 patients with haemochromatosis and chronic periodontitis in order to characterize well-described and newly identified subgingival periodontal pathogens. We used correlation tests and statistical models to assess the association between the periodontal pathogens and mean pocket depth, and to determine the most relevant bacterial biomarkers of periodontitis severity. <u>Results</u> : Based on correlation test results, nine taxa were selected and included in the statistical models. The multiple linear regression models adjusted for systemic and periodontal clinical variables showed that mean pocket depth was negatively associated with Aggregatibacter and Rothia, and positively associated with Porphyromonas. Furthermore, a bacterial ratio that was previously described as a signature of dysbiosis in periodontitis, (%Porphyromonas+%Treponema+%Tannerella)/(%Rothia+%Corynebacterium), was the most significant predictor. <u>Conclusion</u> : In this specific population, we found that the best model in

predicting the mean pocket depth was microbial dybiosis using the dysbiosis ratio taxa formula. While further studies are needed to assess the validity of these results on the general population, such a dysbiosis ratio could be used in the
future to monitor the subgingival microbiota.
SCHOLARONE"
Manuscripts
indituseripes

Periodontal pathogens and clinical parameters in chronic periodontitis

Running Title: Dysbiosis in periodontitis

1	
2 3	
4	
5	
6 7	
, 8	
9	
10	
12	
13	
14 15	
16	
17	
18 19	
20	
21	
22	
24	
25	
26 27	
28	
29	
31	
32	
33 34	
35	
36	
37	
39	
40	
41 42	
43	
44	
45 46	
47	
48	
49 50	
51	
52 52	
55	
55	
56 57	
58	
59	
60	

3	Emile Boyer ¹ ; Bénédicte Martin ¹ ; Sandrine Le Gall-David ¹ ; Shao Bing Fong ¹ ; Yves Deugnier ² ;
4	Martine Bonnaure-Mallet ¹ ; Vincent Meuric ¹
5	¹ INSERM, INRA, Univ Rennes 1, CHU de Rennes, Nutrition Metabolisms and Cancer, Rennes,
6	France
7	² CIC 1414, Inserm, Rennes, 35033, France
8	Corresponding author:
9	Vincent Meuric
10	2, avenue du Professeur Léon Bernard, 35043 Rennes, France
11	Telephone number: +33 2 23 23 49 19 Fax number: +33 2 23 23 49 13
12	E-mail: <u>vincent.meuric@univ-rennes1.fr</u>
13	

14 Abstract

Background: The use of Next Generation Sequencing and bioinformatics have revealed the complexity and richness of the human oral microbiota. While some species are well known for their periodontal pathogenicity, the molecular-based approaches for bacterial identification have raised awareness about new putative periodontal pathogens. Although they are found increased in case of periodontitis, there is currently a lack of data on their interrelationship with the periodontal measures.

<u>*Methods*</u>: We processed the sequencing data of the subgingival microbiota of 75 patients with haemochromatosis and chronic periodontitis in order to characterize well-described and newly identified subgingival periodontal pathogens. We used correlation tests and statistical models to assess the association between the periodontal pathogens and mean pocket depth, and to determine the most relevant bacterial biomarkers of periodontitis severity.

Results: Based on correlation test results, nine taxa were selected and included in the statistical models. The multiple linear regression models adjusted for systemic and periodontal clinical variables showed that mean pocket depth was negatively associated with Aggregatibacter and Rothia, and positively associated with Porphyromonas. Furthermore, a bacterial ratio that was described signature of dysbiosis previously periodontitis, as а in (%Porphyromonas+%Treponema+%Tannerella)/(%Rothia+%Corynebacterium), was the most significant predictor.

33 <u>Conclusion</u>: In this specific population, we found that the best model in predicting the mean
 34 pocket depth was microbial dybiosis using the dysbiosis ratio taxa formula. While further studies
 35 are needed to assess the validity of these results on the general population, such a dysbiosis ratio
 36 could be used in the future to monitor the subgingival microbiota.

37 Keywords

38 Deep sequencing; potential periodontal pathogens; chronic periodontitis; dysbiosis.

to Review Only

1 Introduction

Periodontitis is a worldwide health problem and the 6th most common disease globally (Frencken et al., 2017). It is a chronic inflammation produced in response to a disease-associated multispecies bacterial community with intermittent episodes of remission and relapse, characterized by alveolar bone loss. The current model of periodontitis pathogenesis implies a synergistic polymicrobial community and a dysregulated host inflammatory response (G. Hajishengallis & Lamont, 2012). Indeed, a dysbiosis of the complex and diverse oral microbiota (Paster et al., 2001) that inhabits the sulcus is concomitant with the occurrence of a periodontal pocket. This pathognomonic sign of the periodontitis can be quantified as pocket depth (PD) and clinical attachment loss (CAL) (Lamont, Koo, & Hajishengallis, 2018).

Subgingival colonization by specific species, including the red complex bacterial species, i.e., Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola, has been reported to be related to PD and bleeding as determined by DNA-DNA checkerboard analysis (Socransky, Haffajee, Cugini, Smith, & Kent, 1998). P. gingivalis is also considered as the keystone pathogen of the disease, that is able to disrupt the immune system allowing for dysbiosis to arise (George Hajishengallis, Darveau, & Curtis, 2012; Jiao, Hasegawa, & Inohara, 2014). The development of contemporary molecular methods (Next Generation of Sequencing – NGS) has expanded the list of potential periodontal pathogens. The review by Pérez-Chaparro *et al.* identified in the literature 29 species and one genus putatively implicated in chronic periodontitis that have been cited by at least two different authors (Pérez-Chaparro et al., 2014). However, these results are usually based on health *versus* disease comparisons. The relationship between periodontitis measures and these taxa are lacking. Using NGS data from the literature, we have previously defined a bacterial ratio

as a signature of periodontitis that could score the dysbiosis of the subgingival microbiota (Meuric, Le Gall-David, et al., 2017). However, its relationship with the global periodontal status remains to be tested.

We have worked on a cohort of patients with chronic periodontitis and hemochromatosis. that allowed us to show worsened periodontal measures in case of iron overload (Meuric, Lainé, et al., 2017). An early study (with 66 patients) of their subgingival microbiota showed a potential impact of iron through correlations between transferrin saturation and the abundance of Porphyromonas and Treponema (Boyer et al., 2018). Nevertheless, an extensive characterization of their microbiota (including dysbiosis) has not been performed yet, regarding occurrence, abundance and relationship of well-known and newly identified potential periodontal pathogens with periodontal measures.

The purpose of this study is three-fold. 1: to characterize all the well-described and newly identified periodontal pathogens (bacterial complexes described by Socransky et al. and reviewed by Pérez-Chaparro et al.) in the deepest pocket using the NGS in patients with chronic periodontitis (Pérez-Chaparro et al., 2014; Socransky et al., 1998). Two genera usually cited as implicated in health, Corynebacterium and Rothia, are also analyzed, as a part of the bacterial dysbiosis ratio. 2: to determine the association between the well-described, newly discovered periodontal pathogens and the periodontitis severity through continuous measures (mean PD, sites with $PD \ge 4$ mm). 3: to propose multiple regression linear models to refine the bacterial biomarkers of periodontitis severity adjusted for demographic and clinical data (including iron overload).

- Materials and methods
- Participants and collection of clinical data

Page 7 of 29

Molecular Oral Microbiology

The participants corresponded to a group of 75 patients with hemochromatosis and chronic periodontitis previously studied by Meuric et al. (Meuric, Lainé, et al., 2017). This case study series was approved by the local ethical committee (CPP Ouest V - 10/02-744). The informed consent for study participation has been obtained from all patients and all research was performed in accordance with relevant guidelines and regulations. These patients were recruited at the University Hospital of Rennes and benefited from a full-mouth periodontal examination, including PD, CAL, papillary bleeding index, gingival index, plaque score (Barnett, Ciancio, & Mather, 1980; Lobene, Weatherford, Ross, Lamm, & Menaker, 1986; Silness & Loe, 1964) and evaluation of periodontitis severity according to the case definitions from the Centers for Disease Control and Prevention and the American Association of Periodontology (CDC/AAP) (Eke, Page, Wei, Thornton-Evans, & Genco, 2012). For the 2018 classification, the algorithms considered the interdental CAL on two non-adjacent teeth and tooth loss (Graetz et al., 2019; Tonetti, Greenwell, & Kornman, 2018). The exclusion criteria were pregnancy, periodontal therapy within the last 12 months and treatment with either systemic antibiotics or drugs known to cause gingival hyperplasia within the last 3 months. Clinical data recorded at the time of dental examination included smoking status, alcohol consumption (non-drinkers, former drinkers, occasional drinkers and regular drinkers evaluated quantitatively in dose/day), number of dental visits per year and transferrin saturation. The assays of serum iron and transferrin were performed on an automated analyzer AU2700 Olympus (Beckman Coulter). Serum iron levels were measured by a standard colorimetric method that used TPTZ[2,4,6-Tri-(2-pyridyl)-5-triazine] as a chromogen and serum transferrin concentrations were quantified by immunoturbidimetry. Test procedures were conducted as described in the manufacturer's standard operating manual. Total iron binding capacity (TIBC) was calculated using the formula: (transferrin in g/l x 25) and transferrin saturation is calculated as follows: (iron in μ mol/l / TIBC) × 100.

111 After supra-gingival plaque removal, sterile endodontic paper points (Henry Schein, France) were 112 inserted into one of the deepest periodontal pockets for 30s. The material was transferred into a 113 sterile tube with 100 μ L of sterile distilled water and kept at 4°C overnight before DNA 114 extraction.

115 DNA extraction and sequencing

DNA extraction from supernatants was performed using QIAamp DNA Mini Kit (Qiagen, France) according to the manufacturer's recommendations. DNA was then kept frozen at -80°C until amplification. The V3-V4 regions of the 16S rRNA gene were amplified with the primers (5'-ACTCCTACGGGAGGCAGCAG-3') (Huse et al., 338F 2008) and 802R (5'-TACNVGGGTATCTAATCC-3') (Claesson et al., 2009) using 25 amplification cycles with an annealing temperature of 45°C. PCR products were sequenced with the Illumina MiSeq of the Get-PlaGe platform (Toulouse, France). The sequence data were submitted to the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/Traces/ study/) under BioProject accession number PRJNA416501 and PRJNA517671.

125 <u>Microbiological analysis</u>

Taxonomy at the genus level was assigned using the "Visualization and Analysis of Microbial Population Structures" (VAMPS) analysis pipeline web tool (Huse et al., 2014). Default parameters were used for assignment to the genus level with the Ribosomal Database Project (RDP) classification (Cole et al., 2014). Through the Global Alignment for Sequence Taxonomy (GAST) process of VAMPS, the best taxonomic hit was assigned for each read. Reads identified as Archaea, Eukarya, Organelle and unknown were excluded from further analysis. Taxonomy at

the species level was assigned using the "Quantitative Insights Into Microbial Ecology" (OIIME 1.9.1) package software (Caporaso et al., 2010) with the default parameters (script: pick open reference otus.py, uclust as the clustering method) and the Human Oral Microbiome Database (HOMD, version 15.1) as the reference database (Chen et al., 2010). The mean unassigned reads per sample was 6.4 $\% \pm 6.2 \%$. These reads have been excluded from further species analyses. To challenge the assignment capacities used in this study, the V3V4 sequences of each taxon were extracted from the HOMD and entered in the previously described pipeline. Only three species (Mogibacterium timidum, Anaeroglobus geminatus and Enterococcus *faecalis*) could not be correctly classified by the pipeline, among all the putative periodontal pathogens described.

142 <u>Statistics</u>

Data were studied using R (version 3.3.3) (R. Core Team, 2015) with the RStudio software (RStudio Team, 2016). Statistical tests were considered significant for p < 0.05. Results of the descriptive analysis are presented as mean \pm standard error (SE) and with prevalence (%). Distribution of continuous variables was assessed using the Shapiro-Wilk test. The mean PD was analyzed according to the demographic and clinical data using the Mann-Whitney test. Correlations between taxa relative abundance and clinical parameters were calculated using Spearman correlation coefficient. Multiple linear regression models were used to study the association between periodontal parameters (i.e. mean PD and percentage of site with PD₂4mm) and bacterial taxa presenting a significant correlation ($r \ge 0.20$, p < 0.05) or the dysbiosis ratio (% Porphyromonas + % Treponema + % Tannerella) / (% Rothia + % Corynebacterium) described in Meuric et al. 2017 (Meuric, Le Gall-David, et al., 2017), adjusted for confounding factors including age, gender, smoking status, alcohol intake, plaque index, transferrin saturation,

gingival and bleeding index. The stepwise AIC algorithm was performed using the 'MASS' package for R (Venables & Ripley, 2002). Final regression coefficients, standard errors (SE) and statistics for each variable adjusted for confounding factors, with the characteristics of the models (adjusted R^2 , *p*-value) are presented.

 3 Results

161 - <u>Clinical data</u>

Based on the CDC/AAP case definition (Eke et al., 2012), all patients presented periodontitis; 44.3 % and 54.4 % had moderate and severe periodontitis, respectively. On average, 50. 0 ± 1.96 % of sites per mouth had CAL \geq 3 mm, 10.7 ±1.43 % of sites had CAL \geq 5 mm, and 12.5 ± 1.15 % of sites had PD \geq 4 mm. The full description of the periodontal measures is available in Table S1. According to the 2018 classification, and using CAL and tooth loss as the main available criteria, 3/75 (4%) patients were classified as stage II, 59/75 (79%) as stage III and 13/75 (17%) as stage IV (Tonetti et al., 2018). A minority (9/75, 12%) was classified as localized. The average age was 50.6 ± 8 years. Males represented 56 %, and former or current smokers and drinkers were 62.6% and 80%, respectively. Most of the patients visited their dentist at least once a year (78.6%). Table 1 presents the statistics exploring the potential impact of each demographic on the mean PD. The mean PD was increased in former or current drinkers, compared to patients who had never experienced alcohol abuse (p=0.028).

49 174

Prevalence and proportion of bacteria

176 The average of reads per sample was $14\ 980 \pm 740$ (minimum = 5 298 reads). From the 74 genera 177 and species related to periodontitis and described by Socransky *et al.*, Pérez-Chaparro *et al.* and

Meuric et al., 47 taxa (64.4%) have been identified in this study (Table 2). All the bacteria belonging to the orange and red complexes described by Socransky *et al.* were found. The lowest prevalence was Fusobacterium periodonticum (6.4%), while Fusobacterium nucleatum and T. forsythia were found in all patients. For the 8 species which have been reported as highly implicated in periodontitis (reported by ≥ 4 studies according to the systematic review of the literature by Pérez-Chaparro *et al.*), five of them were found: i.e. *Porphyromonas endodontalis* (prevalence = 88.6 %), Treponema lecithinolyticum (62 %), Treponema medium (55.7 %), Filifactor alocis (40.5%) and Eubacterium saphenum (11.4%), while two were not detected (Fretibacterium OT360 and Selenomonas sputigena). For the taxa cited 3 times, the following were not detected: Bacteroidales OT274, Peptostreptococcus stomatis, Desulfobulbus OT041 and phylum TM7. Species with lower evidence of implication with periodontitis (cited only by two studies) or belonging to the purple or green complex (Actinomyces odontolyticus, Veillonella parvula and Eikenella corrodens respectively) were not, or hardly detected (Table 2). Mogibacterium timidum, Anaeroglobus geminatus and Enterococcus faecalis were not found due to methodological limitations.

193 The most abundant genus was *Fusobacterium* (mean = 26.1 ± 1.7 %) followed by *Prevotella* 194 (12 ± 1 %), *Porphyromonas* (7 ± 0.8 %), *Streptococcus* (5.8 ± 0.7 %), *Treponema* (5.6 ± 0.6 %) and 195 *Tannerella* (5.4 ± 0.6 %). The most abundant species were *F. nucleatum* (15.3 ± 1.4 %) followed by 196 *T. forsythia* (4.5 ± 0.5 %), *P. endodontalis* (3.1 ± 0.4 %), and *P. gingivalis* (2.8 ± 0.6 %).

Microbiological correlation with periodontal data

199 Analysis showed that the species exhibiting significant correlations with periodontal clinical data 200 were *P. gingivalis* (r=0.33 and 0.41, with mean PD and percentage of sites with PD \geq 4 mm, 201 respectively), *T. forsythia* (r=0.25 and 0.30, respectively), and *E. nodatum* (r=0.35 and 0.34,

respectively). Desulfobulbus, Treponema and Porphyromonas genera were also found significantly correlated with these periodontal data. Significant inverse correlations were found for the taxa of *Campylobacter*, Aggregatibacter and Rothia. No correlation was found between the sampled pocket depth and any of the bacterial taxa. Finally, the calculated dysbiosis ratio corresponding to (%Porphyromonas + %Treponema + %Tannerella) / (%Rothia + %Corvnebacterium) showed the highest correlation with mean PD (r=0.37, p=0.001) and with the percentage of sites with PD \geq 4 mm (r=0.35, p=0.0017), as compared with individual taxa. Table 3 presents all the significant results of the correlation between bacterial/clinical data and mean PD, which were further used in the following statistical experiments. As shown in Table 3, the periodontal indices rating the plaque, gingival inflammation and bleeding on probing, were found significantly correlated with the mean PD. Therefore, they were also used in the statistical models as confounding variables.

- - 215 <u>Multiple linear regression analyses</u>

The multiple linear regression was performed with the stepwise method which selects the most significant variables affecting the mean pocket depth. After adjustment for age, sex, smoking status, alcohol status, plaque index, transferrin saturation, bleeding index and gingival index, the mean PD was found independently associated with Aggregatibacter, Rothia and Porphyromonas genera (β =-0.053, -0.063 and 0.051, respectively, p<0.05) in the first model (adjusted R² = 0.3798, p < 0.001). The most significant data were obtained in the second model using the dysbiosis ratio and Aggregatibacter as independent variables with $\beta=0.062$ (p<0.001) and $\beta=-$ 0.053 (p < 0.01) respectively and an adjusted R² = 0.4868 (p < 0.00001) (Table 4). By using another

1		
2		
3 4	224	р
5		_
6	225	d
7		
8	226	
9		
10	227	
11		
12	228	
14		
15	229	р
16		
17	230	ic
18		
19	231	p
20	-01	Р
21	232	n
22	252	Р
23	222	
24 25	233	51
25	224	4 1
27	234	u
28	225	
29	235	
30	224	1
31	236	d
32		
33	237	Si
34 35		
36	238	b
37		
38	239	р
39		
40	240	fð
41		
42	241	li
43		
44 45	242	d
45 46		
40 47	243	e
48	210	U
49	24.4	~
50	644	0
51	215	т
52	240	1
53	246	
54 57	240	p
22		

periodontal measure as the outcome variable – the percentage of sites with $PD \ge 4 \text{ mm}$ –, the dysbiosis ratio (p < 0.001 – Table S2) remained as the most significant predictor.

4 Discussion

Owing to decades of oral microbiology studies, some species are well known for their periodontal pathogenicity. Culture-independent and molecular-based approaches to bacterial identification has highlighted new putative periodontal pathogens, that were found increased in periodontitis patients. However, few studies assess the correlation between the severity of periodontal disease and the abundance of these taxa. The identification of taxa that are significantly associated with the periodontal status is the first step in the search for biomarkers that could predict the onset of the disease.

Most of the periodontal pathogens were identified in the analyses due to the sequencing depth. However, six species were not detected *Bacteroidales OT274*, *Peptostreptococcus stomatis*, *Desulfobulbus OT041*, *Fretibacterium sp.*, TM7 and *Selenomonas Sputigena* could not be found while they are highly cited (\geq 3 times) in the literature as being implicated in periodontitis. Only three (*Mogibacterium timidum*, *Anaeroglobus geminatus* and *Enterococcus faecalis*) could not be identified through the pipeline of assignation, due to methodological limitations. The lack of detection of *Fretibacterium* is surprising since it was proposed as a diagnostic bacterial biomarker for periodontitis screening in a Japanese population (Khemwong et al., 2019). Indeed, *Fretibacterium* was not detected in our Caucasian population. As for the other five remaining taxa, we have confirmed that they were not detected in this population. Thus, different population could carry different taxon associated with periodontitis. However, previous works on periodontitis microbiota analysis through NGS have used a binary approach (healthy or disease) to determine in which condition taxon are increased (Abusleme et al., 2013;

58 59 60

56

Kirst et al., 2015). In this study, we have performed correlation analyses between the abundance of species considered as periodontal pathogens (from previous studies) and continuous periodontal measures, in haemochromatosis patients with periodontitis to assess the current weight of their implication in the disease. Adjusted regression models were then used to determine the best bacterial biomarkers of periodontitis severity. Taxa already well described as implicated in the disease were found correlated with the mean PD or the percentage of sites with PD > 4 mm; *P. gingivalis* and *T. forsythia* (red complex) and *E. nodatum* (orange complex) (Socransky et al., 1998). From the newly identified putative periodontal pathogen, only Desulfobulbus genus (Pérez-Chaparro et al., 2014) was found correlated with the mean pocket depth. Correlation coefficients between periodontal data and bacterial taxa remained inferior to 0.4, similarly to other microbiota analyses in chronic periodontitis (Khemwong *et al.*, 2019; Lourenço et al., 2014; Silva-Boghossian, Cesário, Leão, & Colombo, 2018). Finding associations is especially more difficult when looking at the species level. However, it is not surprising as this disease is a multifactorial pathology, as each taxon might be partially implicated in the disease. For instance, T. denticola which belongs to the red complex (Socransky et al., 1998), was not correlated with the mean PD. Nevertheless, the Treponema genus, which contains T. denticola and other species highly suspected to be periodontal pathogens (i.e. T. vincentii, T. lecithinolyticum and T. medium, as reported in the systematic review by Pérez-Chaparro et al.), was found correlated with the PD measures.

Surprisingly, Campylobacter was negatively associated with the mean PD, while *Campylobacter* species are mainly classified as periodontal pathogens (i.e.: C. showae, C. gracilis, and C. rectus belonging to the orange complex and C. concisus to the green complex (Socransky *et al.*, 1998)). However, this repartition of species is controversial: C. gracilis belongs to the orange complex, though it was found increased in healthy subjects (Henne, Fuchs, Kruth,

Molecular Oral Microbiology

Horz, & Conrads, 2014) and decreased in severe periodontitis (Macuch & Tanner, 2000). Finally, the *Campylobacter* genus was still negatively associated with percentage of PD \geq 4mm in the regression model.

This analytical approach is also interesting when focused on the prevalence or occurrence of periodontal pathogens in patients. Taxa are not found in all patients. Even the prevalence of *P. gingivalis*, which is well described as one of the keystone pathogens in the disease (George Hajishengallis et al., 2012), was only found at 62% in this study. Moreover, P. gingivalis has been detected in 25% of healthy Caucasian (Griffen, Becker, Lyons, Moeschberger, & Leys, 1998), up to 40% in healthy Japanese (Kato, Imai, Ochiai, & Ogata, 2013) and not higher than 79% in chronic periodontitis (Griffen et al., 1998). The chronic nature and clinical course of the disease, with alternating episodes of exacerbation and remission (Goodson, Tanner, Haffajee, Sornberger, & Socransky, 1982), could explain that the bacteria are more difficult to detect. The single sampling could also be a potential modifier of the microbiota and a limitation, as different periodontal pockets in the same patient could show various microbiota composition. Long-term follow-up with regular and multi-site sampling is certainly needed, which could address these issues. As such studies are difficult to implement, a ratio of taxa was developed (Meuric, Le Gall-David, et al., 2017) to explore the periodontal dysbiosis responsible for chronic periodontitis (Kilian et al., 2016). This dysbiosis ratio showed the highest and the most significant correlation with mean PD in this study (p=0.001) and could be calculated for each patient, whereas P. gingivalis could only be detected in 62% of the patients. Thereafter, in the first multiple regression model, each taxon found significantly correlated with mean PD was entered, while Porphyromonas, Treponema, P. gingivalis, T. forsythia and Rothia were replaced in the second model by the dysbiosis ratio which is computed with their relative abundance. The models were

refined with the stepwise method (Venables & Ripley, 2002) adjusted with the clinical and demographic data as for example, alcohol intake, which is considered as a risk factor for periodontitis (Wang, Lv, Wang, & Jiang, 2016) and a worsened mean PD in our population. Since the patients come from a sub-cohort of a previous study where high transferrin saturation (specifically for the hemochromatosis disease) was implicated in explaining clinical severity of periodontitis, we have added the transferrin saturation into the multiple linear regression models as a confounding factor. As described previously, periodontitis through mean PD was associated with transferrin saturation in the two models (Meuric, Lainé, et al., 2017). For each dependent variable (mean PD or percentage of sites with $PD \ge 4$ mm), the most significant result, appeared in the second model with the dysbiosis ratio (p < 0.001) which is compiled with majority of the periopathogens (i.e.: Porphyromonas, Treponema and Tannerella genera) and the genera usually found in health (Meuric, Le Gall-David, et al., 2017). This result is independent from the transferrin saturation (reflecting the iron status of the hemochromatosis disease). Thus, the dysbiosis ratio confirmed its utility in exploring the microbial dysbiosis to explain periodontal disease (Meuric, Le Gall-David, et al., 2017). Similar microbial dysbiosis ratios have also been calculated in Crohn's disease (Gevers et al., 2014) and in peri-implantitis (Kröger et al., 2018).

These results are based on a specific population suffering from haemochromatosis. Extrapolations outside of this population need to be validated, though the iron status of the patients has been included as a confounding variable in statistical models. Nonetheless, these results are in accordance with the existing view that periodontitis or peri-implantitis are orchestrated by a bacterial consortium rather than a single pathogen (Darveau, 2010).

Conclusion

In this study, we have confirmed that most of the well-described and newly identified

periodontal pathogens can be found in the deepest periodontal pocket of patients suffering from

haemochromatosis and periodontitis. Yet, only few taxa were correlated to mean pocket depth.

The relative abundance of these taxa was therefore used as a predictor, with confounding

variables, in multiple linear regression models that allowed for the prediction of mean pocket

depth. While Porphyromonas, Aggregatibacter and Rothia returned significant results, the

previously described dysbiosis ratio – (% Porphyromonas + % Treponema + % Tannerella) / (%

Rothia + % *Corvnebacterium*) – was the most reliable biomarker to predict periodontitis severity

in our patients. Longitudinal studies in patients with no systemic disease, as well as several

sampling sites and time points during the episodes of disease-active periodontitis should be

considered to further validate and generalize the findings of this study.

Molecular Oral Microbiology

Acknowledgments: The authors are indebted to the medical and nursing staff for participating in the recruitment and follow-up of patients. We thank the GeT-PlaGe facility and the NED team for sequencing the samples. We also thank Adina Pascu (INSERM U1241) for her contribution to this work and the "Direction de la Recherche et de l'Innovation" at the University hospital of Rennes for their support. The study was funded by the Comité de la Recherche Clinique of the University Hospital (2009-A00998-49) of Rennes and the University of Rennes 1. The authors report no conflicts of interest related to this study.

337 Data availability: The sequence data were deposited to the NCBI Sequence Read Archive
338 (https://www.ncbi.nlm.nih.gov/Traces/study/) under BioProject accession numbers
339 PRJNA416501 and PRJNA517671. The detailed periodontal and demographic data for every
340 patient can be accessed in Table S3.

1 2 3 4 5	342	References
6	343	Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Diaz,
7	344	P. I. (2013). The subgingival microbiome in health and periodontitis and its relationship with
8 9	345	community biomass and inflammation. The ISME Journal, 7(5), 1016–1025.
10	346	https://doi.org/10.1038/ismej.2012.174
11 12	347	Barnett, M., Ciancio, S., & Mather, M. (1980). The Modified Papillary Bleeding Index—
13	348	Comparison with Gingival Index During the Resolution of Gingivitis. Journal of Preventive
14 15	349	<i>Dentistry</i> , <i>6</i> (2), 135–138.
16	350	Boyer, E., Gall-David, S. L., Martin, B., Fong, S. B., Loréal, O., Deugnier, Y., Meuric, V.
17	351	(2018). Increased transferrin saturation is associated with subgingival microbiota dysbiosis and
10	352	severe periodontitis in genetic haemochromatosis. Scientific Reports, 8(1), 15532.
20	353	https://doi.org/10.1038/s41598-018-33813-0
21 22	354	Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K.,
23	355	others. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature
24 25	356	Methods, 7(5), 335–336.
26	357	Chen, T., Yu, WH., Izard, J., Baranova, O. V., Lakshmanan, A., & Dewhirst, F. E. (2010). The
27	358	Human Oral Microbiome Database: A web accessible resource for investigating oral microbe
28 29	359	taxonomic and genomic information. <i>Database</i> , 2010, bag013.
30	360	https://doi.org/10.1093/database/baq013
31 22	361	Claesson M. I. O'Sullivan O. Wang O. Nikkilä I. Marchesi, I. R. Smidt H. O'Toole P.
33	362	W (2009) Comparative Analysis of Pyrosequencing and a Phylogenetic Microarray for
34	363	Exploring Microbial Community Structures in the Human Distal Intestine PLOS ONE 4(8)
35	364	e6669 https://doi.org/10.1371/journal.pone.0006669
36 37	504	
38	365	Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., Tiedje, J. M. (2014).
39	366	Ribosomal Database Project: Data and tools for high throughput rRNA analysis. <i>Nucleic Acids</i>
40 41	367	Research, 42(Database issue), D633-642. https://doi.org/10.1093/nar/gkt1244
42	368	Darveau, R. P. (2010). Periodontitis: A polymicrobial disruption of host homeostasis. Nature
43	369	Reviews Microbiology, 8(7), 481-490. https://doi.org/10.1038/nrmicro2337
45	370	Eke, P. I., Page, R. C., Wei, L., Thornton-Evans, G., & Genco, R. J. (2012). Update of the case
46	371	definitions for population-based surveillance of periodontitis. Journal of Periodontology, 83(12),
47 48	372	1449–1454. https://doi.org/10.1902/jop.2012.110664
49 50	373	Frencken, J. E., Sharma, P., Stenhouse, L., Green, D., Laverty, D., & Dietrich, T. (2017). Global
50 51	374	epidemiology of dental caries and severe periodontitis – a comprehensive review. Journal of
52	375	Clinical Periodontology, 44(S18), S94–S105. https://doi.org/10.1111/jcpe.12677
53 54	376	Gevers D. Kugathasan S. Denson I. A. Vázquez-Raeza V. Van Treuren W. Ren R
54 55 56	377	Xavier, R. J. (2014). The treatment-naive microbiome in new-onset Crohn's disease. <i>Cell Host &</i>
57 58		

2						
- 3 4	378	Microbe, 15(3), 382-392. https://doi.org/10.1016/j.chom.2014.02.005				
5 6 7 8	379 380 381	Goodson, J. M., Tanner, A. C., Haffajee, A. D., Sornberger, G. C., & Socransky, S. S. (1982). Patterns of progression and regression of advanced destructive periodontal disease. <i>Journal of Clinical Periodontology</i> , <i>9</i> (6), 472–481.				
9 10 11 12	382 383 384	Graetz, C., Mann, L., Krois, J., Sälzer, S., Kahl, M., Springer, C., & Schwendicke, F. (2019). Comparison of periodontitis patients' classification in the 2018 versus 1999 classification. <i>Journal of Clinical Periodontology</i> . https://doi.org/10.1111/jcpe.13157				
13 14 15 16 17	385 386 387	Griffen, A. L., Becker, M. R., Lyons, S. R., Moeschberger, M. L., & Leys, E. J. (1998). Prevalence of Porphyromonas gingivalis and periodontal health status. <i>Journal of Clinical Microbiology</i> , <i>36</i> (11), 3239–3242.				
18 19 20 21 22 23	388 389 390 391	Hajishengallis, G., & Lamont, R. J. (2012). Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. <i>Molecular Oral Microbiology</i> , <i>27</i> (6), 409–419. https://doi.org/10.1111/j.2041- 1014.2012.00663.x				
23 24 25 26 27	392 393 394	Hajishengallis, George, Darveau, R. P., & Curtis, M. A. (2012). The keystone-pathogen hypothesis. <i>Nature Reviews. Microbiology</i> , <i>10</i> (10), 717–725. https://doi.org/10.1038/nrmicro2873				
28 29 30 31 32 33 34 35 36	395 396 397	Henne, K., Fuchs, F., Kruth, S., Horz, HP., & Conrads, G. (2014). Shifts in Campylobacter species abundance may reflect general microbial community shifts in periodontitis progression <i>Journal of Oral Microbiology</i> , <i>6</i> , 25874. https://doi.org/10.3402/jom.v6.25874				
	398 399 400	Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A., & Sogin, M. L. (2008). Exploring Microbial Diversity and Taxonomy Using SSU rRNA Hypervariable Tag Sequencing. <i>PLOS Genet</i> , 4(11), e1000255. https://doi.org/10.1371/journal.pgen.1000255				
37 38 39 40	401 402 403	Huse, S. M., Mark Welch, D. B., Voorhis, A., Shipunova, A., Morrison, H. G., Eren, A. M., & Sogin, M. L. (2014). VAMPS: A website for visualization and analysis of microbial population structures. <i>BMC Bioinformatics</i> , <i>15</i> , 41. https://doi.org/10.1186/1471-2105-15-41				
41 42 43 44	404 405 406	Jiao, Y., Hasegawa, M., & Inohara, N. (2014). The Role of Oral Pathobionts in Dysbiosis during Periodontitis Development. <i>Journal of Dental Research</i> , <i>93</i> (6), 539–546. https://doi.org/10.1177/0022034514528212				
45 46 47 48 49	407 408 409	Kato, A., Imai, K., Ochiai, K., & Ogata, Y. (2013). Higher Prevalence of Epstein–Barr Virus DNA in Deeper Periodontal Pockets of Chronic Periodontitis in Japanese Patients. <i>PLoS ONE</i> , <i>8</i> (8), e71990. https://doi.org/10.1371/journal.pone.0071990				
50 51 52 53	410 411 412	Khemwong, T., Kobayashi, H., Ikeda, Y., Matsuura, T., Sudo, T., Kano, C., Izumi, Y. (2019). Fretibacterium sp. Human oral taxon 360 is a novel biomarker for periodontitis screening in the Japanese population. <i>PloS One</i> , <i>14</i> (6), e0218266. https://doi.org/10.1371/journal.pone.0218266				
54 55 56 57	413 414	Kilian, M., Chapple, I. L. C., Hannig, M., Marsh, P. D., Meuric, V., Pedersen, A. M. L., Zaura, E. (2016). The oral microbiome – an update for oral healthcare professionals. <i>British</i>				
58 59		10				

1 2 3	41 E	D_{ental} I_{ourmal} 221(10) 657 666 https://doi.org/10.1028/si.hdi 2016.865
4 5 6 7 8 9	415 416 417 418 419	 Kirst, M. E., Li, E. C., Alfant, B., Chi, YY., Walker, C., Magnusson, I., & Wang, G. P. (2015). Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. <i>Applied and Environmental Microbiology</i>, <i>81</i>(2), 783–793. https://doi.org/10.1128/AEM.02712-14
10 11 12 13 14 15	420 421 422 423	Kröger, A., Hülsmann, C., Fickl, S., Spinell, T., Hüttig, F., Kaufmann, F., Kebschull, M. (2018). The severity of human peri-implantitis lesions correlates with the level of submucosal microbial dysbiosis. <i>Journal of Clinical Periodontology</i> , <i>45</i> (12), 1498–1509. https://doi.org/10.1111/jcpe.13023
16 17 18 19	424 425 426	Lamont, R. J., Koo, H., & Hajishengallis, G. (2018). The oral microbiota: Dynamic communities and host interactions. <i>Nature Reviews. Microbiology</i> , <i>16</i> (12), 745–759. https://doi.org/10.1038/s41579-018-0089-x
20 21 22	427 428	Lobene, R. R., Weatherford, T., Ross, N. M., Lamm, R. A., & Menaker, L. (1986). A modified gingival index for use in clinical trials. <i>Clinical Preventive Dentistry</i> , 8(1), 3–6.
23 24 25 26 27	429 430 431	Lourenço, T. G. B., Heller, D., Silva-Boghossian, C. M., Cotton, S. L., Paster, B. J., & Colombo, A. P. V. (2014). Microbial signature profiles of periodontally healthy and diseased patients. <i>Journal of Clinical Periodontology</i> , <i>41</i> (11), 1027–1036. https://doi.org/10.1111/jcpe.12302
28 29 30 31	432 433 434	Macuch, P. J., & Tanner, A. C. (2000). Campylobacter species in health, gingivitis, and periodontitis. <i>Journal of Dental Research</i> , <i>79</i> (2), 785–792. https://doi.org/10.1177/00220345000790021301
32 33 34 35 36 37	435 436 437 438	Meuric, V., Lainé, F., Boyer, E., Le Gall-David, S., Oger, E., Bourgeois, D., Deugnier, Y. (2017). Periodontal status and serum biomarker levels in HFE hemochromatosis patients. A case series study. <i>Journal of Clinical Periodontology</i> , <i>44</i> (9), 892–897. https://doi.org/10.1111/jcpe.12760
38 39 40 41	439 440 441	Meuric, V., Le Gall-David, S., Boyer, E., Acuña-Amador, L., Martin, B., Fong, S. B., Bonnaure-Mallet, M. (2017). Signature of Microbial Dysbiosis in Periodontitis. <i>Applied and</i> <i>Environmental Microbiology</i> , <i>83</i> (14). https://doi.org/10.1128/AEM.00462-17
42 43 44 45 46	442 443 444	Paster, B. J., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., Dewhirst, F. E. (2001). Bacterial diversity in human subgingival plaque. <i>Journal of Bacteriology</i> , <i>183</i> (12), 3770–3783. https://doi.org/10.1128/JB.183.12.3770-3783.2001
47 48 49 50	445 446 447	Pérez-Chaparro, P. J., Gonçalves, C., Figueiredo, L. C., Faveri, M., Lobão, E., Tamashiro, N., Feres, M. (2014). Newly identified pathogens associated with periodontitis: A systematic review. <i>Journal of Dental Research</i> , <i>93</i> (9), 846–858. https://doi.org/10.1177/0022034514542468
51 52 53	448 449	R. Core Team. (2015). R: A language and environment for statistical computing. <i>R Foundation for Statistical Computing, Vienna, Austria</i> . Retrieved from https://www.R-project.org/
54 55 56 57 58	450 451	RStudio Team. (2016). RStudio: Integrated development for R. RStudio, Inc., Boston, MA. URL Http://Www.Rstudio.Com.

2 3 4	452 453	Silness, J., & Loe, H. (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. <i>Acta Odontologica Scandinavica</i> 22, 121–135
5 6 7 8 9	454 455 456	Silva-Boghossian, C. M., Cesário, P. C., Leão, A. T. T., & Colombo, A. P. V. (2018). Subgingival microbial profile of obese women with periodontal disease. <i>Journal of</i> <i>Periodontology</i> , <i>89</i> (2), 186–194. https://doi.org/10.1002/JPER.17-0236
10 11 12	457 458	Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., & Kent, R. L. (1998). Microbial complexes in subgingival plaque. <i>Journal of Clinical Periodontology</i> , <i>25</i> (2), 134–144.
13 14 15 16 17	459 460 461	Tonetti, M. S., Greenwell, H., & Kornman, K. S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. <i>Journal of Clinical</i> <i>Periodontology</i> , <i>45 Suppl 20</i> , S149–S161. https://doi.org/10.1111/jcpe.12945
18 19 20	462 463	Venables, W. N., & Ripley, B. D. (2002). <i>Modern Applied Statistics with S</i> (4th ed.). Retrieved from https://www.springer.com/gp/book/9780387954578
20 21 22 23 24 25 26 27 28 29 30 31 23 34 35 36 37 38 30 41 42 43 44 546 47 48 950 51 52	464 465 466 467	Wang, J., Lv, J., Wang, W., & Jiang, X. (2016). Alcohol consumption and risk of periodontitis: A meta-analysis. <i>Journal of Clinical Periodontology</i> , <i>43</i> (7), 572–583. https://doi.org/10.1111/jcpe.12556
53 54 55		
56 57 58 59		ე 1
60		21

Page 23 of 29

Demographics	Mean PD (mm)	<i>p</i> -value
Gender		
Male $(n = 42)$	2.32 ± 0.06	
Female $(n = 33)$	2.29 ± 0.06	0.863
Smoking status		
Never $(n = 28)$	2.29 ± 0.04	
Former $(n = 30)$ and Current $(n = 17)$	2.31 ± 0.06	0.81
Alcohol status		
Never $(n = 15)$	2.12 ± 0.07	
Former $(n = 2)$ and Current $(n = 58)$	2.35 ± 0.05	0.028
Frequency of dentist visits		
Once or twice a year $(n = 59)$	2.32 ± 0.06	
Less than once a year $(n = 16)$	2.27 ± 0.09	0.535

Table 1: Mean probing depth (PD) according to demographic data. Data are presented as mean ± standard error (SE). Bold *p*-values indicate significant Mann-Whitney test.

Phylum	Genera	Species	НОТ	Author	Year	Identidied	Mean %	Mean %	+/-SE	Occurence
						in	genera	species		(%)
Actinobacteria	Corynebacteria			Meuric	2017	Health	0.143		0.028	83.5
Actinobacteria	Rothia			Meuric	2017	Health	0.215		0.050	73.4
Firmicutes	Streptococcus			Socransky	1998	yellow complex	5.782		0.728	100
Firmicutes	Veillonella	parvula	161	Socransky	1998	purple complex	ND			
Actinobacteria	Actinomyces						0.237		0.054	77.2
Actinobacteria	Actinomyces	odontolyticus	701	Socransky	1998	purple complex	ND			
Proteobacteria	Eikenella	corrodens	577	Socransky	1998	green complex	ND			
Bacteroidetes	Capnocytophaga						1.667		0.262	98.7
Bacteroidetes	Capnocytophaga	gingivalis	337	Socransky	1998	green complex		0.002	0.001	11.4
Bacteroidetes	Capnocytophaga	sputigena	775	Socransky	1998	green complex		0.509	0.087	83.5
Bacteroidetes	Capnocytophaga	ochracea	700	Socransky	1998	green complex		0.002	0.001	19.0
Bacteroidetes	Campylobacter						4.279		0.365	100.0
Bacteroidetes	Campylobacter	concisus	575	Socransky	1998	green complex		0.190	0.041	77.2
Bacteroidetes	Porphyromonas					•	7.054		0.833	98.7
Bacteroidetes	Porphyromonas	gingivalis	619	Socransky	1998	red complex		2.769	0.626	62.0
Spirochaetes	Treponema			-		-	5.591		0.584	100
Spirochaetes	Treponema	denticola	584	Socransky	1998	red complex		1.061	0.170	87.3
Bacteroidetes	Tannerella			-		-	5.368		0.584	100
Bacteroidetes	Tannerella	forsythia	613	Socransky	1998	red complex		4.522	0.531	100
Firmicutes	Eubacterium						1.415		0.200	97.6
Firmicutes	Eubacterium	nodatum	694	Socransky	1998	orange complex		0.177	0.043	62.0
Fusobacteria	Fusobacterium						26.140		1.733	100
Fusobacteria	Fusobacterium	nucleatum	200. 202. 420. 698	Socransky	1998	orange complex		15.292	1.458	100
Fusobacteria	Fusobacterium	periodonticum	201	Socransky	1998	orange complex		0.0003	0.0002	6.3
Firmicutes	Parvimonas	-				- · ·	1.019		0.186	92.4
Firmicutes	Parvimonas	micra	111	Socransky	1998	orange complex		1.015	0.176	91.1
Bacteroidetes	Prevotella					- · ·	12.006		1.043	100
Bacteroidetes	Prevotella	intermedia	643	Socransky	1998	orange complex		0.774	0.228	58.2
Bacteroidetes	Prevotella	nigrescens	693	Socransky	1998	orange complex		1.558	0.290	82.3
Firmicutes	Streptococcus	constellatus	576	Socransky	1998	orange complex		0.789	0.177	82.3
Proteobacteria	Campylobacter	gracilis	623	Socransky	1998	orange complex		1.363	0.202	92.4
Proteobacteria	Campylobacter	rectus	748	Socransky	1998	orange complex		1.740	0.240	94.9
Proteobacteria	Campylobacter	showae	763	Socransky	1998	orange complex		0.012	0.002	53.2
Proteobacteria	Aggregatibacter			5		0 1	0.851		0.167	86.1
Proteobacteria	Aggregatibacter	actinomycetemcomitans	531	Socransky	1998			0.027	0.012	17.7
Bacteroidetes	Prevotella	denticola	291	Perez-Chapparo	2014	2 studies		0.868	0.226	75.9
Bacteroidetes	Allonrevotella	tannerae	466	Perez-Channaro	2014	2 studies	ND			

Molecular Oral Microbiology

Ratio	(% Porphyromonas + % Treponet	ma + % Tannerella) / (% C	Corynebacterium + % Rothia)	Meuric	2017	61 81	6	+/- 19	257	100
Dysbiosis						Mean	%	+/-\$	SE	Occurence
Spirochaetes	Treponema	medium	667	Perez-Chapparo	2014	5 studies		0.044	0.010	55.7
Firmicutes	Selenomonas	sputigena	151	Perez-Chapparo	2014	5 studies	ND			
Firmicutes	Filifactor	alocis	539	Perez-Chapparo	2014	5 studies		0.041	0.011	40.5
Firmicutes	Filifactor			11			2.202		0.305	97.5
Firmicutes	Eubacterium [XI] [G-5]	saphenum	759	Perez-Chapparo	2014	5 studies		0.002	0.001	11.4
Synergistetes	Fretibacterium	oral taxon 360	360	Perez-Chapparo	2014	4 studies	ND			
Spirochaetes	Treponema	lecithinolyticum	653	Perez-Chapparo	2014	4 studies		0.696	0.158	62
Firmicutes	Enterococcus	faecalis	604	Perez-Chapparo	2014	4 studies	ND			
Bacteroidetes	Porphyromonas	endodontalis	273	Perez-Chapparo	2014	4 studies		3.105	0.421	88.6
TM7	TM7 [G-5]	oral taxon 356	3.56	Perez-Chapparo	2014	3 studies	ND			
Synergistetes	Fretibacterium	fastidiuosum	363	Perez-Chapparo	2014	3 studies	ND			
Synergistetes	Fretibacterium	oral taxon 362	362	Perez-Chapparo	2014	3 studies	ND			
Spirochaetes	Treponema	vincentii	0 29	Perez-Chapparo	2014	3 studies		0.034	0.009	41.8
Proteobacteria	Desulfobulbus	sp. oral taxon 041	0 41	Perez-Chapparo	2014	3 studies	ND			- 1.0
Proteobacteria	Desulfobulbus	0		i cici chuppuro		5 stadios	1.515		0.285	81.0
Firmicutes	Anaeroglobus	geminatus	121	Perez-Chapparo	2014	3 studies	ND			
Firmicutes	Pentostrentococcus	stomatis	112	Perez-Chapparo	2014	3 studies	ND		0.010	,,
Firmicutes	Pentostrentococcus			refez chapparo	2017	5 studies	0.192		0.040	77.2
Firmicutes	Mogihacterium	timidum	0.42	Perez-Channaro	2014	3 studies	ND		0.057	1.1
Firmicutes	Mogihacterium	01 ai tax011 2 / 7	- 277	r erez-Chapparo	2017	5 studies	0 293		0.037	93 7
Bacteroidetes	Bacteroidales [G-2]	oral taxon 274	274	Perez-Channaro	2014	3 studies	ND			
SR1	SR1 [G-1]	oral taxon 345	345	Perez-Chapparo	2014	2 studies	ND			
TM7	TM7 [G-1]	oral taxon 346 340	346 349	Perez-Channaro	2014	2 studies	ND			
Synergistetes	Fretihacterium	oral taxon 359	350	Perez-Chapparo	2014	2 studies	ND	1.100	0.174	00.7
Spirochaetes	Treponema	nhvlogroun II	584 743	Perez-Channaro	2014	2 studies		1 106	0 194	86 7
Proteobacteria	Escherichia	coli	574	Perez-Channaro	2014	2 studies	ND		0.010	55.1
Proteobacteria	Escherichia	ounnunnu	557	refez chapparo	2017	2 5100105	0.065		0.016	55 7
Proteobacteria	Acinetobacter	haumannii 🦱	554	Perez-Channaro	2014	2 studies	ND		0.050	45.0
Proteobacteria	Acinetobacter	preamosinies	/50	r erez-enapparo	2017	2 studies	0.081		0.030	45.6
Firmicutes	Dialister	nnaumosintas	736	Perez-Channaro	2014	2 studies	1.510 ND		0.17/	24.7
Firmieutes	Dialiston	<i>0141 14X011 300</i>	500	relez-Chapparo	2014	2 studies	1.518		0 107	04.0
Firmieutes	Lachnospingeogo [C 8]	oral taxon 500	500	Perez-Chapparo	2014	2 studies	ND			
Firmicules	Eubacterium [X1] [G-3]	brachy	35/	Perez-Chapparo	2014	2 studies	ND	0.005	0.001	30.4
Firmicutes	Johnsonella	oral taxon 166	160	Perez-Chapparo	2014	2 studies	ND	0.005	0.001	20.4
Finneinster		1 <i>((</i>	639. 892. 919. 920. 936. 937	Dana Channan	2014	2	ND			
Firmicutes	Selenomonas	genus	136. 137. 138. 139. 146. 149. 388. 442. 478. 479. 481. 501.	Perez-Chapparo	2014	2 studies	ND			
			124. 125. 126. 130. 133. 134.							

Correlation with mean PD	Rho	<i>p</i> -value	Mean ± SE	Occurrence (%)
Bacterial data				
Dysbiosis ratio	0.37	0.001	3 573 ± 202	100
E. nodatum	0.35	0.01	0.18 ± 0.04	62
P. gingivalis	0.33	0.01	2.77 ± 0.63	62
Desulfobulbus	0.28	0.05	1.52 ± 0.29	81
Treponema	0.26	0.05	5.59 ± 0.58	100
T. forsythia	0.25	0.05	4.52 ± 0.53	100
Porphyromonas	0.23	0.05	7.05 ± 0.83	98.7
Aggregatibacter	-0.31	0.01	0.85 ± 0.17	86.1
Campylobacter	-0.27	0.05	4.28 ± 0.36	100
Rothia	-0.26	0.05	0.22 ± 0.05	73.4
Clinical data				
Plaque index	0.25	0.03	0.19 ± 0.03	
Gingival index	0.35	0.002	0.41 ± 0.03	
Papillary bleeding index	0.36	0.002	0.4 ± 0.03	

Table 3: Correlation between mean probing depth (PD) and bacterial or clinical data. Only significant correlations are presented.

Dysbiosis ratio: (%*Porphyromonas*+%*Treponema*+%*Tannerella*)/(%*Rothia*+%*Corynebacterium*); SE: standard error. Plaque index from Silness & Loe. 1964, Gingival index from Lobene et al. 1986 and Papillary bleeding index from Barnett et al. 1980.

Model 1(Intercept)1Transferrin saturation (binary)0Plaque index-CPapillary bleeding index0log($Aggregatibacter$)-Clog($Rothia$)-Clog($Porphyromnas$)0Model 21Log(Dysbiosis ratio)0Log($Aggregatibacter$)-CPapillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0Table 4: Multiple linear adjusted regression models with bacteria positioredictors of mean periodontal pocket depth, adjusted for age, sex, snndex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.37Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.48	timate	Std. Error	t value	p-value
(Intercept)1Transferrin saturation (binary)0Plaque index-(Papillary bleeding index0log(Aggregatibacter)-(log(Rothia)-(log(Porphyromnas)0Model 21Log(Dysbiosis ratio)0Log(Aggregatibacter)-(Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0Table 4: Multiple linear adjusted regression models with bacteria positioredictors of mean periodontal pocket depth, adjusted for age, sex, snndex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.458Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.458				
Transferrin saturation (binary)0Plaque index-(Papillary bleeding index0 $log(Aggregatibacter)$ -($log(Rothia)$ -($log(Porphyromnas)$ 0Model 21(Intercept)1 $Log(Dysbiosis ratio)$ 0 $Log(Aggregatibacter)$ -(Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0Table 4: Multiple linear adjusted regression models with bacteria positi predictors of mean periodontal pocket depth, adjusted for age, sex, snndex and bleeding index.Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	1.484	0.221	6.730	3.54e-0
Plaque index-(Papillary bleeding index0 $log(Aggregatibacter)$ -($log(Rothia)$ -($log(Porphyromnas)$ 0Model 21(Intercept)1 $Log(Dysbiosis ratio)$ 0 $Log(Aggregatibacter)$ -(Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0Table 4: Multiple linear adjusted regression models with bacteria positi predictors of mean periodontal pocket depth, adjusted for age, sex, snIndex and bleeding index.Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	0.198	0.076	2.612	0.05
Papillary bleeding index0 $log(Aggregatibacter)$ -($log(Rothia)$ -($log(Porphyromnas)$ 0 Model 2 1 $(Intercept)$ 1 $Log(Dysbiosis ratio)$ 0 $Log(Aggregatibacter)$ -(Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positionpredictors of mean periodontal pocket depth , adjusted for age, sex, smindex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.4573Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.458	0.049	0.195	-0.252	NS
$\log(Aggregatibacter)$ -($\log(Rothia)$ -($\log(Porphyromnas)$ 0 $Model 2$ 1 $(Intercept)$ 1 $Log(Dysbiosis ratio)$ 0 $Log(Aggregatibacter)$ 0Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positionpredictors of mean periodontal pocket depth , adjusted for age, sex, smindex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.4573Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.458	0.301	0.161	1.872	NS
$\log(Rothia)$ -($\log(Porphyromnas)$ 0 $Model 2$ 1 $(Intercept)$ 1 $\log(Dysbiosis ratio)$ 0 $\log(Aggregatibacter)$ -(Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positioredictors of mean periodontal pocket depth , adjusted for age, sex, smIndex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.37Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.48	0.053	0.021	-2.492	0.05
$\log(Porphyromnas)$ 0Model 21 $(Intercept)$ 0 $Log(Dysbiosis ratio)$ 0 $Log(Aggregatibacter)$ 0Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positiopredictors of mean periodontal pocket depth, adjusted for age, sex, snindex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.48Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.48	0.063	0.025	-2.482	0.05
Model 2(Intercept)1Log(Dysbiosis ratio)0Log(Aggregatibacter)-CPapillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth, adjusted for age, sex, smindex and bleeding index.Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	0.051	0.025	2.035	0.05
Model 21Log(Dysbiosis ratio)0Log(Aggregatibacter)-CPapillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth, adjusted for age, sex, snindex and bleeding index.Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$				
(Intercept) 1 Log(Dysbiosis ratio) 0 Log(Aggregatibacter) -C Papillary bleeding index 0 Transferrin saturation (binary) 0 Male 0 Alcohol 0 Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth , adjusted for age, sex, smindex and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.488$				
Log(Dysbiosis ratio) 0 Log(Aggregatibacter) 0 Papillary bleeding index 0 Transferrin saturation (binary) 0 Male 0 Alcohol 0 Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth , adjusted for age, sex, sm index and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	1,280	0.147	8.686	4.39e-1
Log(<i>Aggregatibacter</i>) Papillary bleeding index 0 Transferrin saturation (binary) 0 Male 0 Alcohol 0 Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth , adjusted for age, sex, srindex and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	0.062	0,016	3.791	0.001
Papillary bleeding index0Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positipredictors of mean periodontal pocket depth , adjusted for age, sex, srindex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.37Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.48	0.053	0.018	-2.895	0.01
Transferrin saturation (binary)0Male0Alcohol0 Table 4: Multiple linear adjusted regression models with bacteria positipredictors of mean periodontal pocket depth , adjusted for age, sex, srindex and bleeding index.Characteristics of the model 1: multiple R ² = 0.4573, adjusted R ² = 0.37Characteristics of the model 2: multiple R ² = 0.5349, adjusted R ² = 0.48	0.349	0.128	2.732	0.01
Male 0 Alcohol 0 Table 4: Multiple linear adjusted regression models with bacteria positi predictors of mean periodontal pocket depth , adjusted for age, sex, sr index and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	0.159	0.069	2.309	0.05
Alcohol 0 Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth , adjusted for age, sex, srindex and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$. Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$.	0.171	0.069	2.457	0.05
Table 4: Multiple linear adjusted regression models with bacteria position predictors of mean periodontal pocket depth , adjusted for age, sex, srindex and bleeding index. Characteristics of the model 1: multiple $R^2 = 0.4573$, adjusted $R^2 = 0.37$ Characteristics of the model 2: multiple $R^2 = 0.5349$, adjusted $R^2 = 0.48$	0.121	0.088	1.373	NS
	moking status 798, F-statisti 868, F-statisti	c = 5.899 , <i>P</i> -value = $c = 11.12$, <i>P</i> -value = 3	ferrin saturation, pl 0.0001569. (42 deg 3.219e-08. (58 deg	laque index, g rees of freedo rees of freedo