Description of the Subgingival Microbiota of Periodontally Untreated Mexican Subjects: Chronic Periodontitis and Periodontal Health

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Background: Recent studies have suggested that changes in the prevalence and/or proportion of distinct microorganisms characterize the subgingival microbial profiles of populations around the world. At present, no information is available on the subgingival microbiota of Mexican subjects. The purpose of the present study was to determine the microbial composition of subgingival plaque in Mexican subjects with untreated chronic periodontitis.

Methods: A total of 44 chronic periodontitis and 20 periodontally healthy subjects (who were currently non-smokers) were selected. Clinical measurements including plaque accumulation, gingival erythema, bleeding on probing, suppuration, probing depth, and attachment level were recorded at six sites of every tooth. Up to 28 subgingival plaque samples were obtained from each subject and individually analyzed to determine the levels, proportion, and prevalence of 40 microbial species using the checkerboard DNA-DNA hybridization technique.

Results: *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythensis* were the only species that presented higher mean levels in periodontitis subjects. The proportions of *P. gingivalis* ($P < 0.001$), *T. forsythensis* ($P < 0.01$), and red complex species (*P. gingivalis*, *T. forsythensis*, and *T. denticola*; $P < 0.001$) as a group were also significantly higher in periodontitis subjects. Periodontally healthy subjects harbored a significantly larger proportion of *Actinomyces* species ($P < 0.05$). No significant differences were detected in the percentage of carriers of any of the species tested.

Conclusions: Our results revealed that the subgingival microbiota of untreated chronic periodontitis Mexican subjects was characterized by increases in the level, prevalence, and proportion of classic periodontal pathogens. However, the prevalence and proportion of specific microbial species varied significantly from the results of other reports on subjects from different geographical locations. *J Periodontol 2006;77:460-471.*

KEY WORDS

Dental plaque/microbiology; DNA probes; periodontal diseases/microbiology; periodontitis/microbiology.

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Periodontal diseases are distributed worldwide and represent a major oral health concern for both industrialized and developing countries. The role of subgingival microbial species in the etiology of periodontal diseases has been extensively documented.[1-3] The current body of knowledge indicates that specific microorganisms or groups of species, including *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola* occur more frequently and/or in higher levels and proportions in periodontitis sites and subjects, whereas others, such as members of the *Actinomyces* genus, are primarily associated with periodontal health.[4-6]

However, most of the available information on the microbial composition of subgingival plaque is based on studies from industrialized countries in which preventive and therapeutic oral-healthcare regimens are available to significant proportions of the population. Thus, only limited data are available on the subgingival microbiota of subjects in developing countries and on the undisturbed (pretreatment) microbial composition of periodontitis patients globally. Several recent studies have

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provided information which suggests that, although similar microbial species can be detected in populations around the world, significant differences in their prevalence and/or proportion, characterize the subgingival microbial profile of subjects in specific geographic locations.[7-14] Such findings raise the concern that the results from studies reporting the efficacy of different forms of periodontal treatments in a given population may not be directly extrapolated to subjects in other locations around the world.

Using the checkerboard DNA-DNA hybridization technique, Lopez et al.[12] compared the microbial composition of subgingival plaque between Chilean and North American (from the United States of America [U.S.]) subjects with chronic periodontitis. Their results indicated that Chilean subjects harbored a significantly larger proportion of a number of periodontal pathogens including *A. actinomycetemcomitans*, *T. forsythensis*, *P. gingivalis*, and *T. denticola*. Haffajee et al.[11] evaluated the subgingival microbiota of 300 chronic periodontitis subjects from Sweden, U.S., Chile, and Brazil using the checkerboard technique. Brazilian subjects harbored significantly larger proportions of *Actinomyces naeslundii* serotype 1 (*A. naeslundii* 1), *Streptococcus intermedius*, *Streptococcus sanguinis*, *Streptococcus gordonii*, *Streptococcus constellatus*, *Eubacterium nodatum*, and *T. denticola* than the other three populations evaluated. Other significant differences included higher proportions of *Capnocytophaga gingivalis* and *Leptotrichia buccalis* in the U.S. population, of *P. gingivalis* in Chilean subjects, and of *Micromonas micros* in subjects from Sweden. Furthermore, in comparison to Brazilian and Chilean subjects, the U.S. and Swedish populations presented significantly lower proportions of *T. denticola* and *P. gingivalis*.

Ellwood et al.[15] evaluated 527 adolescents of three U.S. ethnic populations (Indo-Pakistani, white, and Afro-Caribbean) who were either periodontally healthy or had aggressive periodontitis. The percentage of carriers of *P. gingivalis*, *A. actinomycetemcomitans*, and *Prevotella intermedia* was determined by enzyme-linked immunosorbent assay (ELISA). Their results suggested that Indo-Pakistani subjects had a 3.1 to 4.8 higher risk of harboring *P. gingivalis* and that significantly more subjects of this ethnic group were carriers of the same species than white individuals. Using 16S rRNA sequencing, Sirinian et al.[16] determined the percentage of carriers of four periodontal pathogens in white, Hispanic, and Asian periodontally healthy children from the U.S. Their results indicated that a higher percentage of Hispanic and Asian children were carriers of *A. actinomycetemcomitans* and *P. gingivalis* than whites. Furthermore, a higher percentage of Hispanic children were also carriers of *T. denticola* than Asian and white subjects. By cultural methods, Ali et al.[17] determined the prevalence and proportion of five periodontal pathogens in 25 Sudanese and 18 Norwegian chronic periodontitis subjects. Norwegian subjects harbored a significantly higher proportion of *P. gingivalis*, *A. actinomycetemcomitans*, and *Fusobacterium nucleatum*, whereas Sudanese subjects had a significantly greater percentage of sites colonized by *P. intermedia* and *Capnocytophaga* species.

Although it is becoming increasingly apparent that there are substantial differences in the subgingival microbiota in subjects from various geographical locations, a global understanding of the microbiota associated with different forms of periodontal disease and of the possible clinical and therapeutic implications of microbial changes in subgingival plaque has not been achieved. The reported variations in subgingival microbial profiles in populations around the world reflect the need for a global understanding of the bacterial ecology of periodontal diseases which may enable the establishment of specific preventive and therapeutic strategies for disease control.

The purpose of the present study was to determine the subgingival microbial composition of Mexican subjects with untreated chronic periodontitis using the checkerboard DNA-DNA hybridization technique.[18]

MATERIALS AND METHODS

Subject Population

The present study received approval from the ethics committee for human studies of the Division of Postgraduate Studies and Research, School of Dentistry, National University of Mexico (UNAM). All subjects were asked to sign informed consent forms, with which they acknowledged their willingness to participate.

Forty-four subjects with chronic periodontitis and 20 periodontally healthy subjects were included in the study. Subjects were randomly selected for the study and recruited from the population of individuals seeking consults and/or treatment at the Periodontology Department, Division of Postgraduate Studies and Research, School of Dentistry, UNAM, from February 1999 to September 2003. Every subject who fit the entry criteria was included in the study. All of the subjects selected were currently non-smokers, who had not received any form of periodontal therapy in the past other than professional supragingival plaque removal and had at least 20 natural teeth (excluding third molars). All subjects were born and lived in Mexico and were of Mexican descent, i.e., both of their parents and at least two of their grandparents were born and lived in Mexico. Chronic periodontitis subjects had at least eight sites with an attachment level ≥5 mm and were >30 years of age. Periodontally healthy subjects had less than three sites with an attachment level of 4 mm, no sites with an attachment level ≥5 mm, and were ≥20 years of age. Exclusion

Table 1.

Clinical Characteristics of the Subject Population

	Periodontitis (N = 44)		Health (N = 20)	
	Mean ± SEM	Range	Mean ± SEM	Range
Age (years)*	44.9 ± 1.7	31-75	27.5 ± 1.3	22-51
Number of missing teeth*	3.5 ± 0.3	0-8	1 ± 0.3	0-4
Gender (% females)	61.4		45	
Mean probing depth (mm)*	3.9 ± 0.2	2.3-7.4	2 ± 0.03	1.7-2.3
Mean attachment level (mm)*	4.2 ± 0.2	2.7-9.0	2 ± 0.03	1.7-2.3
% sites with:				
Plaque accumulation*	51.9 ± 5.7	0-100	11.7 ± 3.7	0-72
Gingival erythema†	27.5 ± 5.3	0-100	3.6 ± 2.2	0-38
Bleeding on probing*	47 ± 3.9	4.5-100	2.7 ± 1.1	0-22.7
Suppuration*	6.1 ± 1.3	0-36.9	0 ± 0	0

* $P < 0.001$; Mann-Whitney test.
† $P < 0.01$; Mann-Whitney test.

criteria included pregnancy, lactation, antibiotic therapy in the previous 3 months, and any systemic condition that could influence the course of periodontal disease, such as diabetes, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), or autoimmune diseases.

Clinical Monitoring and Sample Collection

Clinical measurements were taken at six sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual) at all teeth excluding third molars (a maximum of 168 sites per subject) as previously described.[19] Clinical assessment included plaque accumulation (0/1, undetected/detected), gingival erythema (0/1), bleeding on probing (0/1), suppuration (0/1), probing depth, and attachment level. Probing depth and attachment level measurements were taken twice by the same examiner, and the average of the pair of measurements was used for analysis. Such measurements were recorded to the nearest millimeter using a periodontal probe.† Table 1 summarizes the clinical features of the 64 subjects.

Samples of subgingival plaque were obtained from the mesio-buccal site of ≤28 teeth in each subject. After drying and isolation with cotton rolls, supragingival plaque was removed from the sample sites, and subgingival samples were taken with individual sterile curets.‡ The samples were placed in individual tubes containing 150 μl TE buffer§ (10 mM Tris-HCl and 0.1 mM EDTA; pH 7.6). Samples were dispersed, and 100 μl 0.5 M NaOH‖ was added to each tube. All samples were stored at –20°C until processing.

Microbial Assessment

Bacterial strains. Table 2 presents a list of the 40 bacterial strains employed for the preparation of DNA probes. Lyophilized bacterial stocks¶ were rehydrated in *Mycoplasma* broth base.# All strains, with the exception of *T. forsythensis*, *Campylobacter* spp., and *T. denticola*, were grown on *Mycoplasma* agar base** supplemented with 5% defibrinated sheep blood, 0.3 μg/ml menadione,†† and 5 μg/ml hemin‡‡ at 35°C under anaerobic conditions (80% N_2, 10% CO_2, and 10% H_2). The medium for *T. forsythensis* was also supplemented with 10 μg/ml N-acetyl muramic acid§§ (NAM). The media for *Campylobacter* spp. was supplemented on the surface with 200 μl sodium formate/fumaric acid‖‖ solution (6% each; pH 7.0). *T. denticola* was grown in *Mycoplasma* broth base supplemented with 0.05 mg/ml thioglycolic acid,¶¶

† North Carolina periodontal probe, Hu-Friedy, Chicago, IL.
‡ Gracey curets, Hu-Friedy.
§ Sigma-Aldrich, St. Louis, MO.
‖ Sigma-Aldrich.
¶ American Type Culture Collection, Rockville, MD.
# BBL, Becton Dickinson, Sparks, MD.
** BBL, Becton Dickinson.
†† Sigma-Aldrich.
‡‡ Sigma-Aldrich.
§§ Sigma-Aldrich.
‖‖ Sigma-Aldrich.
¶¶ Sigma-Aldrich.

Table 2.

Reference Strains Employed for the Development of DNA Probes

Species	Strain*	Complex†	Species	Strain*	Complex†
A. actinomycetemcomitans	‡	Ungrouped	*M. micros*	33270	Orange
A. georgiae	49285	*Actinomyces*	*N. mucosa*	19696	Other
A. israelii	12102	*Actinomyces*	*Porphyromonas endodontalis*	35406	Other
A. naeslundii 1	12104	*Actinomyces*	*P. gingivalis*	33277	Red
Actinomyces odontolyticus	17929	Purple	*P. intermedia*	25611	Orange
A. viscosus	43146	*Actinomyces*	*Prevotella melaninogenica*	25845	Other
Campylobacter gracilis	33236	Orange	*P. nigrescens*	33563	Orange
C. rectus	33238	Orange	*Propionibacterium acnes*	6919	Other
Campylobacter showae	51146	Orange	*Selenomonas artemidis*	43528	Other
C. gingivalis	33624	Green	*S. noxia*	43541	Ungrouped
C. ochracea	27872	Green	*Streptococcus anginosus*	33397	Yellow
Capnocytophaga sputigena	33612	Green	*S. constellatus*	27823	Orange
C. matruchotii	14266	Other	*S. gordonii*	10558	Yellow
E. corrodens	23834	Green	*S. intermedius*	27335	Yellow
E. saburreum	33271	Other	*S. mitis*	49456	Yellow
E. sulci	35585	Other	*S. oralis*	35037	Yellow
F. nucleatum	§	Orange	*S. sanguinis*	10556	Yellow
F. periodonticum	33693	Orange	*T. forsythensis*	43037	Red
Gemella morbillorum	27824	Other	*T. denticola*	35405	Red
L. buccalis	14201	Other	*V. parvula*	10790	Purple

* American Type Culture Collection, Rockville, MD.
† Strains were grouped according to the description of microbial complexes in subgingival plaque by Socransky et al.[23] with the following exceptions: *A. georgiae, A. israelii, A. naeslundii* 1, and *A. viscosus* were grouped as "*Actinomyces*"; *C. matruchotii, E. saburreum, E. sulci, G. morbillorum, L. buccalis, N. mucosa, P. endodontalis, P. melaninogenica, P. acnes*, and *S. artemidis* were grouped as "Other."
‡ DNA from serotypes a (43717) and b (43718) was combined to generate a single DNA probe.
§ DNA from subspecies *nucleatum* (25586), *polymorphum* (10953), and *vincentii* (49256) was combined to generate a single DNA probe.

1 mg/ml L-cysteine-HCl,## 0.026 mg/ml L-asparagine,*** 2.1 mg/ml glucose,††† 0.3% thiamine pyrophosphate solution‡‡‡ (2 mg/ml cocarboxylase), 0.2% VFA solution§§§ (isobutyric acid, methylbutyric acid, isovaleric acid, and valeric acid; 0.5% each in 0.1 M NaOH), and 2% rabbit serum.‖‖‖

Preparation of DNA probes. The growth from 3- to 7-day cultures was harvested and placed in tubes containing 1 ml of TE buffer (pH 7.6). Cells were washed twice and lysed at 37°C for 1 hour with either 10% sodium dodecyl sulfate¶¶¶ (SDS) and proteinase K### (20 mg/ml) (for Gram-negative strains) or lysozyme**** (15 mg/ml) and achromopeptidase†††† (5 mg/ml) (for Gram-positive strains). DNA was isolated and purified using the method described by Smith et al.[20] Whole-genomic DNA probes were prepared for each species by labeling 1 μg DNA with digoxigenin‡‡‡‡ using a random primer technique.[21]

Sigma-Aldrich.
*** Sigma-Aldrich.
††† Sigma-Aldrich.
‡‡‡ Sigma-Aldrich.
§§§ Sigma-Aldrich.
‖‖‖ GIBCO, Invitrogen, Grand Island, NY.
¶¶¶ Sigma-Aldrich.
Sigma-Aldrich.
**** Sigma-Aldrich.
†††† Sigma-Aldrich.
‡‡‡‡ Roche Diagnostics, Mannheim, Germany.

Before the microbial detection in clinical samples, the specificity and sensitivity of the 40 DNA probes were assessed by hybridizing each DNA probe against individual pure cultures of all of the test species adjusted to 10^4, 10^5, 10^6, and 10^7 cells. The sensitivity of the assay was set to allow the detection of approximately 10^4 cells of a given species by adjusting the concentration of each individual DNA probe.

DNA-DNA hybridization. Samples were individually analyzed using the checkerboard DNA-DNA hybridization technique.[18] Samples were thawed at room temperature, boiled for 10 minutes, and neutralized with 800 μl 5 M ammonium acetate.§§§§ The released DNA from each sample was then placed into individual lanes,|||||| concentrated onto a 15 × 15-cm positively charged nylon membrane,¶¶¶¶ and fixed to the membrane by cross-linking under ultraviolet light. Two lanes on each membrane contained standards consisting of a mixture at 10^5 and 10^6 cells of each bacterial species tested.

The membranes were prehybridized at 42°C for 2 hours in 50% formamide,#### 5 × standard saline citrate [SSC] (1 × SSC = 150 mM NaCl***** and 15 mM Na citrate;†††††† pH 7.0), 1% casein,‡‡‡‡‡ 5 × Denhardt's solution, 25 mM sodium phosphate§§§§§ (pH 6.5), and 0.5 mg/ml yeast RNA.|||||| Each membrane was placed in a second device¶¶¶¶¶ with the sample lanes rotated 90° to the channels of the apparatus. The probes were diluted to ~20 ng/ml in hybridization solution (45% formamide, 5 × SSC, 1 × Denhardt's solution, 20 mM Na phosphate [pH 6.5], 0.2 mg/ml yeast RNA, 10% dextran sulfate,##### and 1% casein), placed in individual channels of the device, and hybridized overnight at 42°C. Probes were hybridized in four sets of 10 consecutive channels, leaving one empty channel (hybridization solution only) between each set to allow noise and background correction of signals. The membranes were washed twice at high stringency for 20 minutes each time at 68°C in phosphate buffer (0.1 × SSC and 0.1% SDS).

Detection and enumeration of taxa. Membranes were blocked by 1-hour incubation in blocking buffer containing 1% casein in maleate buffer (100 mM maleic acid****** and 150 mM NaCl, pH 7.5). Hybrids were detected by exposing the membranes to a 1:50,000 dilution of antidigoxigenin antibody conjugated to alkaline phosphatase††††††† for 30 minutes, using a modification previously described.[22] Signals were detected by chemiluminescence. In brief, membranes were incubated in a chemiluminescent agent‡‡‡‡‡‡ for 5 minutes at room temperature and exposed to films in autoradiographic cassettes for 10 minutes. Films were developed and then photographed using a digital photodocumentation system.§§§§§§ Signals were detected and analyzed with specialized software,|||||||| adjusted by subtracting the average plus two standard deviations of the noise and background detected in the three empty lanes, and converted to absolute counts by comparison with the standards on each membrane. Failure to detect a signal was recorded as zero.

Statistical Analysis

Microbiological data available for each subject were the absolute counts of each of the 40 test species from ≤28 subgingival plaque samples (mean = 25.3 samples per subject; total = 1,617 samples analyzed). The analyses compared the composition of subgingival plaque in periodontally healthy and chronic periodontitis subjects. The data are presented as mean ± standard error of the mean (SEM) levels (DNA probe counts × 10^5), proportion (percentage of the total DNA probe count), and prevalence (percentage of sites colonized). To compare the levels, proportion, and prevalence of every bacterial species, each type of data was recorded at each site, averaged within a subject, and then across subjects in each clinical group. The percentage of carriers was computed by determining the presence or absence of every species in each sample. Subjects in whom a given species was detected in at least one sample were considered carriers of that particular microorganism. Percentages for each microbial species tested were determined on the basis of the total number of subjects in each clinical group. The proportion of groups of microorganisms was determined for healthy and periodontitis subjects by grouping the 40 test species as similarly as possible to the description of subgingival microbial complexes by Socransky et al.;[23] exceptions have been noted (Table 2). Significance of differences between clinical groups in the levels, proportion, prevalence, and percentage of carriers of each species or microbial group was determined using the Mann-Whitney test after adjusting for multiple comparisons.[24]

RESULTS

Figure 1 summarizes the mean levels (× 10^5 ± SEM) of the 40 individual test species in 1,617 subgingival plaque samples from currently non-smoking periodontally healthy and chronic periodontitis subjects.

§§§§ Sigma-Aldrich.
|||||| Minislot-30, Immunetics, Cambridge, MA.
¶¶¶¶ Roche Diagnostics.
Sigma-Aldrich.
***** Sigma-Aldrich.
†††††† Sigma-Aldrich.
‡‡‡‡‡ Sigma-Aldrich.
§§§§§ Sigma-Aldrich.
|||||||| Roche Diagnostics.
¶¶¶¶¶ Miniblotter-45, Immunetics.
Sigma-Aldrich.
****** Sigma-Aldrich.
††††††† Roche Diagnostics.
‡‡‡‡‡‡ CDP-Star, Roche Diagnostics.
§§§§§§ DigiDoc, BioRad Laboratories, Hercules, CA.
|||||||| Quantity One, BioRad Laboratories.

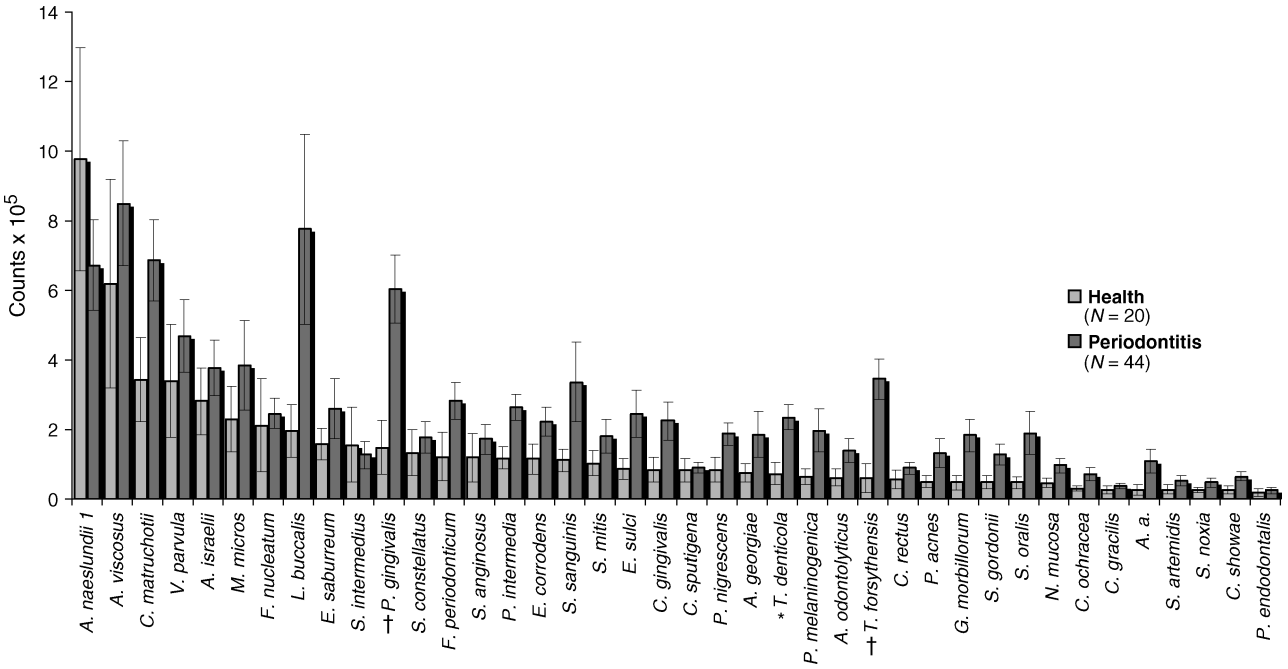

Figure 1.

*Bar chart of the mean levels (DNA probe count × 10^5 ± SEM) of each of the 40 test species in 1,617 subgingival plaque samples from 20 periodontally healthy and 44 chronic periodontitis subjects. The levels of each species were computed in each sample and averaged within a subject and then across subjects in each clinical group. The data are presented in decreasing order based on the levels detected in periodontally healthy subjects (*P <0.05; †P <0.001; Mann-Whitney test after adjusting for multiple comparisons). (Based on the format and style of figures developed by Socransky et al.[23,24])*

All of the species tested were detected in both clinical groups. Only *A. naeslundii* 1 and *S. intermedius* presented higher mean levels in periodontally healthy subjects; the other 38 test species presented higher mean levels in the periodontitis group. The differences were only statistically significant for *P. gingivalis* (health = 1.5 ± 0.8 × 10^5; periodontitis = 6.1 ± 0.9 × 10^5; $P<0.001$), *T. denticola* (health = 0.7 ± 0.3 × 10^5; periodontitis = 2.4 ± 0.3 × 10^5; $P<0.05$), and *T. forsythensis* (health = 0.6 ± 0.4 × 10^5; periodontitis = 3.5 ± 0.6 × 10^5; $P<0.001$).

The mean proportion (±SEM) of individual species in each clinical group is summarized in Figure 2. *A. naeslundii* 1, *Actinomyces viscosus*, *Cornyebacterium matruchotii*, *Actinomyces israelii*, and *Veillonella parvula* represented the highest proportion of species in healthy subjects (14.7% ± 2.5%, 10.1% ± 2.2%, 6.2% ± 1.4%, 4.4% ± 1.6%, and 4.2% ± 1.5%, respectively). Such species were also detected in high proportions in periodontitis subjects (9.6% ± 2.0%, 7.7% ± 1.4%, 7.1% ± 1.0%, 2.9% ± 0.7%, and 3.5% ± 0.8%, respectively). However, in contrast to periodontally healthy subjects, it was notable that *P. gingivalis* ($P<0.001$) and *T. forsythensis* ($P<0.01$) were among the six species that represented the highest proportion in samples (8.3% ± 1.3% and 4.3% ± 0.7%, respectively) in the periodontitis group. The differences between healthy and periodontitis subjects were statistically significant only for these two species.

Figure 3 summarizes the mean prevalence (±SEM) of the 40 individual test species in subgingival plaque samples from periodontally healthy and chronic periodontitis subjects. On average, none of the test species in either clinical group colonized more than 52% of sites. *A. naeslundii* 1, *A. viscosus*, *Eubacterium saburreum*, and *M. micros* were the only species that colonized a greater percentage of sites in periodontally healthy subjects (health = 51.5% ± 5.3%, 45.4% ± 5.4%, 32.0% ± 6.1%, and 29.7% ± 5.9%; periodontitis = 44.5% ± 5.4%, 41.6% ± 4.8%, 23.0% ± 4.6%, and 25.2% ± 4.4%, respectively). The other 36 test species (90%) colonized a greater percentage of sites in chronic periodontitis subjects. The differences between clinical groups were statistically significant only after adjusting for multiple comparisons, for *P. gingivalis* (health = 19.4% ± 5.4%; periodontitis = 47.6% ± 4.3%; $P<0.01$), *T. forsythensis* (health = 13.5% ± 4.3%; periodontitis = 40.6% ± 3.7%; $P<0.01$), *Prevotella nigrescens* (health = 13.0% ± 4.3%; periodontitis = 31.0% ± 3.6%; $P<0.05$), *T. denticola* (health = 12.1% ± 4.1%; periodontitis = 31.7% ± 3.7%; $P<0.01$), and *Fusobacterium periodonticum* (health = 9.3% ± 3.1%; periodontitis = 36.5% ± 4.5%; $P<0.01$). *P. gingivalis* was one of the most prevalent species in chronic

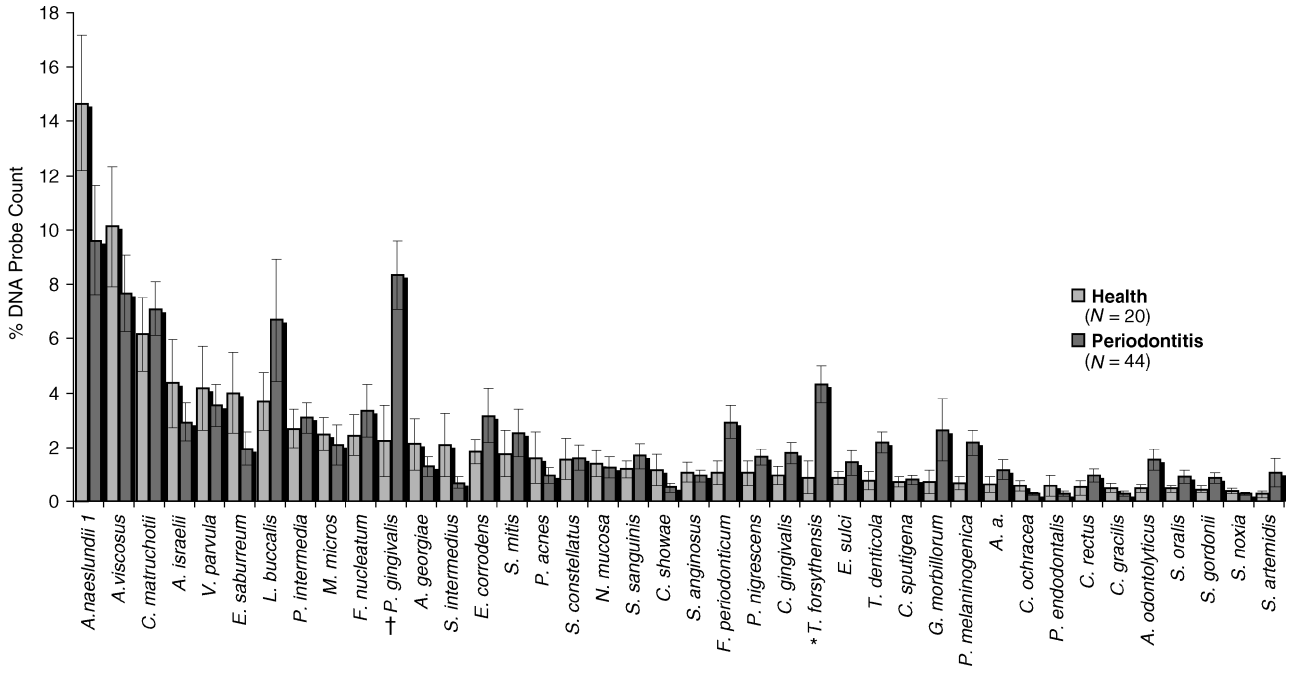

Figure 2.

*Bar chart of the mean proportion (percentage of the total DNA probe count ± SEM) of each of the 40 test species in 1,617 subgingival plaque samples from 20 periodontally healthy and 44 chronic periodontitis subjects. The proportion of each species was computed in each sample and averaged within a subject and then across subjects in each clinical group. The data are presented in decreasing order based on the proportions detected in periodontally healthy subjects (*P <0.01; †P <0.001; Mann-Whitney test after adjusting for multiple comparisons).*

periodontitis subjects, second only to *C. matruchotii* in that particular clinical group.

The percentage of carriers of each individual test species in chronic periodontitis and periodontally healthy subjects is presented in Table 3. From 73.7% to 97.7% of periodontitis subjects and 65% to 100% of periodontally healthy individuals were carriers of each species. In both clinical groups, the percentage of carriers of *A. naeslundii* 1, *A. israelii*, *A. viscosus*, and *C. matruchotii* was above 94%. A larger percentage of periodontitis subjects were carriers of a number of periodontal pathogens, including *P. gingivalis*, *T. denticola*, *P. nigrescens*, *F. nucleatum*, and *P. intermedia*, whereas healthy subjects were more frequently carriers of *Actinomyces georgiae*, *Capnocytophaga ochracea*, *Neisseria mucosa*, *S. constellatus*, *Streptococcus mitis*, and *Eubacterium sulci*. The differences in the percentage of healthy and periodontitis carriers were not statistically significant for any of the species tested.

The mean proportion of groups of microorganisms in each clinical group is summarized in Figure 4. The 40 test species were grouped as similarly as possible to the description of microbial complexes in subgingival plaque by Socransky et al.;[23] exceptions are noted in Table 2. The areas of the pies were adjusted to reflect the mean total levels (mean total DNA probe count) of species in each clinical group (health = $53.3 \pm 16.1 \times 10^5$; periodontitis = $96.1 \pm 13.7 \times 10^5$, $P < 0.01$). The major differences in the proportion of microorganisms were increases in the *Actinomyces* group in periodontally healthy subjects ($P < 0.05$) and in red-complex species (*P. gingivalis*, *T. forsythensis* and *T. denticola*) in chronic periodontitis subjects ($P < 0.001$). No significant differences in the proportion of the other six microbial groups were detected.

DISCUSSION

The present study compared the subgingival microbial composition of currently non-smoking Mexican subjects with untreated chronic periodontitis and periodontal health. To our knowledge, this is the first report in which the microbiota of subgingival plaque samples has been comprehensively examined in the Mexican population. Taken together, our results indicated that there were significant differences in the microbiota of subgingival plaque between chronic periodontitis and periodontally healthy subjects in the Mexican population. Although the microbiota in both clinical groups seemed to be dominated in levels, proportion, and prevalence by specific microbial species such as *A. naeslundii* 1, *A. israelii*, *A. viscosus*, *C. matruchotii*, *L. buccalis*, and *V. parvula*, a number of species previously associated with periodontal subjects and/or sites in other populations[4,6,8-10,25] were also dominant in Mexican subjects with chronic

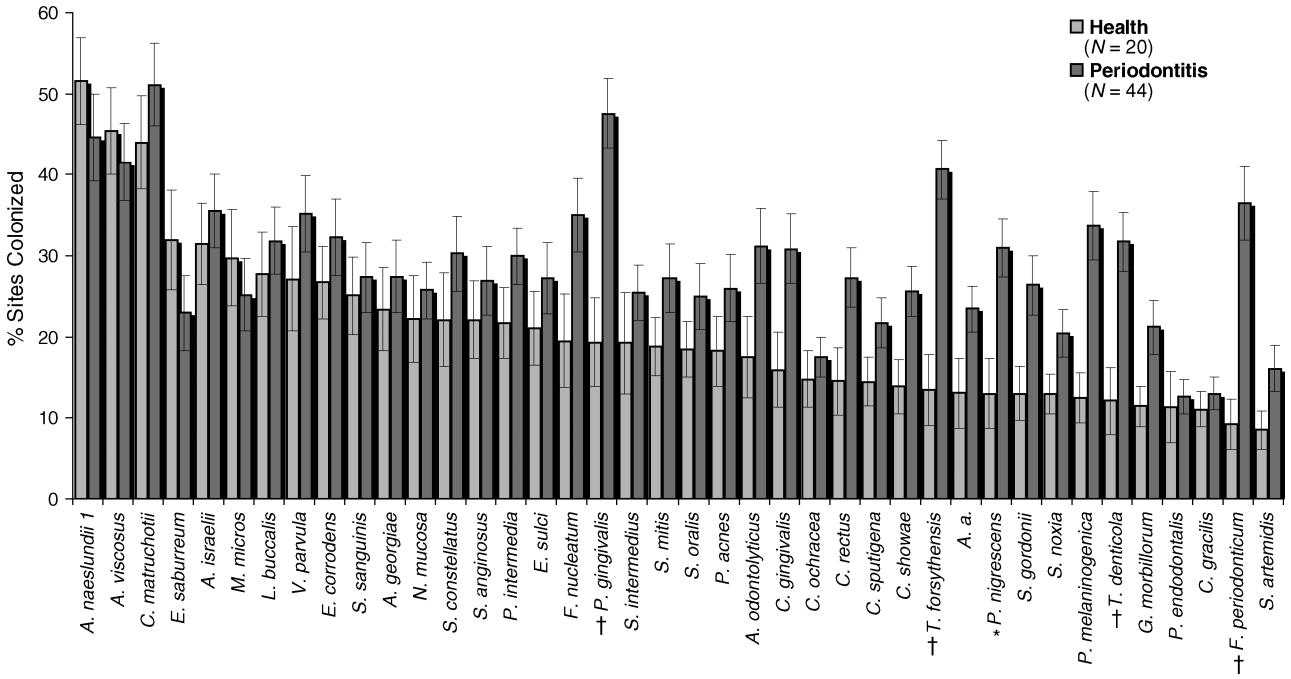

Figure 3.

*Bar chart of the mean prevalence (percentage of sites colonized ± SEM) of each of the 40 test species in 1,617 subgingival plaque samples from 20 periodontally healthy and 44 chronic periodontitis subjects. The prevalence of each species was computed in each sample and averaged within a subject and then across subjects in each clinical group. The data are presented in decreasing order based on the prevalence detected in periodontally healthy subjects (*P <0.05;† P <0.01; Mann-Whitney test after adjusting for multiple comparisons).*

periodontitis. Such species included *P. gingivalis*, *T. denticola*, and *T. forsythensis*. Furthermore, the periodontal pathogens included in the red complex represented a significantly larger proportion of species in subgingival plaque samples from periodontitis subjects than from healthy individuals, which seemed to be primarily related to a significant decrease in *Actinomyces* species. Such shifts in the proportion of red-complex and *Actinomyces* species in healthy and disease populations have also been reported in other populations in the past.[6] It is noteworthy that all of the species detected in periodontitis subjects were also present in periodontally healthy subjects. In fact, the percentage of healthy and periodontitis carriers of all putative periodontal pathogens was not significantly different between clinical groups, and ≥65% of periodontally healthy subjects were carriers of all of such species. This finding is consistent with the notion that both pathogenic and health-compatible bacterial species are indigenous residents of the subgingival microbiota.[6]

The increasing number of studies being published that report the subgingival microbial composition of different populations around the world has provided significant insight into the microbial shifts that occur in subjects from various geographical locations. However, only a limited number of research groups have been able to study more than two different populations under similar experimental conditions within the same research facilities.[11,15,16] Unable to overcome the logistic and financial difficulties of carrying out such studies, the majority of reports, including the present investigation, have reported the microbiota of subjects in a single location. However, comparing the results from different studies is a difficult task because most of the reports share few similarities in the general characteristics of the subject populations, such as mean probing depth and/or attachment level, previous periodontal treatment, and smoking history. Also, a diversity of methods for sample collection, microbiological processing, and data analysis have been employed, and the number of samples, subjects and/or bacterial species evaluated vary widely from one study to another. Due to these and other difficulties in comparing studies carried out by different research groups, the results from most of the available reports, while valuable in their own right, contribute only marginally to a global understanding of the microbial composition of subgingival plaque. In the hopes of setting our findings in perspective with other reports, an effort to compare a number of previous studies is outlined in the following paragraphs. Careful consideration was given to clinical, statistical, and methodological differences between studies, and only reports

Table 3.

Percentage of Carriers of Individual Species in Subjects With Chronic Periodontitis (N = 44) and Periodontal Health (N = 20)

Species	Periodontitis	Health	Species	Periodontitis	Health
A. actinomycetemcomitans	86.4	70	*M. micros*	80.5	88.9
A. georgiae	86.4	90	*N. mucosa*	88.4	100
A. israelii	94.9	94.1	*P. endodontalis*	76.7	65
A. naeslundii 1	97.6	100	*P. gingivalis*	97.7	85
A. odontolyticus	90.2	82.4	*P. intermedia*	95	73.7
A. viscosus	97.5	94.7	*P. melaninogenica*	83.7	68.4
C. gracilis	83.3	78.9	*P. nigrescens*	93	73.7
C. rectus	90.9	85	*P. acnes*	79.5	85
C. showae	88.4	80	*S. artemidis*	73.8	66.7
C. gingivalis	82.9	77.8	*S. noxia*	86	89.5
C. ochracea	83.7	90	*S. anginosus*	79.5	85
C. sputigena	88.4	90	*S. constellatus*	88.6	94.1
C. matruchotii	97.6	100	*S. gordonii*	85.7	65
E. corrodens	86.4	89.5	*S. intermedius*	86.4	75
E. saburreum	73.7	89.5	*S. mitis*	83.3	94.4
E. sulci	79.1	100	*S. oralis*	92.7	85
F. nucleatum	92.7	83.3	*S. sanguinis*	83.7	85
F. periodonticum	87.2	83.3	*T. forsythensis*	95.5	90
G. morbillorum	79.5	75	*T. denticola*	95.1	80
L. buccalis	91.9	89.5	*V. parvula*	88.1	78.9

Subjects in whom a given species was detected in at least one sample were considered carriers of that particular microorganism. Percentages were determined based on the total number of subjects in each clinical group. No significant differences between clinical groups were found using the Mann-Whitney test after adjusting for multiple comparisons.

that employed whole-genomic DNA probes for the enumeration of taxa were used for comparison. Furthermore, no attempts were made to compare the levels of microbial species, because such results tend to be highly dependent on sample size. Instead, only ≥2.5-fold differences in proportion and prevalence (expressed both as percentage of sites colonized and percentage of carriers) have been considered.

Lopez et al.[12] reported the proportion and prevalence (percentage of sites colonized) of 40 subgingival species in 26 Chilean subjects with untreated chronic periodontitis. On average, Chilean subjects presented a proportion of *A. actinomycetemcomitans* and *Campylobacter rectus* approximately three times greater than Mexican subjects with chronic periodontitis. A 2.7 to 4.3 times greater percentage of sites were also colonized by the same species and by *M. micros*, *Selenomonas noxia*, all tested *Streptococcus* species (except *S. constellatus*), and all tested *Capnocytophaga* species (except *C. gingivalis*) in Chilean subjects. Mexican subjects, on the other hand, presented substantially larger proportions of *Eikenella corrodens* (4.5 times), *A. naeslundii* 1 (3.2 times), and *A. viscosus* (2.7 times). However, the prevalence and proportion of a number of other important periodontal species, such as *P. gingivalis*, *T. denticola*, *T. forsythensis*, and *P. intermedia* and of *Actinomyces*

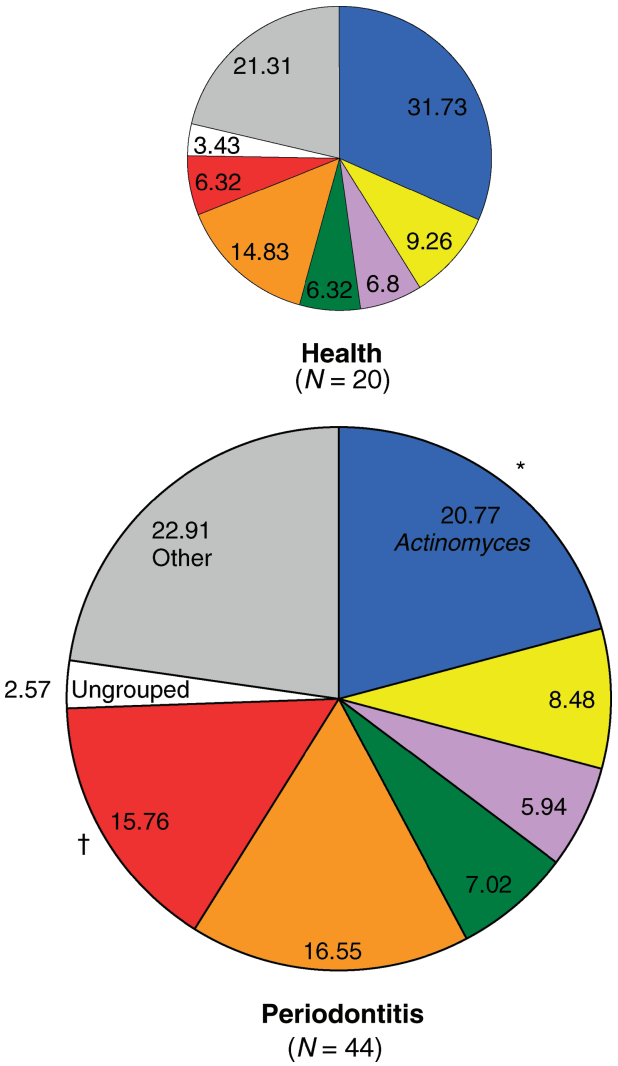

Figure 4.
*Pie charts of the mean proportion (percentage of the total DNA probe count) of microbial groups in 1,617 samples from 20 periodontally healthy and 44 chronic periodontitis subjects. The areas of the pies were adjusted to reflect the mean total levels of species in each clinical group (*P <0.05; †P <0.001; Mann-Whitney test after adjusting for multiple comparisons).*

and yellow, purple, green, orange, and red complex species were similar in Mexican and Chilean subjects. Such similarities were somewhat surprising because there were distinct differences between both subject populations: 46% of Chilean subjects were current-smokers, and our periodontitis group had a greater mean full-mouth probing depth.

In a different study,[11] the subgingival microbiota of 101 chronic periodontitis subjects from Sweden was evaluated. Although Swedish subjects had a greater mean full-mouth probing depth than our periodontitis group, had received previous periodontal treatment, and current smokers comprised 62% of the population (none were included in the present study), the proportions of the majority of bacterial species tested were similar to those detected in Mexican periodontitis individuals. Only *P. gingivalis* and *A. naeslundii* 1 represented a larger proportion of the species tested in Mexico (5.2 and 3.1 times, respectively), and *C. rectus* and *M. micros* in Sweden (4.2 and 2.9 times, respectively). By DNA probe analysis, Ali et al.[17] evaluated the prevalence of five periodontal pathogens in 25 periodontally untreated Sudanese subjects with severe chronic periodontitis (mean full-mouth probing depth = 6.8 mm). Despite the differences in mean full-mouth probing depth, Sudanese subjects had a 4.6 to 5.4 times lower prevalence (percentage of sites colonized and percentage of carriers, respectively) of *A. actinomycetemcomitans* than Mexican subjects with chronic periodontitis. However, the prevalence of *P. gingivalis*, *T. forsythensis*, *P. intermedia*, and *F. nucleatum* was similar in both populations.

In comparison to studies on periodontally healthy subjects from Cameroon[25] and Guatemala,[13] a larger percentage of healthy Mexican subjects were carriers of *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, and *T. forsythensis* than Cameroonian and Guatemalan subjects. A lower percentage of healthy Cameroonian subjects were also carriers of *C. rectus*, *E. corrodens*, *M. micros*, *S. noxia*, and *S. intermedius* than healthy Mexican individuals. The percentage of chronic periodontitis carriers of the 18 bacterial species tested in the Cameroonian study was similar to that detected in Mexico for the same clinical group. In contrast, a larger percentage of Mexican subjects with chronic periodontitis were carriers of *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythensis*, *F. nucleatum*, *M. micros*, and *P. nigrescens* than periodontitis subjects from Guatemala. Importantly, *A. actinomycetemcomitans* was not detected in any periodontitis or healthy Guatemalan subject included in that study (N = 114 subjects). In a different study of 15 currently non-smoking periodontally healthy subjects from Saudi Arabia,[26] the reported percentage of carriers of *P. gingivalis*, *T. denticola*, *C. rectus*, *E. corrodens*, and *S. noxia* was 3 to 12.7 times lower than in healthy Mexican subjects. However, the percentage of periodontally healthy carriers of other subgingival species, such as *A. actinomycetemcomitans*, *T. forsythensis*, *P. intermedia*, *P. nigrescens*, *F. nucleatum*, *M. micros*, and *S. intermedius* was similar in Mexico and Saudi Arabia.

The proportion of 40 microbial species in subgingival plaque samples from 58 Brazilian subjects with untreated chronic periodontitis was evaluated in a study by Haffajee et al.[11] The reported proportions of *T. denticola*, *P. nigrescens*, *C. rectus*, *S. gordonii*, and *S. intermedius* were 3.1 to 4.6 times greater than those detected in the present study for Mexican

periodontitis subjects. In terms of the prevalence of species (percentage of sites colonized) in healthy and periodontitis subjects, the results of a different study[8] were similar to ours in both clinical groups for all of the species tested in common, except *S. gordonii*, *S. mitis*, *Streptococcus oralis*, and *S. sanguinis*, which on average colonized a 2.6 to 3.5 times greater percentage of sites in periodontally healthy Brazilian subjects than in healthy Mexican individuals.

Perhaps the greatest similarities to the subgingival microbial profile of chronic periodontitis Mexican subjects were seen in subjects from the U.S. In terms of the proportion of microbial complexes, Mexican subjects with untreated chronic periodontitis presented similar proportions of *Actinomyces* and yellow, purple, green, orange, and red complex species to those reported in U.S. chronic periodontitis subjects.[6,12] Surprisingly, such similarities were present despite the fact that our periodontitis group had a greater mean full-mouth probing depth than subjects included in the cited studies and that 27% of U.S. subjects in one of the reports were current smokers.[12] Individually, only the proportion of *P. nigrescens* was 2.7[12] times greater, and that of *A. naeslundii* 1 was ~3.4 times lower in U.S. subjects. In addition, the proportion of red-complex species in periodontally healthy Mexican subjects was almost three times greater than in healthy subjects from the U.S.[6] However, comparisons with populations from the U.S. tend to be among the most difficult because subject populations include individuals from a number of racial and ethnic backgrounds in most cases. Thus, unless such groups are separated in the analysis of data (which they seldom are), it is unclear if the results reflect a true homogeneity in the distribution of species among racial and ethnic groups or if differences between such groups have been masked by a generalized analysis. Furthermore, the question remains of the suitability of arbitrary methods for grouping individuals from different racial and ethnic origins into single, broad categories. For example, the currently accepted grouping of individuals within the highly diverse Hispanic/Latino ethnic group has led a number of U.S. investigators[16,27-34] to assume questionable similarities among natives of over 20 different countries, including Mexico, Uruguay, Brazil, Venezuela, Costa Rica, Puerto Rico, Cuba, Belize, Panama, Peru, and Argentina. Many such countries share few cultural, dietary, historical, socioeconomic, racial, and genetic characteristics. To date, the influence of such factors on the microbial composition of subgingival plaque are not clearly understood; thus, a more specific separation of this particular ethnic group is warranted. Other research groups have also expressed concern about the adequacy of the "Hispanic" designation.[11]

CONCLUSIONS

The subgingival microbiota of untreated chronic periodontitis Mexican subjects was characterized by significant increases in the levels, prevalence, and proportion of classic periodontal pathogens. However, our results suggested that, whereas similar species can be detected in subgingival plaque samples in subjects from different geographical locations, their prevalence and/or proportion may vary significantly from one population to another. The therapeutic implications of such changes in microbial profiles and the cumulative microbial effects of periodontal therapy in populations around the world remain unclear for the most part. Therefore, further studies are necessary before a global understanding of the distribution of microbial species in subgingival plaque can be achieved.

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