Subgingival microbiological profile of periodontitis patients in Dominican Republic

James R. Collins[1], Sofía Chinea[3], Rosanna J. Cuello[1], Alba P. Florian[1], Patricia Palma[2], Nagore Ambrosio[3], María J. Marín[3], Elena Figuero[3], David Herrera[3]

[1] *Pontificia Universidad Católica Madre y Maestra (PUCMM), Escuela de Odontología, Departamento de Periodoncia, Santo Domingo, República Dominicana.*

[2] *Universidad de Chile, Escuela de Odontología, Departamento de Patología y Medicina Oral, Santiago, Chile.*

[3] *Universidad Complutense de Madrid, Facultad de Odontología, Grupo de investigación ETEP (Etiología y Terapéutica de las Enfermedades Periodontales), Departmento de Especialidades Clínicas Odontológicas, Madrid, Spain.*

ABSTRACT

Several studies have tried to associate the presence of different pathogens with the onset and progression of periodontitis, reporting a wide variety of results from different populations and environments. The aim of this study was to determine the main periodontal pathogens present in the subgingival biofilm of Dominican patients with periodontitis, by using specific microbiological culturing techniques. Periodontitis patients were selected after a full-mouth periodontal evaluation, and assigned to different periodontitis groups based on percentage of affected locations. Subgingival samples were collected and analyzed by means of specific culture techniques. Anaerobic counts, frequency of detection and proportions of target pathogens were calculated. Variables were analyzed by means of Student's T-test or chi-square test. Twenty-nine subjects were recruited, of whom 17 were diagnosed with generalized periodontitis (GenP) and 12 with localized periodontitis (LocP). The most prevalent bacterial species in both groups was Prevotella intermedia (94.1% in GenP and 91.7% in LocP), followed by Porphyromonas gingivalis (88.2% in GenP and 83.3% in LocP). Total microbiota in subgingival samples was 1.3 x10^7 colony-forming units (CFU)/mL (standard deviation, SD= 1.5 x10^7) and 9.6x10^6 CFU/mL (SD= 1.1 x10^7) in GenP and LocP subjects, respectively, though differences were not statistically significant (p= 0.222). The highest counts were observed for P. gingivalis in both groups, with mean concentration 2.5x10^6 CFU/mL (6.1x10^6) in GenP and 2.9x10^6 CFU/mL (5x10^6) in LocP, with no statistically significant difference (p=0.879). These results suggest that relevant periodontal pathogens are found with diversity and abundance in the subgingival microbiota of adult Dominican patients with periodontitis.

Received: February 2019; Accepted: March 2019.

Key words: *microbiology, culture techniques, Dominican Republic, periodontitis.*

Perfil microbiológico subgingival de pacientes con periodontitis en República Dominicana

RESUMEN

Varios estudios han tratado de asociar la presencia de diferentes patógenos con el inicio y la progresión de la periodontitis, mostrando una gran variedad de resultados en diferentes poblaciones y entornos. El objetivo del presente estudio fue determinar los principales patógenos periodontales presentes en la biopelícula subgingival de pacientes dominicanos con periodontitis, utilizando técnicas específicas de cultivo microbiológico. Los pacientes con periodontitis se seleccionaron después de una evaluación periodontal de boca completa y se asignaron a diferentes grupos de periodontitis según el porcentaje de localizaciones afectadas. Las muestras subgingivales fueron recolectadas y analizadas mediante técnicas de cultivo específicas. Se calcularon los recuentos anaeróbicos, la frecuencia de detección y las proporciones de los patógenos seleccionados. Las variables se analizaron mediante la prueba T de Student o la prueba de chi-cuadrado. Se reclutaron veintinueve sujetos, 17 diagnosticados como periodontitis generalizada (GenP) 12 con periodontitis localizada (LocP). La especie bacteriana más prevalente en ambos grupos fue Prevotella intermedia (94.1% y 91.7%, respectivamente) y seguida de Porphyromonas gingivalis (88.2% y 83.3%, respectivamente). La microbiota total en muestras subgingivales fue 1.3 x10^7 unidades formadoras de colonias (CFU)/mL (desviación estándar, SD= 1.5 x10^7) y 9.6x10^6 CFU / mL (SD= 1.1 x10^7) en sujetos GenP y LocP, respectivamente, pero no hubo diferencias estadísticamente significativas (p= 0.222). Los recuentos más altos se observaron para P. gingivalis en ambos grupos, con una concentración media de 2.5x10^6 CFU/mL (6.1x10^6) en GenP y 2.9x10^6 CFU/mL (5x10^6) en LocP, sin diferencias estadísticamente significativas (p= 0.879). Estos resultados sugieren que se encuentran patógenos periodontales relevantes con diversidad y abundancia en la microbiota subgingival de pacientes adultos dominicanos con periodontitis.

Palabras clave: *microbiología, técnicas de cultivo, República Dominicana, periodontitis.*

INTRODUCTION

Periodontal diseases are a group of diseases of infectious origin that occur with the inflammation of the tissues supporting the teeth. They are currently highly prevalent worldwide[1,2], and represent one of the main factors leading to tooth loss[3-6].

The primary etiological factor of periodontitis is the presence of bacteria organized in biofilms, which develop as interactive communities of microorganisms. The relationships between the bacteria embedded in a biofilm can be symbiotic, when there is a beneficial relationship among the bacteria that make up the biofilm and between them and the host, or dysbiotic, when there is a change in the community of microorganisms that leads to the development of pathology[7].

Several studies, mainly based on microbiological culturing for analysis of the samples, have tried to associate the presence of different pathogens with the diagnosis of periodontitis, reporting a wide variety of results from different populations and environments[8-11]. One of these studies by Sanz *et al.* (2000)[11] compared patients with periodontitis in Spain and the Netherlands, finding significant differences between the microbiological profiles. *Aggregatibacter actinomycetemcomitans* was found to be more prevalent in Dutch than in Spanish patients (23% versus 3%), whereas *Porphyromonas gingivalis* was found to be more prevalent in Spanish than in Dutch patients (65% versus 37%). These differences may be explained by patients' genetic profiles or as a result of the difference in the use of antibiotics between these two countries[12].

In Latin America, the "red complex" of bacteria (*P. gingivalis, Tannerella forsythia* and *Treponema denticola*) is found in high levels in patients with periodontitis [13, 14, 9, 10.] In Brazil, black-pigmented bacteria of the species *Porphyromonas* was detected in patients with periodontitis (89.4%), gingivitis (30%) and healthy patients (8%)[13]. In another study of the Brazilian population, *Porphyromonas* has prevalence of 74% in patients with periodontitis[14].

In the Dominican Republic to date, only two studies have been conducted analyzing subgingival microflora of Dominican patients with periodontitis, and they report different results. Slots et al.[15] performed the first study in which direct microscopic examination revealed that nonmotile organisms and cocci made up 85% of total microorganisms, while spirochetes only accounted for 3%. Non-selective culturing showed 53% Gram-negative organisms, 15% *Fusobacterium nucleatum*, 7% black-pigmented anaerobes and 10% *Parvimonas micra*. In contrast, a recent study using polymerase chain reaction (PCR) found prevalence of "red complex" bacteria, with approximately 90%, in patients with periodontitis, especially *T. forsythia*, differing from reports published for other Latin American countries[16].

The primary aim of the current study was to determine the main periodontal pathogens present in the subgingival biofilm of patients with periodontitis in the Dominican Republic, by using specific microbiological culturing techniques. The secondary aim was to compare these pathogens between patients with generalized and localized periodontitis.

MATERIALS AND METHODS

Study design

This cross-sectional study was approved by the institutional ethical committee of Pontificia Universidad Católica Madre y Maestra (PUCMM). All participants signed written informed consent. Study procedures were conducted according to the Declaration of Helsinki, the UNESCO Universal Declaration and the requirements of the Dominican Republic legislation.

Participants

Patients at the PUCMM dental clinic (Santo Domingo, Dominican Republic) who met the inclusion criteria were invited to participate in the study. All subjects provided informed consent. The screening period lasted from January 2014 to August 2015.

Inclusion criteria were: (1) age 18 years or older; (2) non-smokers; (3) at least 15 teeth present; (4) had not received periodontal treatment in the 12 months prior to the study; (5) were free of systemic diseases that could affect the tissue response (such as diabetes or immune diseases); (6) presented periodontitis, defined as the presence of at least 3 interproximal non-adjacent sites with probing pocket depth (PPD) of 4 mm or greater and (7) radiographic evidence of alveolar bone loss. Exclusion criteria were: (1) pregnant women and (2) having taken antibiotics and/or anti-inflammatory drugs in the previous month.

Clinical outcomes

A full-mouth clinical examination was performed on each patient and the following parameters were recorded at six sites per tooth using a North

Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA): (1) Plaque index (PlI) in percentage of sites, following O'Leary[17]; PPD; recession (REC) and clinical attachment loss (CAL) in millimeters; and bleeding on probing (BOP) as present/absent 30 seconds after probing, following Ainamo & Bay[18]. Based on their periodontal information (proportions of affected sites), patients were assigned to the generalized or localized periodontitis groups.

Microbiological sampling

In each patient, four sampling sites were selected, one in each quadrant, choosing those most accessible and with deepest probing depth and bleeding on probing[19]. At the selected sites, supragingival plaque was removed and the sites were dried with sterile cotton roles and air. Two consecutive sterile paper points (#30, Maillefer, Ballaigues, Switzerland) were inserted as deep as possible into the pocket, and left in place for 10 seconds. The paper points were transferred to a vial containing 2 ml of reduced transport fluid[20], and pooled with all the other paper points. The vial was kept at 4 ºC and sent to the laboratory at the Complutense University of Madrid (Spain) and processed within 24-36 hours.

Culture analyses

Vials were vortexed (30 seconds), serially diluted in phosphate-buffered saline, and plated on two different media: (1) blood agar medium (Blood Agar Base II®, Oxoid, Basingstoke, United Kingdom) supplemented with 5% horse blood, haemin (5 mg/l) and menadione (1 mg/l), and (2) Dentaid-1 medium[21]. After 4-14 days of anaerobic incubation (80% N_2, 10% CO_2 and 10% H_2), the plates were examined for the identification of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, P. micra, Campylobacter rectus, F. nucleatum, Capnocytophaga* spp. and *Eikenella corrodens*, based on different microbiological procedures: colony morphology, Gram-staining, catalase test, N-benzoyl-dL-arginine-2-napthylamide, indole and alpha -glucosidase activity; and standard biochemical test (RapIDTM ANA II System; Remel, Lenexa, KS, USA). Bacterial counts were expressed in colony-forming units (CFU) per mL of the original sample and total anaerobic counts were calculated, as well as counts of the detected periodontal pathogens. In addition to the quantitative microbiological data, the frequency of detection and proportions for each bacterial species were calculated.

Statistical analysis

Sample size calculation could not be performed. A convenience sample of 29 subjects was therefore selected based on previous microbiological studies[8-11]. The primary outcome variable was total anaerobic count (CFU/mL). Secondary outcome variables included all other microbiological variables, including frequency of detection of target pathogens, counts of each study pathogen, proportions of flora of each pathogen, and all clinical variables (PPD, BOP, REC, PlI, CAL). A subject-level analysis was performed for each study parameter. Data were expressed as means and standard deviation (SD), prevalence and proportions (%) for all variables. Total anaerobic counts and counts of each study pathogen were log transformed to fit a normal distribution. After evaluating the normality of the distribution (assessed by the Shapiro Wilk test), differences between different periodontal diagnosis groups were compared by Student´s T or Mann-Whitney U-test for quantitative variables, and chi-square or Fisher tests for categorical variables. The level of statistical significance was set at $p<0.05$. A statistical software package IBM® SPSS Statistics 25.0 (IBM Corporation, Armonk, NY, USA) was used for data analysis.

Table 1: Demographic data for GenP and LocP periodontitis groups.

	GenP (n= 17)	LocP (n=12)	*P value*
Age (years) *[mean (SD)]*	42.3(9.9)	46.2 (10.7)	*0.31*
Sex *[n (%)]*			*0.14*
Male	5 (29.4)	7 (58.3)	
Female	12 (70.6)	5 (41.7)	

SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis

RESULTS

Patient sample description

Twenty-nine patients were recruited, of whom 17 were diagnosed with generalized periodontitis (GenP) and 12 with localized periodontitis (LocP). Table 1 shows patient demographics. No statistically significant difference was found between groups. Mean age was 42.3 and 46.2 years, and percentage of males was 29.4% and 58.4% in the GenP and LocP groups, respectively.

Periodontal outcome variables

Table 2 shows clinical outcome variables. PPD and BOP values were significantly higher in GenP patients than in LocP patients ($p<0.05$). Mean PPD was 2.9 mm (SD=0.6) in the GenP versus 2.1 mm (SD=0.2) in LocP subjects. Similarly, BOP percentages were significantly higher in the GenP group (60.7% [SD=26.4%] versus 33.0% [SD=17.1%]). Table 3 shows the frequency distribution of PPD.

Table 4 shows data from the sampling locations. Plaque was 100%, BOP 85.7% and 62.5%, and mean PPD 5.8 and 5.0 mm in GenP and LocP, respectively. Statistically significant differences were found for PD values between groups ($p=0.037$).

Table 2: Mean values and standard deviation (SD) for clinical variables.

	GenP (n= 17)	LocP (n=12)	*P value*
	Mean (SD)	Mean (SD)	
Number of teeth	21.6 (4.1)	21.7 (3.7)	*0.969*
Number of sites	129.6 (24.7)	130.0 (22.05)	*0.968*
PII (%)	59.9 (32.1)	58.9 (32.5)	*0.720*
PPD (mm)			
All	2.9 (0.6)	2.1 (0.2)	*<0.001**
Upper	3.3 (0.7)	2.4 (0.3)	*<0.001**
Lower	2.5 (0.6)	1.9 (0.3)	*0.006**
Interproximal	3.2 (0.6)	2.4 (0.2)	*<0.001**
Vestibular/lingual	2.2 (0.5)	1.7 (0.3)	*0.019**
REC (mm)			
All	0.3 (0.7)	0.3 (0.9)	*0.793*
Upper	0.1 (0.7)	0.4 (0.9)	*0.372*
Lower	0.4 (0.7)	0.3 (0.9)	*0.806*
Interproximal	0.1 (0.7)	0.2 (0.9)	*0.929*
Vestibular/lingual	0.5 (0.7)	0.7 (0.9)	*0.554*
CAL (mm)			
All	3.2 (1.2)	2.5 (0.9)	*0.150*
Upper	3.4 (1.3)	2.8 (1.1)	*0.206*
Lower	3.0 (1.2)	2.3 (1.0)	*0.145*
Interproximal	2.5 (0.7)	1.9 (0.6)	*0.035**
Vestibular/lingual	2.1 (0.5)	1.8 (0.6)	*0.417*
BOP (%)			
All	60.7 (26.4)	33.0 (17.1)	*0.004**
Upper	68.3 (21.4)	38.1 (19.4)	*0.001**
Lower	54.0 (34.6)	27.9 (15.9)	*0.022**
Interproximal sites	60.8 (28.7)	32.4 (20.0)	*0.007**
Vestibular/lingual	60.6 (27.2)	34.1 (16.5)	*0.006**

BOP: bleeding on probing; CAL: clinical attachment loss; PPD: probing pocket depth; PII: plaque index; REC: recession; SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Subgingival samples

Table 5 shows data on detection of pathogens from subgingival samples, including their frequency of detection, mean concentrations and proportions.

The most prevalent bacterial species in GenP subjects were *P. intermedia* (94.1%) and *F. nucleatum* (94.1%) followed by *P. gingivalis* (88.2%). In LocP patients *P. intermedia* (91.7%) and *P. gingivalis* (83.3%) were the most prevalent bacteria, followed by *F. nucleatum* (58.3%). Statistically significant differences were only found for *F. nucleatum* ($p=0.019$).

Total microbiota counts in subgingival samples were 1.3×10^7 CFU/mL (SD=1.5×10^7) and 9.6×10^6 CFU/mL (SD=1.1×10^7) in GenP and LocP subjects, respectively, but differences were not statistically significant ($p=0.222$). The highest counts were observed for *P. gingivalis* in both groups, with a mean concentration of 2.5×10^6 CFU/mL (6.1×10^6) in GenP groups and 2.9×10^6 CFU/mL (5×10^6) in LocP, followed by *P. intermedia* [with mean concentration 7.2×10^5 CFU/mL (1.4×10^6) in GenP

Table 3: Frequency distribution of probing depth in both groups.

	GenP (n= 17)	LocP (n=12)	*P value*
PPD (% of sites)			
< 4 mm	71.4 (17.5)	90.6 (4.9)	<0.001*
4-6 mm	25.9 (15.9)	8.6 (4.7)	<0.001*
> 6 mm	2.7 (2.5)	0.8 (1.9)	0.040*

PPD: probing depth; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Table 4: Mean values and standard deviation (SD) of clinical variables at sites selected for microbial sampling.

	GenP (n= 17)	LocP (n=12)	*P value*
	Mean (SD)	Mean (SD)	
PII (%)	100 (0)	100 (0)	
PPD (mm)	5.8 (1.1)	5.0 (0.8)	*0.037**
BOP (%)	85.7 (23.8)	62.5 (38.3)	*0.081*
REC (mm)	0.2 (1.0)	0.2 (0.9)	*0.963*

BOP: bleeding on probing; PII: plaque index; PD: probing depth; REC: recession; SD: standard deviation; GenP: generalized periodontitis; LocP: localized periodontitis; *: Statistically significant differences ($p<0.05$).

Table 5: Frequency of detection (%), mean counts (in colony forming units, CFU/mL) and mean proportions of microbiota (%) of periodontal pathogens in subgingival samples.

	- Aa	- Pg	- Pi	- Fn	- Cr	- Ec	- Tf	- Capno	- Pm
Prevalence n (%)									
- GenP	- 2 (11.8)	- 15 (88.2)	- 16 (94.1)	- 16 (94.1)	- 2 (11.8)	- 6 (35.3)	- 10 (58.8)	- 2 (11.8)	- 3 (17.6)
- LocP	- 1 (8.3)	- 10 (83.3)	- 11 (91.7)	- 7 (58.3)	- 1 (8.3)	- 2 (16.7)	- 5 (41.7%)	- 1 (8.3)	- 4 (33.3)
p value	*0.765*	*0.708*	*1.000*	*0.019**	*0.763*	*0.408*	*0.362*	*1.000*	*0.403*
Concentration CFU/ml [Mean (SD)]									
GenP	$2.1x10^4$	$2.5x10^6$	$7.2x10^5$	$1.5x10^5$	$2.3x10^4$	$3.0x10^5$	$4.5x10^5$	$1.2x10^4$	$9.4x10^3$
	($8.7x10^4$)	($6.1x10^6$)	($1.4x10^6$)	($2.2x10^5$)	($6.6x10^4$)	($5.8x10^4$)	($8.4x10^5$)	($4.8x10^4$)	($2.6x10^4$)
LocP	$5 x10^2$	$2.7x10^6$	$2.7x10^5$	$2.1x10^5$	$3.3x10^4$	$1.6x10^4$	- $3.1x10^5$	$4.5x10^4$	$3.9x10^4$
	($1.8x10^3$)	($5x10^6$)	($4.1x10^5$)	($3.8x10^5$)	($1.2x10^5$)	($3.6x10^4$)	($5.9x10^5$)	($1.5x10^5$)	($7.7x10^4$)
P value	*0.222*	*0.403*	*0.403*	*0.238*	*0.397*	*0.599*	*0.478*	*0.523*	*0.551*
Mean proportions of microflora % [Mean (SD)]									
- GenP	- 0.6 (2.7)	- 6.3 (11.2)	- 2.2 (6.8)	- 0.4 (0.7)	- 0.01 (0.03)	- 0.5 (1.7)	- 1.2 (3.7)	- 0.01 (0.03)	- 0.1 (0.7)
- LocP	- 0.1	- 3.2 (5.7)	- 0.7 (1.2)	- 2.0 (4.4)	- 0.02 (0.08)	- 0.03 (0.09)	- 0.02 (0.03)	- 0.5 (1.8)	- 0.3 (0.6)
- p value	*0.84*	*- 0.74*	*0.49*	*0.44*	*0.91*	*0.39*	*0.30*	*0.91*	*0.44*

-Aa: Aggregatibacter actinomycetemcomitans; Pg: Porphyromonas gingivalis; Pi: Prevotella intermedia; Fn: Fusobacterium nucleatum; Cr: Campylobacter rectus; Ec: Eikenella corrodens; Tf: Tannerella forsythia; Capno: Capnocytophaga spp.; Pm: Parvimonas micra.
-GenP: generalized periodontitis; LocP: localized periodontitis; SD: Standard deviation; *: Statistically significant differences ($p<0.05$).

groups and $2.7x10^5$ CFU/mL ($4.1x10^5$) in LocP] and *T. forsythia* [with mean concentration $4.5x10^5$ CFU/mL ($8.4x10^5$) in GenP groups and $3.1x10^5$ CFU/mL ($5.9x10^5$) in LocP]. Statistically significant differences were not found between groups for any individual bacteria counts. In terms of proportions, similar mean values were found between groups without statistically significant differences between them.

DISCUSSION

In the present study, subgingival samples from 29 Dominican patients with periodontitis were analyzed using microbiological culturing in specific non-selective and selective media. The bacterial species with highest frequency of detection were *P. intermedia, P. gingivalis* and *F. nucleatum* (93.1%, 86.2% and 79.3%, respectively). No difference was detected when localized and generalized periodontitis patients were compared. There are few previous studies on the Dominican population using cultures. The results reported by Slots et al. [15] are consistent with ours (*P. gingivalis, F. nucleatum* and *P. intermedia* as the most prevalent species), but the frequencies of detection are different, almost certainly attributable to the use of different transport media [reduced transport fluid (RTF) or Viability Medium, Göteborg, Anaerobically prepared (VMGA) III]. Collins *et al.*[16] studied gingivitis, periodontitis and healthy participants from the Dominican Republic using specific PCR, finding frequencies of 93.3% for *P. gingivalis,* 53.3% for *P. intermedia* and proportions greater than 80% for other species such as *F. nucleatum, P. micra and E. corrodens.* They detected these three species in a greater number of patients compared to the current study, possibly due to the lower detection limits of molecular techniques compared to culture.

In the general population, the reported prevalence (86.2%) of *P. gingivalis* agrees with the current evidence, with this species being one of the most frequently detected species in subgingival samples in patients with periodontitis. In Spain, this bacterial species was found in 64.5%[11] and 77.8%[10] of the population. In Chile and Colombia, its prevalence has been reported as 83.8% and 65.9%, respectively. All these studies used similar methodologies and the populations analyzed had similar clinical features. In all cases, the samples were processed between 24 and 48 hours after being taken.

In our study, *P. intermedia*, a Gram-negative bacillus, was also found in a high percentage of cases, having been recovered in the cultures from 93.1% of the

subjects. These findings are similar to those from Spain and Chile, where the occurrence of *P. intermedia* was 97.2% and 72.5%, respectively. However, in a Colombian population the frequency was lower, at 19.4%[10]. These differences could be attributed to the different lifestyles of people living in each geographical location or to the clinical presentations of periodontitis[22,23]. On the other hand, differences could also be explained, not only by genetic differences[24] , but also by epigenetic differences, which could be related to variations in the abundance and distribution of microbial species in populations[25].

It should be highlighted that *T. forsythia* was detected in more than 50% of the patients in the present study, but in 90% of the subjects in the PCR-based study[16]. The greater frequency of detection using molecular methodologies evidences the greater sensitivity of the PCR technique[26-28], though it should also be considered that detection and abundance of this anaerobic species, which is difficult to culture, could have been underestimated due to the time elapsed between sampling and processing. However, although *T. forsythia* may be partly underestimated, its detection by culture highlights its high frequency in Dominican patients compared to other populations, with reported frequencies of 16.2%, 39.0% and 36.1%, in Chile, Colombia and Spain, respectively.

A. actinomycetemcomitans has been associated by various authors, such as Jardim *et al*.[29] or Sulugodu *et al*.[30], with a higher rate of progression in periodontitis. In the present study, its occurrence was 11.8% and 8.3% for GenP and LocP, respectively, which was low compared to other studies, such as Mullally *et al*.[31], which reported a detection frequency of 19%, and higher in patients with LocP.

It should be considered that results for bacterial abundance may be a direct consequence of the sampling and processing protocol. The volume of transport medium in which the sample was placed, and from which dilutions are subsequently prepared for inoculation into the culture media, as well as the number of sites from which the sample was collected, will reflect the total counts of viable cells present in these samples. Both these factors may make comparisons between studies difficult. However, in the present study, the most abundant species in relative terms were *P. gingivalis* and *P. intermedia,* followed by *F. nucleatum* and *T. forsythia*, similarly to Slots *et al*. [15], with the exception of *T. forsythia,* which was not evaluated.

The advantages of detecting bacteria by means of culture techniques are that it enables determination of number of viable cells in a sample, detection of species that could be present unusually, analysis of susceptibility to antimicrobials and characterization of the microbiota associated with oral diseases[32]. Microbiological culturing techniques are fundamental and basic diagnostic methods widely used as research tools in molecular biology. In addition, these methods are still considered the "gold standard" methods of reference in periodontal microbiology, to which other microbiological identification procedures are compared[33]. However, culturing can be affected by the handling and processing of the sample, especially in the current study, in which sampling and analysis were performed in different countries. These aspects, along with the relatively small sample size, should be considered as potential limitations of the study.

In the present study, periodontal diagnosis was initially based on the 1999 classification of periodontal diseases[34]. In the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, the terms "aggressive" and "chronic" have disappeared, and the condition "periodontitis" should be now categorized with a multidimensional system of stages (I, II, III, IV) and grades (A, B, C). The stages will capture the severity of the disease and the anticipated complexity of the therapy, while the grades consider the identification of risk factors that may impact general health and the progression of the disease[35-37]. This new approach may allow the development of meticulous treatment strategies according to the specific needs of each patient (precision medicine). With regard to the extension and distribution of periodontitis, minor differences are expected with the new classification. To determine a case of LocP or GenP, the presence of <30% or >30%, respectively, of affected sites was used in the 1999 classification[34], while with the 2018 approach, LocP is described as <30% of the teeth involved and >30% for GenP.

The strength of this study is that samples were analyzed in an experienced laboratory, which has already processed samples from distinct geographical populations (Spain, The Netherlands, Chile, Colombia) in different international research projects. This enables comparison of the results of

the present study (and population) to previous studies which followed identical methodology and were analyzed in the same laboratory[10,11]. It was possible to detect and identify nine bacterial species that are part of the subgingival microbiota and that are closely associated with the dysbiotic phenomena that trigger periodontal diseases. Therefore, this study constitutes an approximation of the bacterial composition in both diversity and abundance of the subgingival microbiota of patients with periodontitis in the Dominican Republic.

CONCLUSION

Within the limitations of the present study, the subgingival microbial composition in patients with periodontitis from Dominican Republic showed a high frequency of detection of *P. intermedia, P. gingivalis* and *F. nucleatum.* Our data suggest that periodontal pathogens in the subgingival microbiota of adult Dominican patients with periodontitis have overall similar diversity and abundance to those in other geographical populations, but with higher frequency of detection of *T. forsythia*.

FUNDING

None

CORRESPONDENCE

Dra. Elena Figuero.
Departamento de Especialidades Clínicas Odontológicas
Facultad de Odontología.
Universidad Complutense de Madrid
Plaza Ramón y Cajal s/n (Ciudad Universitaria).
28040 Madrid, España
elfiguer@ucm.es

REFERENCES

1. Pihlstrom B, Michalowicz B, Johnson N. Periodontal Diseases. Lancet. 2005; 19:1809-1820.
2. Papapanou PN. The prevalence of periodontitis in the US: forget what you were told. J Dent Res 2012; 91:907-908.
3. Kassebaum N, Bernabé E, Dahiya M, Bhandari B, Murray C, Marcenes W. Global burden of severe tooth loss: A systematic review and meta-analysis. J Dent Res 2014; 93(Suppl 7):20-28.
4. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. Periodontol 2000. 2012; 60:15-39.
5. Heitz-Mayfield L, Schätzle M, Löe H, Bürgin W, Anerud A, Boysen H, Lang NP. Clinical course of chronic periodontits. II. Incidence, characteristics and time of occurrence of the initial periodontal lesion. J Clin Periodontol 2003; 30:902-908.
6. Schätzle M, Löe H, Lang N, Heitz-Mayfield L, Bürgin W, Anerud A, Boysen H. Clinical course of chronic periodontitis. III. Patterns, variations and risks of attachment loss. J Clin Periodontol 2003; 30:909-918.
7. Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H, Ellwood R, Giacaman RA et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017; 44(Suppl18):s5-s11.
8. Haffajee A, Bogren A, Hasturk H, Feres M, Lopez N, Socransky S. Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol. 2004; 31:996-1002.
9. Gajardo M, Silva N, Gomez L, Leon R, Parra B, Contreras A, Gamonal J. Prevalence of periodontopathic bacteria in aggressive periodontitis patients in a Chilean population. J Periodontol. 2005; 76:289-294.
10. Herrera D, Contreras A, Gamonal J, Oteo A, León Berríos R, Jaramillo A, Silva N, Sanz M et al. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. J Clin Periodontol 2008; 35:106-113.
11. Sanz M, van Winkelhoff A, Herrera D, Dellemijn-Kippuw N, Simón R, Winkel E. Differences in the composition of the subgingival microbiota of two periodontitis populations of different geographical origin. A comparison between Spain and The Netherlands. Eur J Oral Sci 2000; 108:383-392.
12. van Winkelhoff A, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. J Clin Periodontol 2005; 32:893-898.
13. Missailidis C, Umeda J, Ota-Tsuzuki C, Anzai D, Mayer M. Distribution of fimA genotypes of Porphyromonas gingivalis in subjects with various periodontal conditions. Oral Microbiol Immunol 2004; 19:224-229.
14. Cortelli J, Cortelli S, Jordan S, Haraszthy V, Zambon J. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol 2005; 32:860-866.
15. Slots J, Rams T, Feik D, Taveras H, Gillespie G. Subgingival microflora of advanced periodontitis in the Dominican Republic. J Periodontol 1991; 62:543-547.
16. Collins J, Arredondo A, Roa A, Valdez Y, León R, Blanc V. Periodontal pathogens and tetracycline resistance genes in subgingival biofilm of periodontally healthy and diseased Dominican adults. Clin Oral Invest 2016; 20:349-356.
17. O'Leary, TJ, Drake R, Naylor J. The plaque control record. J Periodontol 1972; 43:38.
18. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975; 25:229-235.
19. Mombelli A, McNabb H, Lang N. Black-pigmenting gram-negative bacteria in periodontal disease. II. Screening

strategies for detection of *P. gingivalis*. J Periodontal Res 1991; 26:308-313.
20. Syed S, Loesche W. Survival of human dental plaque flora in various transport media. Appl Microbiol 1972; 24:638-644.
21. Alsina M, Olle E, Frias J. Improved, low-cost selective culture medium for *Actinobacillus actinomycetemcomitans*. J Clin Microbiol 2001; 39:509-513.
22. Gjermo P, Rosing C, Susin C, Oppermann R. Periodontal diseases in Central and South America. Periodontol 2000. 2002; 29:70-78.
23. Sheiham A, Netuveli G. Periodontal diseases in Europe. Periodontol 2000. 2002; 29:104-121.
24. Loos B, John R, Laine M. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol 2005; 32(Suppl 6):s159–s179.
25. Maudet C, Mano M, Eulalio A. MicroRNAs in the interaction between host and bacterial pathogens. FEBS Lett 2014; 588:4140-4147.
26. Jervoe-Storm P, Koltzscher M, Falk W, Dorfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. J Clin Periodontol 2005; 32:778-783.
27. Eick S, Pfister W. Comparison of microbial cultivation and a commercial PCR based method for detection of periodontopathogenic species in subgingival plaque samples. J Clin Periodontol 2002; 29:638-644.
28. Bankur P, Nayak A, Bhat K, Bankur R, Naik R, Rajpoot N. Comparison of culture and polymerase chain reaction techniques in the identification of *Tannerella forsythia* in periodontal health and disease, an *in vitro* study. J Indian Soc Periodontol 2014; 18:155-160.
29. Jardim E, Bosco J, Lopes A, Landucci L, Jardim E, Carneiro S. Occurrence of *Actinobacillus actinomycetemcomitans* in patients with chronic Periodontitis, Aggressive periodontitis, Healthy subjects and children with Gingivitis in two cities of the state of São Paulo, Brazil. J Appl Oral Sci 2006; 14:153-156.
30. Sulugodu S. Low levels of caries in aggressive periodontitis: A literature review. Saudi Dent J 2014; 26: 47-49.
31. Mullally B, Dace B, Shelburne C, Wolff F, Coulter W. Prevalence of periodontal pathogens in localized and generalized forms of early-onset periodontitis. J Periodontal Res 2000; 35:232-241.
32. Tanner AC. Anaerobic culture to detect periodontal and caries pathogens. J Oral Biosci 2015; 57:18-26.
33. Yang H, Huang Y, Chou M. Occurrence of *Porphyromonas gingivalis and Tannerella forsythensis* in Periodontally Diseased and Healthy Subjects. J Periodontol 2004; 75:1077-1083.
34. Armitage, GC. Development of a Classification System for Periodontal Diseases and Conditions. Ann Periodontol. 1999; 4:1-6.
35. Caton JG, Armitage GC, Berglundh T, Chapple I, Jepsen S, Kornman KS et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol 2018; 45(Suppl 20):S1-S8.
36. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018; 45(Suppl 20): S162-S170.
37. Tonetti M S, Greenwell H, Kornman K S. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Clin Periodontol 2018; 45 Suppl 20: S149–S161.