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


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, χ², 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 available.

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 Cienci y Tecnología: Fondecyt Número 1050518”. 

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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 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 naeslundii 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 (PII 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 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% N₂; 10% H₂; 10% CO₂ at 37 °C), and the Dentaid-1 plates after 3–5 days of 37 °C incubation in air with 5% CO₂. Plates were carefully examined for the identification of A. actinomycescomitans, 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. actinomycescomitans 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-CO₂ 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 χ² 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 χ² test. The proportions of bacterial pathogens in positive samples were compared as described for the clinical variables. Statistical significance was assumed when P ≤ 0.05.

Table 1. Demographics of the selected populations

<table>
<thead>
<tr>
<th></th>
<th>Chile</th>
<th>Colombia</th>
<th>Spain</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>41</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>44.6</td>
<td>41.5</td>
<td>44.9</td>
<td>One-way ANOVA</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>8.1</td>
<td>10.6</td>
<td>11.7</td>
<td>p = 0.3731</td>
</tr>
<tr>
<td>Maximum</td>
<td>60</td>
<td>64</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>31</td>
<td>25</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>25</td>
<td>20</td>
<td>χ²</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>% Female</td>
<td>67.6</td>
<td>61.0</td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>% Male</td>
<td>32.4</td>
<td>39.0</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>26</td>
<td>37</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>11</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Former smokers</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>Spain–Chile, NS</td>
</tr>
<tr>
<td>% Non-smokers</td>
<td>70.3</td>
<td>90.2</td>
<td>63.6</td>
<td></td>
</tr>
<tr>
<td>% Smokers</td>
<td>29.7</td>
<td>7.3</td>
<td>36.4</td>
<td></td>
</tr>
<tr>
<td>% Former</td>
<td>0.0</td>
<td>2.4</td>
<td>9.1</td>
<td></td>
</tr>
</tbody>
</table>

NS, not statistically significant (p > 0.05).

Results

Demographics of the selected populations (Table 1)

The sample population of moderate to severe chronic periodontitis 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
showed a narrower range of variability. Conversely, Chilean samples harboured more bacterial cells but also differences were detected among the groups. 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.

*A. actinomycetemcomitans* 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Chile</th>
<th>Colombia</th>
<th>Spain</th>
<th>Statistics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>6.688</td>
<td>8.379</td>
<td>7.351</td>
<td>K-W, <em>p</em> &lt; 0.0001</td>
</tr>
<tr>
<td>SD</td>
<td>1.01</td>
<td>0.26</td>
<td>0.54</td>
<td>Colombia–Spain, <em>p</em> &lt; 0.001</td>
</tr>
<tr>
<td>Maximum</td>
<td>9.6</td>
<td>8.8</td>
<td>8.5</td>
<td>Chile–Spain, NS</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.7</td>
<td>7.6</td>
<td>5.9</td>
<td>Colombia–Spain, <em>p</em> &lt; 0.001</td>
</tr>
</tbody>
</table>

*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.

### 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).

### 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.

**Geographic microbiological differences in periodontitis**

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Enteric rods
Eikenella corrodens
Parvimonas micra
Fusobacterium
Capnocytophaga
Tannerella forsythia
Prevotella intermedia
Porphyromonas gingivalis
Aggregatibacter actinomycetemcomitans

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, Sheihem & 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 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.

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

<table>
<thead>
<tr>
<th>Frequency of detection</th>
<th>Spain (%)</th>
<th>Columbia (%)</th>
<th>Chile (%)</th>
<th>p value ($\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>16.7</td>
<td>17.1</td>
<td>19.4</td>
<td>NS</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>77.8</td>
<td>65.9</td>
<td>83.8</td>
<td>NS</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>97.2</td>
<td>72.5</td>
<td>19.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>36.1</td>
<td>39.0</td>
<td>16.2</td>
<td>NS</td>
</tr>
<tr>
<td>Capnocytophaga spp.</td>
<td>16.7</td>
<td>9.8</td>
<td>13.5</td>
<td>NS</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>100.0</td>
<td>82.9</td>
<td>63.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parvimonas micra</td>
<td>61.1</td>
<td>2.4</td>
<td>29.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eikenella corrodens</td>
<td>11.1</td>
<td>27.5</td>
<td>34.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Enteric rods</td>
<td>0.0</td>
<td>36.6</td>
<td>17.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Mean percentage of flora in positive sites</th>
<th>Kruskal–Wallis p value</th>
<th>p-value (Dunn’s multiple comparison)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chile (%)</td>
<td>Colombia (%)</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>0.1678</td>
<td>0.20</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>0.0091</td>
<td>22.21</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>0.069</td>
<td>6.74</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>0.8997</td>
<td>5.54</td>
</tr>
<tr>
<td>Capnocytophaga spp.</td>
<td>0.0155</td>
<td>0.77</td>
</tr>
<tr>
<td>Fusobacterium spp.</td>
<td>0.0107</td>
<td>5.31</td>
</tr>
<tr>
<td>Parvimonas micra</td>
<td>0.0118</td>
<td>2.41</td>
</tr>
<tr>
<td>Eikenella corrodens</td>
<td>0.0025</td>
<td>0.83</td>
</tr>
<tr>
<td>Enteric rods</td>
<td>NA</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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


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.

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