J Clin Periodontol 2008; 35: 106–113 doi: 10.1111/j.1600-051X.2007.01170.x

Journal of Clinical Periodontology

Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain

David Herrera[1], Adolfo Contreras[2], Jorge Gamonal[3], Alfonso Oteo[1], Adriana Jaramillo[2], Nora Silva[3], Mariano Sanz[1], Javier Enrique Botero[2] and Rubén León[3]

[1]ETEP Research Group, Faculty of Dentistry, University Complutense, Madrid, Spain; [2]Periodontal Medicine Group, School of Dentistry, University del Valle, Cali, Colombia; [3]Faculty of Dentistry, University de Chile, Santiago de Chile, Chile

Herrera D, Contreras A, Gamonal J, Oteo A, Jaramillo A, Silva N, Sanz M, Botero JE, León R. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. J Clin Periodontol 2008; 35: 106–113. doi: 10.1111/j.1600-051X.2007.01170.x.

Abstract

Aim: To investigate the subgingival microbiota of distinct periodontitis patient populations, in Chile, Colombia and Spain, using identical clinical and bacteriological methods.

Material and Methods: In this multicentre study, 114 chronic periodontitis patients were selected. Patients were examined using an identical clinical protocol and pooled subgingival samples were obtained from each patient. Samples were processed in the three laboratories by means of culturing under identical clinical and microbiological protocols. Total anaerobic counts and frequency of detection and proportions of nine periodontal pathogens were calculated. Variables were analysed by means of ANOVA, χ^2, Kruskal–Wallis and Dunn's multiple comparison tests.

Results: The Colombian population demonstrated greater severity of periodontitis, with significantly deeper mean probing pocket depth, and had a significantly lower percentage of current smokers. When comparing samples from the three patient populations, the total counts were significantly higher in the Colombian patients. The numbers of putative pathogens differed among groups. *Tannerella forsythia* was found less frequently in Chilean samples, while *Parvimonas micra* and enteric rods differed significantly among the three population groups.

Conclusion: Significant differences among Chile, Colombia and Spain existed regarding the frequency and proportions of specific periodontal pathogens in the subgingival microbiota of periodontitis patients.

Key words: Chile; Colombia; periodontitis; Spain; subgingival microbiota

Accepted for publication 22 October 2007

Periodontal diseases are widely distributed in the world and represent a major oral health problem both in developed and in developing countries. The role of subgingival microbial species in the etiology of periodontal disease has been extensively documented (Socransky & Haffajee 1994, Curtis et al. 2005, van Winkelhoff & Boutaga 2005). The current body of knowledge indicates that specific microorganisms, including *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*, occur more frequently and/or in higher numbers of organisms in periodontitis sites and subjects, whereas other bacterial species, such as members of the genera *Streptococcus* and *Actinomyces*, have been associated with periodontal health (Haffajee & Socransky 1994, Haffajee et al. 1998).

Knowledge on the microbial risk factors associated with periodontal disease in various populations is limited. Most of the knowledge available on the microbial composition of subgingival plaque is based on studies from the United States, Western Europe and Japan, in where preventive and therapeutic oral care programmes are available to significant proportions of the population. Thus, only limited data are

Conflict of interest and source of funding statement

The authors declares that they have no conflict of interests.
The Chilean group was supported by ''Fondo Nacional Desarrollo Ciencia y Tecnología: Fondecyt Número 1050518''.

available on the subgingival microbial composition of periodontitis subjects in developing countries (Gjermo et al. 2002), and few studies have compared the microbial differences among countries (Ali et al. 1994, Sanz et al. 2000, Haffajee et al. 2004, Lopez et al. 2004) and seldom have investigators using the same technology and previously calibrated methods to compare the microbial composition among different populations. The relative contribution of different bacterial species may vary in geographically or racially different groups. Compiling data from various populations by different researchers will confirm common risk factors or uncover risk factors unique to a specific group in the population. These differences may have an impact on the diagnosis, prognosis and treatment of periodontal disease.

Different studies using both cultural and molecular bacterial detection techniques have tried to associate the presence of different putative pathogens with gingivitis and periodontitis, demonstrating a wide variety of results in different populations from different geographical origins (Sanz et al. 2000, 2004). These studies have concluded that the prevalence of specific periodontal pathogens varies between individuals from the same environment and among different ethnicities and/or countries (Umeda et al. 1998, Haffajee et al. 2004). Such findings raise the concern that results from studies reporting the efficacy of different protocols of periodontal treatment in a given population may not be directly extrapolated to subjects in other locations.

Some studies have revealed differences in microbial profiles when comparing different ethnic groups. Using 16S rRNA-based polymerase chain reaction (PCR), Umeda et al. (1998) demonstrated that Hispanics and Asians living in Los Angeles (USA) harboured higher proportions of *P. gingivalis* and *A. actinomycetemcomitans* than Caucasians. Michalowicz et al. (2000), using the same PCR technology, demonstrated that Jamaicans with localized aggressive periodontitis (formerly localized juvenile periodontitis) had a higher prevalence of *P. gingivalis* and Cytomegalovirus, rather than *A. actinomycetemcomitans*, the aetiologic agent in most forms of localized aggressive periodontitis in subject groups studied in the United States, Finland and Africa.

Other studies have revealed country-specific differences in subgingival microbial composition. Sanz et al. (2000), using cultural methods, compared the microbial composition of subgingival plaque samples from Dutch and Spanish periodontitis patients. The results showed that *A. actinomycetemcomitans* and *Parvimonas micra* (formerly *Micromonas micros*) were more prevalent in the Dutch patients (23.3% *versus* 2.3%; 96.7% *versus* 74.2%), while *P. gingivalis* was more prevalent in the Spanish subjects (64.5% *versus*. 36.7%). In another study from the same research group, Herrera et al. (2000) demonstrated an increase in β-lactamase-producing bacteria among bacterial isolates from the Spanish group. Using the checkerboard DNA–DNA hybridization technique, Lopez et al. (2004) compared the microbial composition of subgingival plaque samples between Chilean subjects and subjects from the USA with chronic periodontitis. Their results indicated that Chilean subjects harboured significantly higher proportions of *A. actinomycetemcomitans, P. gingivalis, T. denticola* and *T. forsythia.* Haffajee et al. (2004) evaluated the subgingival microbiota of 300 chronic periodontitis subjects from Sweden, the United States, Chile and Brazil using the checkerboard technique. Brazilians harboured significantly higher proportions of *Actinomyces naeslundi* genospecies 1 and 2, *Streptococcus intermedius*, *Streptococcus sanguinis, Streptococcus gordonii, Streptococcus constellatus, Eubacterium nodatum* and *T. denticola* than the other study populations. Compared with Brazilians and Chileans, the United States and Swedish populations presented significantly lower proportions of *T. denticola* and *P. gingivalis.*

Enteric rods and pseudomonads are opportunistic organisms that can be detected in the subgingival environment of periodontitis subjects. Their prevalence varies around the world and they are resistant to the majority of adjunctive therapeutic agents (antibiotics) used to treat periodontitis (Slots et al. 1990a, b, Ali et al. 1994, Barbosa et al. 2001, Botero et al. 2007a, b, Lafaurie et al. 2007). Geographical differences in the distribution of these microbes could impact periodontal treatment protocols.

Although it has become apparent that there are substantial differences in the composition of the subgingival microbiota in subjects from various geographical locations, there is a need for a global understanding of the bacterial composition in different periodontal infections, which may enable the establishment of specific preventive and therapeutic strategies.

The objective of this study, therefore, was to investigate the subgingival microbiota of defined periodontitis patient populations in Chile, Colombia and Spain, using identical bacteriological methods.

Material and Methods

Patients diagnosed with chronic periodontitis at the dental clinics in the University Complutense of Madrid (Spain), University of El Valle (Cali, Colombia) and University of Chile (Santiago, Chile) were invited to participate in the study and were recruited into this multicentre investigation if they fulfilled the inclusion criteria and manifested their voluntary participation by signing an Institutional Review Board (IRB) approved informed consent. The IRB of each University had also approved the protocol and study design of the study.

Selection of patients

An initial screening visit was performed to assess whether the patients fulfilled the following inclusion criteria: individuals aged 25 years and older; with at least 12 teeth present; excluding third molars; that had no previous periodontal treatment; and were free of systemic diseases such as diabetes, arthritis, ulcerative colitis, Crohn's disease, HIV infection, cancer and heart disease. Pregnant women were also excluded from participating in the study, in addition to subjects who, in the previous month, had taken antibiotics and/or anti-inflammatory drugs.

Chronic periodontitis was defined according to the American Academy of Periodontology consensus report on the Classification of Periodontal Diseases (Armitage 1999). A full-mouth clinical examination was performed in each patient using a manual probe (UNC 15, Hu Friedy, Chicago, IL, USA) and the following parameters were recorded at six sites per tooth: probing depth (PD in mm), clinical attachment loss (CAL in mm), bleeding on probing (BOP in percentage of sites) and plaque index (PlI in percentage of sites). Patients were selected if they presented four or more sites with a PD of 5 mm or higher

and an attachment loss of 2 mm or higher (Offenbacher et al. 2001). Patients with initial periodontitis were not included in the study.

Selection of sampling sites

Four sites were selected in each quadrant based on the patient's clinical and radiographic periodontal records. Sites presenting PD > 5 mm, CAL > 5 mm and BOP were selected (Lang et al. 1990, Mombelli et al. 1991a, b). The selection of sites was made based on the data recorded during the screening visit. No further evaluation of the selected sites was performed before subgingival sampling.

Microbiological sampling

At selected sites, supragingival plaque was carefully removed to avoid bleeding using sterile gauze and/or curettes. Then, these sites were dried with sterile cotton rolls and gentle air drying. Two consecutive sterile paper points (medium size, Maillefer, Ballaigues, Switzerland) were inserted as deep as possible into the pocket, and left in place for 15 s. The paper points were transferred to a vial containing 1.5 ml of reduced transport fluid (Syed & Loesche 1972), and pooled with all the other paper points. The vial was sent to the laboratory and processed within 24 h.

At the laboratory, the vials were vortexed (30 s), serially diluted and plated in two different media: blood agar medium (No. 2 of Oxoid; Oxoid Ltd, Basingstoke, UK), with 5% horse blood, and haemin (5 mg/l) and menadione (1 mg/l) and Dentaid-1 medium (Alsina et al. 2001). The blood agar plates were studied after 7 and 14 days of anaerobic incubation (80% N_2; 10% H_2; 10% CO_2 at 37°C), and the Dentaid-1 plates after 3–5 days of 37°C incubation in air with 5% CO_2. Plates were carefully examined for the identification of *A. actinomycetemcomitans, P. gingivalis, Prevotella intermedia/nigrescens, T. forsythia, P. micra, Capnocytophaga* spp., *Eikenella corrodens* and *Fusobacterium* spp., based on the morphology of the colony and using different standard biochemical tests to confirm the initial identification (RAPID ANA II). Other relevant colonies (those representing an important proportion of the flora) were also isolated for further characterization. Colonies of each bacterial species were counted, as was the total number of colonies in a representative plate (between 30 and 300 colonies). Counts of *A. actinomycetemcomitans* were performed on Dentaid-1 plates, based on its typical colony morphology, a catalase reaction and a set of specific enzymes. Additionally, any colony growing on Dentaid-1 medium, suspected of being an enteric rod, was isolated. Dentaid-1 medium, as a TSBV medium (Slots 1982), has demonstrated excellent recovery of *Enterobacteriaceae* and *Pseudomonadaceae* species. Suspect non-oral, Gram-negative, facultatively anaerobic rods (Slots et al. 1990b) were subcultured on McConkey agar, purified and classified using a commercial identification kit system (API 20 E, Baxter Healthcare, West Sacramento, CA, USA). The panels and bacterial inoculae were prepared following the recommendations of the manufacturer, and incubated for 18–24 h at 35°C in a non-CO_2 incubator. Bacterial speciation, based on 34 taxonomic test reactions, was performed using the software provided by the manufacturer.

The laboratory technicians at the three study centres had been previously trained and calibrated in the use of the same bacterial culture technology and followed an identical microbiological methodology.

Statistical analysis

Age was compared among groups using ANOVA, while the χ^2 test was used for gender and smoking. PPD and CAL were averaged by patient and group, and the percentage of sites with plaque and BOP were calculated first by patient and then by group. Differences among population groups were determined using the Kruskal–Wallis test and the post-hoc test selected was Dunn multiple comparison test. Total anaerobic counts were log transformed and compared following the same procedures as those applied to the clinical variables. The frequency of detection of different bacterial pathogens was compared by the χ^2 test. The proportions of bacterial pathogens in positive samples were compared as described for the clinical variables. Statistical significance was assumed when $P \leqslant 0.05$.

Table 1. Demographics of the selected populations

	Chile	Colombia	Spain	Statistics
n	37	41	36	
Age				
Mean	44.6	41.5	44.9	One-way ANOVA
Standard deviation	8.1	10.6	11.7	$p = 0.3731$
Maximum	60	64	67	
Minimum	31	25	26	
Gender				
Female	25	25	20	χ^2
Male	12	16	16	NS
% Female	67.6	61.0	55.6	
% Male	32.4	39.0	44.4	
Smoking				
Non-smokers	26	37	21	χ^2
Smokers	11	3	12	Spain–Chile, NS
Former smokers	0	1	3	Spain–Colombia, $p = 0.004$
% Non-smokers	70.3	90.2	63.6	Colombia–Chile, $p = 0.01$
% Smokers	29.7	7.3	36.4	
% Former	0.0	2.4	9.1	

NS, not statistically significant ($p > 0.05$).

Results

Demographics of the selected populations (Table 1)

The sample population of moderate to severe chronic periodontitis patients included 41 patients in Cali (Colombia), 37 patients in Santiago (Chile) and 36 patients in Madrid (Spain). No statistically significant differences for age and gender were observed among the groups. The percentage of smokers was significantly lower in the Colombian population (7.3%), when compared with the Chileans (29.7%, $p = 0.01$) and the Spanish subjects (36.4%, $p = 0.004$).

Clinical data at sampled sites (Table 2)

The mean PD (mm) of the sampled sites was significantly deeper in the

Table 2. Comparison of clinical data at sampled sites: probing pocket depth (PPD), clinical attachment level (CAL), percentage of sites with bleeding on probing (BOP), percentage of sites with plaque (PlI)

	Chile	Colombia	Spain	Statistics*
PPD				
Mean	5.401	7.963	5.757	K–W, $p<0.0001$
SD	0.489	1.303	0.942	Chile–Colombia, $p<0.0001$
Maximum	6.5	11.5	9.3	Chile–Spain, NS
Minimum	5.0	5.2	4.5	Colombia–Spain, $p<0.001$
CAL				
Mean	5.905	8.088	6.458	K–W, $p<0.0001$
SD	0.350	1.679	1.326	Chile–Colombia, $p<0.0001$
Maximum	6.5	12.1	11.5	Chile–Spain, NS
Minimum	5.0	3.1	4.8	Colombia–Spain, $p<0.001$
BOP				
Mean	68.92%	100.00%	90.28%	K–W, $p<0.0001$
SD	22.36%	0.00%	20.07%	Chile–Colombia, $p<0.001$
Maximum	100%	100%	100%	Chile–Spain, $p<0.001$
Minimum	0%	100%	25%	Colombia–Spain, NS
PlI				
Mean	77.03%	59.15%	47.22%	K–W, $p<0.015$
SD	21.55%	28.92%	41.31%	Chile–Colombia, $p<0.05$
Maximum	100%	100%	100%	Chile–Spain, $p<0.01$
Minimum	25%	25%	0%	Colombia–Spain, NS

*Kruskal–Wallis (K–W) test was selected to compare the three groups. As post-hoc test, Dunn's Multiple Comparison test, performed paired comparisons.
NS, not statistically significant ($p>0.05$); SD, standard deviation.

Table 3. Comparison of log of total anaerobic colony forming unit (per ml)

	Chile	Colombia	Spain	Statistics*
Mean	6.688	8.379	7.351	K–W, $p<0.0001$
SD	1.01	0.26	0.54	Chile–Colombia, $p<0.001$
Maximum	9.6	8.8	8.5	Chile–Spain, NS
Minimum	4.7	7.6	5.9	Colombia–Spain, $p<0.001$

*Kruskal–Wallis (K–W) test was selected to compare the three groups. As post-hoc test, Dunn's Multiple Comparison test, performed paired comparisons.
NS, not statistically significant ($p>0.05$); SD, standard deviation.

Colombian sample, when compared with the Chilean and Spanish patients. Similar results were found for the CAL data.

The proportion of sites with BOP was significantly lower in the Chilean when compared with Colombian and Spanish patients. Conversely, the presence of plaque was significantly higher in Chileans when compared with patients from the other two countries.

Total anaerobic counts (Table 3)

Total counts, expressed as log of colony-forming units per ml, were significantly different when comparing the groups, showing a higher amount of colonies in the Colombian when compared with the Chilean and Spanish samples. Colombian patients not only harboured more bacterial cells but also showed a narrower range of variability. Conversely, Chilean samples harboured less bacterial cells and showed a wider range of variability.

Frequency of detection of different bacterial species (Table 4)

The frequency of detection of *A. actinomycetemcomitans* was less than 20% in all groups, and no significant differences were detected among the groups. Conversely, the prevalence of *P. gingivalis* was higher than 65% in all groups, also without significant differences among the three groups.

P. intermedia/nigrescens varied among groups, with a low frequency of detection in the Chilean population, a high frequency in Colombia and almost 100% in the Spanish group. Differences between groups were statistically significant. *T. forsythia* demonstrated a prevalence of 36–39% in Spain and Colombia, while in Chile it showed a lower frequency of detection, and these differences were statistically significant when compared with Spain ($p=0.02$), but not with Colombia ($p=0.05$).

Capnocytophaga sp. demonstrated low prevalences in all groups, and no differences were detected. Conversely, the prevalence of *Fusobacterium* spp. was high in all groups, reaching 100% in Spain, which was significantly higher as compared with the South-American groups.

P. micra demonstrated important and significant differences among groups, with a very low prevalence in Colombia, intermediate in Chile and high in Spain.

E. corrodens showed relatively low proportions in all groups, with statistically significant differences between subjects from Chile and Spain.

Enteric rods demonstrated important and significant differences among groups, with a complete absence in Spain, intermediate levels in Chile and a 36% rate of detection in Colombia.

Proportions in positive samples of different bacterial species (Table 5)

Low proportions of *A. actinomycetemcomitans* were detected in all groups, with the highest proportion detected in the Chilean subjects, although not significantly different. The proportions of *P. gingivalis* were significantly different among groups ($p<0.001$), with a lower percentage in Colombia when compared with the other two groups. *P. intermedia/nigrescens* showed higher proportions in Chile, and very similar percentages in Spain and Colombia. These differences were not significant. *T. forsythia* demonstrated similar proportions in all groups, and no significant differences were detected. *Capnocytophaga* sp. represented low proportions in all groups, but the differences were statistically significant, due to higher values in Chile as compared with Spain ($p<0.05$). The percentages of *Fusobacterium* spp. were significantly different among groups ($p=0.01$), with lower proportions in Chile, which were statistically significant when compared with Colombia ($p>0.05$). The proportions of *P. micra* were lower in samples from Spanish and Colombian subjects compared with samples from Chile ($p=0.01$), due to a significantly lower value in Spanish subjects ($p>0.01$).

Table 4. Comparison of the frequency of detection of different bacterial species

	Frequency of detection			p value (χ^2)		
	Chile (%)	Colombia (%)	Spain (%)	Spain *versus* Chile	Spain *versus* Colombia	Chile *versus* Colombia
Aggregatibacter actinomycetemcomitans	19.4	17.1	16.7	NS	NS	NS
Porphyromonas gingivalis	83.8	65.9	77.8	NS	NS	NS
Prevotella intermedia	19.4	72.5	97.2	<0.001	0.004	<0.001
Tannerella forsythia	16.2	39.0	36.1	NS	NS	0.02
Capnocytophaga spp.	13.5	9.8	16.7	NS	NS	NS
Fusobacterium spp.	63.9	82.9	100.0	<0.001	0.009	NS
Parvimonas micra	29.7	2.4	61.1	0.007	<0.001	<0.001
Eikenella corrodens	34.3	27.5	11.1	0.008	NS	NS
Enteric rods	17.6	36.6	0.0	0.01	<0.001	0.04

NS, not statistically significant ($p > 0.05$).

Table 5. Comparison of the proportions in positive samples of different bacterial species

	Mean percentage of flora in positive sites			Kruskal–Wallis p value	p-value (Dunn's multiple comparison)		
	Chile	Colombia	Spain		Spain *versus* Chile	Spain *versus* Colombia	Chile *versus* Colombia
Aggregatibacter actinomycetemcomitans	2.86	0.22	0.20	0.1678	NS	NS	NS
Porphyromonas gingivalis	34.01	5.49	22.21	<0.0001	NS	<0.01	<0.001
Prevotella intermedia	15.23	6.71	6.74	0.069	NS	NS	NS
Tannerella forsythia	6.43	4.39	5.54	0.8997	NS	NS	NS
Capnocytophaga spp.	4.62	2.00	0.77	0.0155	<0.05	NS	NS
Fusobacterium spp.	2.76	5.08	5.31	0.0107	NS	NS	<0.05
Parvimonas micra	10.83	2.00	2.41	0.0118	<0.01	NS	NS
Eikenella corrodens	10.14	1.77	0.83	0.0025	<0.01	NS	NS
Enteric rods	14.22	8.10	0.00	NA	NS	NA	NA

NS, not statistically significant ($p > 0.05$).
NA, test not possible to be performed.

The same findings were found for *E. corrodens*. Enteric rods were not found in Spain, and its proportions were relatively high in both South-American countries.

Discussion

This study investigated the microbiological composition of the subgingival microbiota in subjects with chronic periodontitis from three different countries. Taken together, the results indicated that the subgingival microbiota from Chile, Colombia and Spain differed with regard to the frequency of detection and the proportions of important periodontal pathogens, especially red complex microorganisms, *P. gingivalis* and *T. forsythia* and enteric rods. While no differences in terms of demographic variables were observed among the groups, the Colombian subjects showed higher mean PD and CAL values at sampled sites in comparison with Chile and Spain (Tables 1 and 2), indicating a more severe level of periodontal disease. Although other demographic variables such as economic status and education level were not studied, it has been observed that specific living conditions particular to a country may account for differences in the clinical presentation of periodontitis (Gjermo et al. 2002, Sheiham & Netuveli 2002). In addition, genetic background may have also been implicated (Loos et al. 2005). Interestingly, cigarette smoking was more frequent in Chile and Spain but was not correlated to increased periodontal destruction as compared with Colombia. Differences in tobacco exposure, host response, oral hygiene habits, oral health care access and microbial composition may help explain these differences in the clinical expression of periodontitis in the three locations studied (Mager et al. 2003, Van der Velden et al. 2003). More detailed studies addressing the relationship between periodontitis and environmental, economic and genetic variables are needed in South America and Europe.

Most studies on the composition of the subgingival microbiota in periodontitis patients have been performed in North American, European and Japanese populations. There are few studies on the subgingival microbial composition in Latin Americans (Lopez 2000, Gajardo et al. 2005, Ximenez-Fyvie et al. 2006a, b, Botero et al. 2007a, b, Lafaurie et al. 2007) and even fewer studies addressing differences between countries (Sanz et al. 2000, Haffajee et al. 2004, Lopez et al. 2004). Owing to this, the study of the subgingival microbiota in a particular country becomes relevant not only for understanding its implications in the pathogenesis of periodontal disease but also to identify its possible impact on outcomes after treatment.

The red complex species *P. gingivalis*, *T. forsythia and T. denticola* are considered important a etiological agents in the pathogenesis of periodontitis (Haffajee et al. 1998, Umeda et al. 1998, Sanz et al. 2000, Gajardo et al. 2005). In the present study, *P. gingivalis* and *T. forsythia* were detected in high frequencies in most of the patients. The prevalence of *P. gingivalis* was high (>50%) in Colombia, Spain and Chile,

while the frequency of *T. forsythia* was lower in Chile than in Colombia and Spain (Table 3). The most frequently detected microorganisms in Chileans were *P. gingivalis, Fusobacterium* spp., *E. corrodens*, *P. micra* and, with the same frequency, *P. intermedia/nigrescens* and *A. actinomycetemcomitans*. Conversely, the least frequently detected species were *Capnocytophaga* spp. On the other hand, the most prevalent bacteria in Colombians were *Fusobacterium* spp., *P. intermedia/nigrescens, P. gingivalis*, *T. forsythia* and Gram-negative enteric rods, and with less frequency *P. micra*. In patients from Spain, *Fusobacterium* spp., *P. intermedia/nigrescens*, *P. gingivalis*, *P. micra* and *T. forsythia* were the most commonly isolated. In contrast, Gram-negative enteric rods were absent in Spanish subjects (Tables 3 and 4). Interestingly, a frequently reported periodontal pathogen, *A. actinomycetemcomitans*, was detected in low proportions and frequency in the three populations studied. Recent studies (Gajardo et al. 2005, Botero et al. 2007a, b, Lafaurie et al. 2007) have reported that *A. actinomycetemcomitans* is not the most prevalent and prominent organism in aggressive periodontitis and in chronic periodontitis among Chileans and Colombians as compared with other North America populations. The same was found for chronic periodontitis in a Spanish population when compared with a Dutch population (Sanz et al. 2000). Conversely, *P. gingivalis* in this study emerged as an important pathogen in chronic periodontitis, similar to other studies conducted in Latin American populations (Michalowicz et al. 2000, Lopez et al. 2004, Cortelli et al. 2005, Gajardo et al. 2005, Ximenez-Fyvie et al. 2006a, b). Subgingival proportions of *P. gingivalis* were significantly different in Chilean and Spanish patients as compared with Colombians (Table 5). This finding is consistent with the studies from Brazil and Chile in subjects with chronic periodontitis (Lopez et al. 2004, Cortelli et al. 2005), in which *P. gingivalis* was a prevalent organism. Furthermore, a previous study showed similar results when Spain was compared with the Netherlands (Sanz et al. 2000). In addition, the higher frequency of detection of *P. micra* in the populations with the highest levels of smoking (Chile and Spain) agrees with previous studies assessing the influence of smoking on the subgingival microflora (van Winkelhoff et al. 2001).

The association between periodontopathic bacteria and superinfecting bacteria has been described previously in the literature (Slots et al. 1990b), although its pathogenic implications are unclear. In this study, Gram-negative enteric rods were prevalent in Colombians and Chilean subjects; however, they were absent in Spanish subjects (Tables 3 and 4). The role of these microorganisms in the pathogenesis of periodontitis is still ill defined, although they are frequently associated as superinfecting microorganisms, or associated with antibiotic resistance when antibiotics, such as amoxicillin and metronidazole, are frequently used in the treatment of periodontitis (Wroblewska et al. 2002, Goncalves et al. 2007). In this study, it is difficult to know whether their presence is the result of an adaptation of the subgingival biofilm or a transient infection. However, poor antibiotic prescription control is frequent in Latin-American countries and this fact may have an influence on antibiotic resistance and thus on the possible overgrowth of these pathogens in periodontal pockets.

Mechanical debridement of the subgingival biofilm is the standard treatment of periodontal infections demonstrating good therapeutic clinical outcomes in most of the cases. The adjunctive use of systemic antibiotics has been usually limited to acute periodontal infections, severe forms of chronic and aggressive periodontitis and whenever there is a risk of systemic complications. Current protocols for antibiotic prescription in periodontal therapy are based on published microbiological studies from Western and Asian populations and therefore, the occurrence of significant differences in the composition of the subgingival flora in different geographical or ethnic populations may increase the risk of developing antibiotic resistance or even promoting a subgingival overgrowth of Gram-negative enteric rods and other superinfecting microorganisms. These problems would be avoided if the selection of antibiotics is based on prior microbiological diagnosis. The results of this study support this approach, because relevant differences in subgingival microbiota have been detected among populations of different geographical origin, in spite of having a similar clinical presentation. For instance, because of the fact that 18% of Chilean and 37% of Colombian patients harboured Gram-negative enteric rods, the appropriate antibiotic would likely be ciprofloxacin in conjunction with mechanical therapy (Slots et al. 1990a). In contrast, the presence of *P. gingivalis, A. actinomycetemcomitans* and *T. forsythia* would require the use of amoxicillin/metronidazole. In patients allergic to penicillin, azithromycin would be the antibiotic of choice (Gomi et al. 2007). The presence of specific or a combination of periodontal and superinfecting pathogens in high levels should determine antibiotic selection. We therefore consider that before systemic antibiotic administration, it is important to determine the composition of the subgingival microbiota.

One concern for the researchers in this study was the methodology used for the microbiological analysis. Owing to this, before the start of the study the three laboratories agreed on an identical protocol and standardized and calibrated their processes in order to ensure the homogeneity and reproducibility of the obtained results. Clinical sampling of subgingival plaque from the selected patients was also identical following a standard approach (Casas et al. 2007) and a specially designed transport and agar media were used for culturing periodontal pathogens, also following current microbiological standards. In this way, we ensured that any variability in the composition of the microbiota that may be the result from microbial processing would be the same in all three laboratories. However, one cannot rule out completely that the reported microbial differences might have resulted from variations in the amounts of plaque recovered. In addition, the differences in disease severity (probing PD) and smoking habits among the populations, detected in the present study, could have had an impact on the microbiological results, because both factors have been shown to have an influence on the subgingival microflora, but their particular impact on the present populations could not be assessed.

In summary, the results from this study support the existence of geographic variations in the subgingival microbiota in chronic periodontitis patients that may be linked to geographic and environmental factors. These results could impact periodontal treatment, mainly with regard to the

selection of adjunctive systemic antibiotics in a given population.

References

Ali, R. W., Bakken, V., Nilsen, R. & Skaug, N. (1994) Comparative detection frequency of 6 putative periodontal pathogens in Sudanese and Norwegian adult periodontitis patients. *Journal of Periodontology* **65**, 1046–1052.

Alsina, M., Olle, E. & Frias, J. (2001) Improved, low-cost selective culture medium for *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Microbiology* **39**, 509–513.

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Barbosa, F. C., Mayer, M. P., Saba-Chujfi, E. & Cai, S. (2001) Subgingival occurrence and antimicrobial susceptibility of enteric rods and pseudomonads from Brazilian periodontitis patients. *Oral Microbiology and Immunology* **16**, 306–310.

Botero, J. E., Arce, R. M., Escudero, M., Betancourth, M., Jaramillo, A. & Contreras, A. (2007a) Frequency of detection of periodontopathic and superinfecting bacteria in HIV-positive patients with periodontitis. *Journal of the International Academy of Periodontology* **9**, 13–18.

Botero, J. E., Contreras, A., Lafaurie, G., Jaramillo, A., Betancourt, M. & Arce, R. M. (2007b) Occurrence of periodontopathic and superinfecting bacteria in chronic and aggressive periodontitis subjects in a Colombian population. *Journal of Periodontology* **78**, 696–704.

Casas, A., Herrera, D., Martin-Carnes, J., Gonzalez, I., O'Connor, A. & Sanz, M. (2007) Influence of sampling strategy on microbiologic results before and after periodontal treatment. *Journal of Periodontology* **78**, 1103–1112.

Cortelli, J. R., Cortelli, S. C., Jordan, S., Haraszthy, V. I. & Zambon, J. J. (2005) Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. *Journal of Clinical Periodontology* **32**, 860–866.

Curtis, M. A., Slaney, J. M. & Aduse-Opoku, J. (2005) Critical pathways in microbial virulence. *Journal of Clinical Periodontology* **32**, 28–38.

Gajardo, M., Silva, N., Gomez, L., Leon, R., Parra, B., Contreras, A. & Gamonal, J. (2005) Prevalence of periodontopathic bacteria in aggressive periodontitis patients in a Chilean population. *Journal of Periodontology* **76**, 289–294.

Gjermo, P., Rosing, C. K., Susin, C. & Oppermann, R. (2002) Periodontal diseases in Central and South America. *Periodontology 2000* **29**, 70–78.

Gomi, K., Yashima, A., Nagano, T., Kanazashi, M., Maeda, N. & Arai, T. (2007) Effects of full-mouth scaling and root planing in conjunction with systemically administered azithromycin. *Journal of Periodontology* **78**, 422–429.

Goncalves, M. O., Coutinho-Filho, W. P., Pimenta, F. P., Pereira, G. A., Pereira, J. A., Mattos-Guaraldi, A. L. & Hirata, R. Jr. (2007) Periodontal disease as reservoir for multi-resistant and hydrolytic enterobacterial species. *Letters of Applied Microbiology* **44**, 488–494.

Haffajee, A. D., Bogren, A., Hasturk, H., Feres, M., Lopez, N. J. & Socransky, S. S. (2004) Subgingival microbiota of chronic periodontitis subjects from different geographic locations. *Journal of Clinical Periodontology* **31**, 996–1002.

Haffajee, A. D., Cugini, M. A., Tanner, A., Pollack, R. P., Smith, C., Kent, R. L. Jr. & Socransky, S. S. (1998) Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. *Journal of Clinical Periodontology* **25**, 346–353.

Haffajee, A. D. & Socransky, S. S. (1994) Microbial etiological agents of destructive periodontal diseases. *Periodontology 2000* **5**, 78–111.

Herrera, D., van Winkelhoff, A. J., Dellemijn-Kippuw, N., Winkel, E. G. & Sanz, M. (2000) Beta-lactamase producing bacteria in the subgingival microflora of adult patients with periodontitis. A comparison between Spain and The Netherlands. *Journal of Clinical Periodontology* **27**, 520–525.

Lafaurie, G. I., Contreras, A., Baron, A., Botero, J., Mayorga-Fayad, I., Jaramillo, A., Giraldo, A., Gonzalez, F., Mantilla, S., Botero, A., Archila, L. H., Diaz, A., Chacon, T., Castillo, D. M., Betancourt, M., Del Rosario-Aya, M. & Arce, R. (2007) Demographic, clinical, and microbial aspects of chronic and aggressive periodontitis in Colombia: a multicenter study. *Journal of Periodontology* **78**, 629–639.

Lang, N. P., Adler, R., Joss, A. & Nyman, S. (1990) Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* **17**, 714–721.

Loos, B. G., John, R. P. & Laine, M. L. (2005) Identification of genetic risk factors for periodontitis and possible mechanisms of action. *Journal of Clinical Periodontology* **32**, 159–179.

Lopez, N. J. (2000) Occurrence of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*, and *Prevotella intermedia* in progressive adult periodontitis. *Journal of Periodontology* **71**, 948–954.

Lopez, N. J., Socransky, S. S., Da, S. I., Japlit, M. R. & Haffajee, A. D. (2004) Subgingival microbiota of chilean patients with chronic periodontitis. *Journal of Periodontology* **75**, 717–725.

Mager, D. L., Haffajee, A. D. & Socransky, S. S. (2003) Effects of periodontitis and smoking on the microbiota of oral mucous membranes and saliva in systemically healthy subjects. *Journal of Clinical Periodontology* **30**, 1031–1037.

Michalowicz, B. S., Ronderos, M., Camara-Silva, R., Contreras, A. & Slots, J. (2000) Human herpesviruses and *Porphyromonas gingivalis* are associated with juvenile periodontitis. *Journal of Periodontology* **71**, 981–988.

Mombelli, A., McNabb, H. & Lang, N. P. (1991a) Black-pigmenting gram-negative bacteria in periodontal disease. I. Topographic distribution in the human dentition. *Journal of Periodontal Research* **26**, 301–307.

Mombelli, A., McNabb, H. & Lang, N. P. (1991b) Black-pigmenting gram-negative bacteria in periodontal disease. II. Screening strategies for detection of *P. gingivalis*. *Journal of Periodontal Research* **26**, 308–313.

Offenbacher, S., Lieff, S., Boggess, K. A., Murtha, A. P., Madianos, P. N., Champagne, C. M., McKaig, R. G., Jared, H. L., Mauriello, S. M., Auten, R. L. Jr., Herbert, W. N. & Beck, J. D. (2001) Maternal periodontitis and prematurity. Part I: obstetric outcome of prematurity and growth restriction. *Annals of Periodontology* **6**, 164–174.

Sanz, M., Lau, L., Herrera, D., Morillo, J. M. & Silva, A. (2004) Methods of detection of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythensis* in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. *Journal of Clinical Periodontology* **31**, 1034–1047.

Sanz, M., van Winkelhoff, A. J., Herrera, D., Dellemijn-Kippuw, N., Simon, R. & Winkel, E. (2000) Differences in the composition of the subgingival microbiota of two periodontitis populations of different geographical origin. A comparison between Spain and The Netherlands. *European Journal of Oral Sciences* **108**, 383–392.

Sheiham, A. & Netuveli, G. S. (2002) Periodontal diseases in Europe. *Periodontology 2000* **29**, 104–121.

Slots, J. (1982) Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Microbiology* **15**, 606–609.

Slots, J., Feik, D. & Rams, T. E. (1990a) In vitro antimicrobial sensitivity of enteric rods and pseudomonads from advanced adult periodontitis. *Oral Microbiology and Immunology* **5**, 298–301.

Slots, J., Feik, D. & Rams, T. E. (1990b) Prevalence and antimicrobial susceptibility of Enterobacteriaceae, Pseudomonadaceae and Acinetobacter in human periodontitis. *Oral Microbiology and Immunology* **5**, 149–154.

Socransky, S. S. & Haffajee, A. D. (1994) Evidence of bacterial etiology: a historical perspective. *Periodontology 2000* **5**, 7–25.

Syed, S. A. & Loesche, W. J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–644.

Umeda, M., Chen, C., Bakker, I., Contreras, A., Morrison, J. L. & Slots, J. (1998) Risk indicators for harboring periodontal pathogens. *Journal of Periodontology* **69**, 1111–1118.

Van der Velden, U., Varoufaki, A., Hutter, J. W., Xu, L., Timmerman, M. F., van Winkelhoff, A. J. & Loos, B. G. (2003) Effect of smoking and periodontal treatment on the subgingival microflora. *Journal of Clinical Periodontology* **30**, 603–610.

van Winkelhoff, A. J., Bosch-Tijhof, C. J., Winkel, E. G. & van der Reijden, W. A. (2001) Smoking affects the subgingival microflora in periodontitis. *Journal of Periodontology* **72**, 666–671.

van Winkelhoff, A. J. & Boutaga, K. (2005) Transmission of periodontal bacteria and models of infection. *Journal of Clinical Periodontology* **32**, 16–27.

Wroblewska, M. M., Marchel, H. & Luczak, M. (2002) Multidrug resistance in bacterial isolates from blood cultures of haematology patients. *International Journal of Antimicrobial Agents* **19**, 237–240.

Ximenez-Fyvie, L. A., Almaguer-Flores, A., Jacobo-Soto, V., Lara-Cordoba, M., Moreno-Borjas, J. Y. & Alcantara-Maruri, E. (2006a) Subgingival microbiota of periodontally untreated Mexican subjects with generalized aggressive periodontitis. *Journal of Clinical Periodontology* **33**, 869–877.

Ximenez-Fyvie, L. A., Almaguer-Flores, A., Jacobo-Soto, V., Lara-Cordoba, M., Sanchez-Vargas, L. O. & Alcantara-Maruri, E. (2006b) Description of the subgingival microbiota of periodontally untreated Mexican subjects: chronic periodontitis and periodontal health. *Journal of Periodontology* **77**, 460–471.

Address:
David Herrera
Faculty of Odontology – Universidad Complutense
Plaza. Ramón y Cajal, s/n
28040 Madrid
Spain
E-mail: davidher@odon.ucm.es

Clinical Relevance

Scientific rationale for the study: The subgingival profile of periodontal infections, in different geographical populations, changes independently of the patient clinical status, and it is, therefore, important to carry out an adequate microbiological diagnosis in order to establish a more effective therapy.

Principal findings: Colombian subjects with chronic periodontitis present higher numbers and proportions of *T. forsythia* and Gram-negative enteric rods than Chilean and Spanish subjects. In addition, Spanish subjects showed higher numbers and proportions of *P. intermedia-nigrescens* than Chilean and Colombian subjects.

Practical implications: The microbiological profile of chronic periodontitis patients varies markedly among geographical locations. The results could impact the choice of periodontal therapy and the adjunctive antibiotic selection in subjects living in specific geographic locations or with different ethnic backgrounds.