The subgingival cultivable bacteria of Albanian subjects with different periodontal status compared to a similar population of Spanish subjects: a case control study

Gerila Tafaj1,2, Margarita Iniesta1*, Mariano Sanz1 and David Herrera1

Abstract

Background: The objective was to qualitatively and quantitatively describe the subgingival cultivable bacteria in Albanian subjects and to compare it with a similar Spanish population.

Materials and methods: Consecutive patients, diagnosed as periodontitis in stages I–II or III–IV, and as periodontally healthy or with gingivitis, were studied clinically and microbiologically by means of microbiological culture, including total anaerobic counts, proportions, and frequency of detection of target species. Outcome variables were analysed by Mann–Whitney, Kruskal–Wallis, ANOVA, ANCOVA and Chi-square tests.

Results: In this cross-sectional study, 83 (Albania) and 90 (Spain) subjects were included. No statistically significant differences were observed between test and control populations regarding demographic variables or smoking habit. Significantly higher total anaerobic counts in the Albanian population \((p = 0.022)\) were observed, especially in the periodontal health/gingivitis group \((p = 0.001)\). In the test population, the proportions of the cultivable bacteria of \(Fusobacterium nucleatum\) were significantly lower in both the healthy/gingivitis \((p = 0.022)\) and stages I–II periodontitis \((p = 0.034)\) groups.

Conclusions: The subgingival cultivable bacteria in both periodontitis and non-periodontitis subjects from Albania showed significantly higher total anaerobic counts and lower proportions of the cultivable bacteria of \(F. nucleatum\) than a similar population of subjects from Spain.

Keywords: Subgingival cultivable bacteria, Periodontitis, Microbiological culture, Albania, Spain

Background

Periodontal diseases are chronic inflammatory conditions affecting the tooth supporting tissues caused by dental biofilms, but modulated by different patient and environment related risk factors. The pathogenesis of both gingivitis and periodontitis result from an imbalance between the infectious challenge (bacterial pathogens organized in dental biofilms) and the host response [1, 2]. Systemic risk factors will influence the host response by the genetic predisposition and systemic health status of the subject, while the environmental conditions as well as by lifestyle factors may influence both the subgingival microbiota and the host response [2]. For example, there is strong evidence that tobacco smoking is a relevant risk factor for the onset and progression of periodontitis, what contributes to the higher prevalence and severity of periodontitis in smokers [3, 4]. Nutrition has also been reported as...
a relevant lifestyle factor in the onset and progression of periodontal diseases, although its role as a true risk factor has not yet been established [5].

Environmental and lifestyle factors vary among countries and subject populations from different geographical areas and these changes may influence the aetiology and progression of periodontal diseases in these specific places. In fact, differences in the composition of the subgingival microbiota have been demonstrated when sampling subjects with similar clinical characteristics, but belonging to different geographical populations [6]. Whereas it is well established that periodontal pathogens such as Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia have demonstrated a strong association with periodontitis [1], and their relative counts and proportions may be different according to different geographical areas [7, 8]. For example, A. actinomycetemcomitans, [9] was reported in high prevalence in the Chinese population, compared to that of European and North American populations. Similarly, in Brazil [10] A. actinomycetemcomitans was found in high numbers in both periodontitis and in healthy subjects. In Morocco, subjects diagnosed of periodontitis demonstrated a significantly higher prevalence of A. actinomycetemcomitans (35.6%) [11], compared to only 5.7% in periodontitis patients in Spain [12].

These studies therefore suggest that differences in the subgingival microbiota composition may occur in populations with different environmental and lifestyle conditions. In fact, differences in the profiles of the subgingival microbiota were also reported by our research group when comparing periodontitis subjects from Spain and The Netherlands [8], and when comparing periodontitis subjects from Colombia, Chile and Spain [13], or more recently, even comparing subjects between Colombia and Spain [14]. Other authors have postulated that beyond the environmental and lifestyle factors, the genetic background of the subjects may also influence the composition of the subgingival microbiota [6, 15]. In fact, it has been reported that specific subgingival microbiomes when comparing two populations sharing a common environment but different genetic background [16],

Albania is a developing country in the western Balkans surrounded by Montenegro, Kosovo, Macedonia and Greece, with a population of approximately 3.1 million people. The drastic change in economic and social political conditions in the last 2 decades has significantly impacted on the socioeconomic and environmental factors that may have an impact on health [17], what may also influence the prevalence and severity of periodontal diseases in this country [18]. Factors such as low socioeconomic status, lack of dental health education and limited access to proper oral-health care in a highly diverse ethnic population may influence the prevalence of periodontal diseases by affecting the composition of the oral microbiota.

Since there are no studies characterising the subgingival microbiota from Albanian subjects, we have designed this case–control study, where consecutive subjects were periodontally diagnosed with the new classification of periodontal diseases [19, 20], and the composition of their subgingival microbiota has been studied using anaerobic culture microbiology. As controls, we have characterised, both clinically and microbiologically, a similar population of Spanish subjects. The working hypothesis is that the clear environmental differences between Albania and Spain will significantly influence the microbial composition of the subgingival cultivable bacteria in subjects with different periodontal health status.

Materials and methods

Study design

This study was designed as a cross-sectional observational study and it is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [21]. The protocol of this investigation was approved by the local ethical committees in the respective countries (references 18/128-E in Spain and 197 in Albania) and considers all aspects of the Helsinki Declaration regarding experimentation involving human.

Patient sample

Subjects seeking dental attending the Faculty of Dentistry in Tirana (Albanian University, Albania) and the Faculty of Dentistry in Madrid [University Complutense of Madrid (UCM), Spain] were screened between April–May 2018 and March 2020 and registered the socio-demographic characteristics of the patients, such as age, gender, origin, smoking, systemic health, medications, and conditions. Patients included in this study met the following criteria: (1) age between ≥30 and ≤60. The following exclusion study criteria were also evaluated: (1) having less than 16 teeth; (2) patient with a periodontal abscess or necrotizing periodontal diseases; (3) use of systemic antibiotics in the previous month; (4) patient with relevant systemic diseases (diabetes, polymorphonuclear neutrophil defects, other immune system disorders); (5) pregnant or lactating patient; and (6) patient with current anti-inflammatories, anticonvulsant, calcium channel blockers, or immunosuppressant treatments, or 6 months prior to the sample.

When subjects fulfilled these criteria, they were verbally informed about the study and were asked to
participate by signing an informed consent. Upon acceptance, each patient was appointed for the study visits.

Clinical and radiological examination
The patients received a complete periodontal and radiographic examination, including gingival recession, probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BoP) [22] using a UNC-15 periodontal probe (HuFriedy, Leinmen, Germany). Plaque index (PII) [23] was evaluated after rinsing the patient with a plaque disclosing solution containing erythrosine (Plac-Control®, Dentaid, Barcelona, Spain).

After this visit, the included subjects were segmented by their periodontal status in three categories using the following criteria [19, 20]: (1) periodontal health and gingivitis: no CAL, no radiographic bone loss (RBL) and PD ≤ 3 mm, assuming no pseudo-pockets; (2) stages I and II periodontitis: PD 4–5 mm, mostly horizontal RBL and no tooth loss due to periodontal reasons. CAL will be 1–2 mm (stage I) or 3–4 mm (stage II), while RBL affects only the coronal third (<15% for stage I and 15–33% for stage II); and (3) stages III and IV periodontitis: At least two non-adjacent sites with CAL ≥ 5 mm or reaching the middle third of the root, with PD ≥ 6 mm. Evidence of tooth loss due to periodontal reasons.

Microbiological procedures

Microbiological sampling
Samples were taken with two consecutive standardized 30# sterile paper points (Maillefer, Ballaigues, Switzerland). Paper points were inserted into the crevice or pocket and left in place for 10 s. Prior to sampling, four sites per patient were selected, one in each quadrant. The selected sites were isolated from saliva and supragingival plaque contamination with the use of cotton rolls and compressed air. In periodontal health/gingivitis subjects, subgingival samples were taken from the mesio-buccal sites of the first molars and, when absent, from the adjacent second molars (the next alternative was the second premolars and from there, any teeth present mesially). In subjects with periodontitis, subgingival samples were taken from the most accessible site with the deepest PD and BoP per quadrant. The eight paper points were transferred into a screw-capped vial, containing 1.5 ml of reduced transport fluid (RTF) [24], so an individual pooled sample was obtained from each patient. Samples were sent directly (Spanish samples) or via courier (Albanian samples) to the Laboratory of Research at UCM, Spain, where they were processed within 24–36 h. RTF was the ideal transport medium, as it has been shown to maintain a good viability of anaerobes up to four days after sample collection [24].

Direct anaerobic culture
At the Laboratory, samples were homogenized by vortexing for 30 s, and serially diluted in phosphate buffer saline (PBS) (dilutions 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴). For each sample, 100 µl of at least two of the dilutions were plated on non-selective blood agar medium (Blood Agar Base II, Oxoid, Basingstoke, England), supplemented with haemin (5 mg/l), menadione (1 mg/l) and 5%, sterile horse blood. Plates were incubated for up to 14 days in anaerobic conditions (80% N₂, 10% CO₂ and 10% H₂) at 37 °C. After 7–14 days of anaerobic incubation, suspected colonies were further identified by microscopy, gram-staining and enzyme activity (see Additional file 1: Table S1). The counts of representative colonies (those with colony morphologies compatible with target pathogen morphology) were carried out.

For isolation and quantification of A. actinomycetemcomitans, another 100 µl of the 10⁻¹ dilution of each sample and 100 µl without dilution were plated onto the selective medium Dentaid-1 [25], that was incubated for 3 days in air with 5% CO₂ at 37 °C.

Data analysis

Sample size calculation
The outcome variable “proportion of the anaerobic cultivable bacteria of P. gingivalis” was selected to calculate the sample size. With a proportion of P. gingivalis in Spain of 22.21% [13] and in order to detect a difference in proportions of 16.72% between Albania and Spain, with a 90% of power and a significance of 95%, at least 88 patients per country were necessary. Besides, and to narrow differences between different age groups in different conditions, the overall sample was a uniformed stratified sampling, in which the same size for all the defined categories were assigned. The desired sampling distribution was 30 patients in each category (periodontal health/gingivitis, stages I–II periodontitis, stages III–IV periodontitis), and 10 patients per age cohort, 30–40 years, 41–50 years, 51–60 years, within each periodontal status category.

Statistical analysis
The statistical unit of the study was the patient. For continuous data, Kolmogorov–Smirnov test and distribution of data were used to assess normality. Data were expressed as means and standard deviations (SD), and as median and interquartile ranges (IQR) for non-parametric data. Categorical data were expressed as percentages. Demographic data and clinical variables were analysed by Student t test, ANOVA test and chi-square test with probability values adjusted with the Bonferroni correction. For microbiological outcome variables, total
anaerobic counts were calculated on blood-agar plates and expressed in total colony-forming units/ml (CFU/ml). Counts for each specific bacterial species, as well as their percentage of total cultivable bacteria, were also calculated for each patient. Counts and proportions were calculated considering all samples. The logarithmic transformation of CFU of bacterial counts was designed to normalise the data distribution. Microbiological variables were compared by t test and ANOVA test, for parametric data, and U Mann–Whitney test or Kruskal–Wallis test with Dunn–Bonferroni post hoc tests, for non-parametric data. Differences between the two countries were further explored by analysis of covariance (ANCOVA), with country as the factor, and PI and PD were entered into the model as co-variates. In this case, ANCOVA model adjusted means and confidence intervals (CI) were calculated. Proportions of target pathogens were log-transformed to achieve homogeneity of variances.

For categorical data, chi-square test was used, with Bonferroni correction for multiplicity when was necessary.

All statistical analyses were performed using SPSS 20 program package (SPSS Inc, Chicago, IL, USA) and the level of significance was set in 0.05.

**Results**

A total of 186 patients were initially recruited (98 in Spain and 88 in Albania). Eight subjects in Spain and five in Albania were excluded due to technical issues in sample transportation and bacteria culturing. A total of 173 subjects, 90 in Spain and 83 in Albania, were recruited, with a similar distribution within the pre-established categories: periodontal health/gingivitis (55 in total, 30 in Spain and 25 in Albania), stages I–II periodontitis (58, 30 and 28, respectively) and stages III–IV periodontitis (60, 30 and 30, respectively). As depicted in Table 1, there were no statistically significant differences between these populations, either for age and gender or among the periodontal categories. Similarly, the percentage of smokers was not significantly different.

Table 2 depicts the clinical variables in the recruited patients within the three established categories. Overall, subjects from Spain showed a statistically significant higher mean PD (p = 0.025). PD was significantly higher in Spanish subjects in stages III–IV periodontitis (p < 0.001) and PI in the periodontal health/gingivitis group (p < 0.001). Conversely, PI was significantly higher in stages III–IV periodontitis (p = 0.036) for Albanian population.

Table 3 presents the detection of different bacterial species in subgingival samples of Albanian population. The most prevalent bacterial species in periodontal health/gingivitis, stages I–II and stages III–IV periodontitis groups were *Fusobacterium nucleatum* (92%, 92.9% and 86.7%, respectively), *P. gingivalis* (68%, 82.1% and 80%, respectively), *Prevotella intermedia* (52%, 71.4% and 76.7%, respectively) and *Eikenella corrodens* (46.7% in stages III–IV). Statistically significant differences between subjects with and without periodontitis were only found for *P. intermedia* (p = 0.048) and *E. corrodens* (p = 0.035). In addition, *E. corrodens* was detected with a higher frequency in stages III–IV than in periodontal health/gingivitis (p = 0.016). In terms of counts and proportions, comparing subjects with and without periodontitis, statistically significant differences were detected for *P. gingivalis* (p = 0.002 for counts and p = 0.016 for proportions), *P. intermedia* (p = 0.002 and p = 0.020, respectively) and *E. corrodens* (p = 0.014 and p = 0.043, respectively). In the analysis by periodontal status, significant differences were only observed in counts for *P. gingivalis* (p = 0.005), *P. intermedia* (p = 0.005) and *E. corrodens* (p = 0.020), between periodontal health/gingivitis and stages III–IV periodontitis. On the other hand, statistically significant higher counts and proportions of *Actinomyces odontolyticus* were detected in patients without periodontitis (p = 0.030 and p = 0.030, respectively), but such differences were not maintained in the analysis by periodontal status.

Table 4 depicts the total anaerobic count results of the test and control group analysed together, and by periodontal status and country. Significantly higher anaerobic counts were detected in the periodontitis categories (stages I–II (p < 0.001) and III–IV (p < 0.001), compared with the periodontal health/gingivitis group. Higher total counts were observed in Albanian subjects (p = 0.022), being this difference statistically significant in the periodontal health/gingivitis group (p = 0.001).

Tables 5, 6 and 7 depict the subgingival cultivable bacteria (target periodontal pathogens) in both country populations segmented by the three categories. Samples from Albanian subjects were characterized by significantly lower proportions of *E. nucleatum* and *P. intermedia*. In periodontal health/gingivitis and in stages I–II periodontitis, *E. nucleatum* was present in significantly lower proportion (p = 0.022 and p = 0.034, respectively) in the Albanian samples. The same was true in the category stages III–IV periodontitis for *P. intermedia* (p = 0.038). However, when the proportions of *P. intermedia* were adjusted for plaque index and probing depth, as possible confounding factors between countries by an ANCOVA model, this difference between countries was no longer observed (Table 8).

No statistically significant differences were found between the countries for counts or frequencies of detection of the target pathogens, although some tendencies were observed. In stages I–II periodontitis,
| Comparison                      | Age | Gender | Smoking |
|--------------------------------|-----|--------|---------|
|                                |     |        |         |
|                                | First | Second | n | Mean (SD) | p value | Female n (%) | Male n (%) | p value | Non smokers n (%) | Smokers n (%) | p value |
| Periodontal status group       |       |        |    |           |         |              |            |         |                  |              |         |
| Health/gingivitis              | 55   | 43.00 (8.54) | 0.159<sup>a</sup> | 32 (58.2) | 23 (41.8) | 0.286<sup>b</sup> | 46 (83.6) | 9 (16.4) | 0.381<sup>b</sup> |
| Periodontitis I–II             | 58   | 44.05 (8.77) | 0.107<sup>c</sup> | 33 (56.9) | 25 (43.1) |               | 43 (74.1) | 15 (25.9) |
| Periodontitis III–IV           | 60   | 46.03 (8.49) | 0.076<sup>d</sup> | 29 (48.3) | 31 (51.7) |               | 46 (76.7) | 14 (23.3) |
| Country                        |       |        |    |           |         |              |            |         |                  |              |         |
| Spain                          | 90   | 45.42 (7.98) | 1.000<sup>a</sup> | 49 (54.4) | 41 (45.6) | 0.976<sup>d</sup> | 68 (75.6) | 22 (24.4) | 0.412<sup>d</sup> |
| Albania                        | 83   | 43.30 (9.23) | 1.000<sup>a</sup> | 45 (54.2) | 38 (45.8) |               | 67 (80.7) | 16 (19.3) |
| Periodontal status by country  |       |        |    |           |         |              |            |         |                  |              |         |
| Health/gingivitis              |       |        |    |           |         |              |            |         |                  |              |         |
| Spain                          | 30   | 44.90 (7.47) | 1.000<sup>a</sup> | 16 (53.3) | 14 (46.7) | 1.000<sup>b</sup> | 25 (83.3) | 5 (16.7) | 1.000<sup>b</sup> |
| Albania                        | 25   | 40.72 (9.31) | 1.000<sup>a</sup> | 16 (64.0) | 9 (36.0)   |               | 21 (84.0) | 4 (16.0)  |
| Periodontitis I–II             |       |        |    |           |         |              |            |         |                  |              |         |
| Spain                          | 30   | 45.47 (8.69) | 1.000<sup>a</sup> | 16 (53.3) | 14 (46.7) |               | 22 (73.3) | 8 (26.7)  |
| Albania                        | 28   | 42.54 (8.75) | 1.000<sup>a</sup> | 17 (60.7) | 11 (39.3) |               | 21 (75.0) | 7 (25.0)  |
| Periodontitis III–IV           |       |        |    |           |         |              |            |         |                  |              |         |
| Spain                          | 30   | 45.90 (7.97) | 1.000<sup>a</sup> | 17 (56.7) | 13 (43.3) |               | 21 (70.0) | 9 (30.0)  |
| Albania                        | 30   | 46.17 (9.12) | 1.000<sup>a</sup> | 12 (40.0) | 18 (60.0) |               | 25 (83.3) | 5 (16.7)  |

n, number of patients; SD, standard deviation

<sup>a</sup> One-way ANOVA test
<sup>b</sup> Chi-square test with Bonferroni correction
<sup>c</sup> Student t test
<sup>d</sup> Chi-square test
| Table 2 | Comparison of clinical parameters among periodontal status groups and between countries |
|---------|--------------------------------------------------------------------------------------------------|
|         | Comparison | n   | Mean (SD) | Mean difference | 95% CI | p value |
|         | Firstb     |     |           |               |        |         |
| PD (mm) | Periodontal status group                        |     |           |               |        |         |
|         | Health/gingivitis                               | 55  | 2.13 (0.30)| −0.77*        | −1.03  | −0.51   | <0.001  |
|         | Periodontitis I–II                              | 58  | 2.91 (0.46)| −0.81†        | −1.06  | −0.56   | <0.001  |
|         | Periodontitis III–IV                            | 60  | 3.72 (0.78)| −1.58‡        | −1.83  | −1.33   | <0.001  |
|         | Country                                           |     |           |               |        |         |
|         | Spain                                             | 90  | 3.08 (0.90)| 0.28ское  | 0.03   | 0.54    | 0.025   |
|         | Albania                                           | 83  | 2.79 (0.77)| 0.28oneksi  | 0.03   | 0.54    | 0.025   |
|         | Periodontal status by country                    |     |           |               |        |         |
|         | Health/gingivitis                               | 30  | 2.20 (0.31)| 0.14§         | −0.01  | 0.31    | 0.072   |
|         | Spain                                             | 25  | 2.05 (0.28)| 0.14koneksi  | −0.01  | 0.31    | 0.072   |
|         | Albania                                           | 30  | 2.98 (0.47)| 0.15§        | −0.08  | 0.40    | 0.195   |
|         | Periodontitis III–IV                            | 28  | 2.82 (0.44)| 0.15§        | −0.08  | 0.40    | 0.195   |
|         | Country                                           |     |           |               |        |         |
|         | Spain                                             | 90  | 2.70 (1.93)| −0.00§       | −0.59  | 0.58    | 0.985   |
|         | Albania                                           | 83  | 2.71 (1.98)| −0.00§       | −0.59  | 0.58    | 0.985   |
| CAL (mm)| Periodontal status group                        |     |           |               |        |         |
|         | Health/gingivitis                               | 55  | 0.25 (0.25)| −2.80*       | −3.15  | −2.46   | <0.001  |
|         | Periodontitis I–II                              | 58  | 3.06 (0.47)| −1.56†       | −1.89  | −1.22   | <0.001  |
|         | Periodontitis III–IV                            | 60  | 4.62 (1.16)| −4.36‡       | −4.70  | −4.02   | <0.001  |
|         | Country                                           |     |           |               |        |         |
|         | Spain                                             | 90  | 2.70 (1.93)| −0.00§       | −0.59  | 0.58    | 0.985   |
|         | Albania                                           | 83  | 2.71 (1.98)| −0.00§       | −0.59  | 0.58    | 0.985   |
| BoP (%) | Periodontal status group                        |     |           |               |        |         |
|         | Health/gingivitis                               | 55  | 9.87 (10.57)| −25.10*      | −34.69 | −15.51  | <0.001  |
|         | Periodontitis I–II                              | 58  | 34.97 (21.19)| −29.04†     | −38.43 | −19.65  | <0.001  |
|         | Periodontitis III–IV                            | 60  | 64.02 (27.28)| −54.14‡     | −63.66 | −44.63  | <0.001  |
|         | Country                                           |     |           |               |        |         |
|         | Spain                                             | 90  | 37.70 (29.69)| 1.33§       | −7.85  | 10.52   | 0.775   |
|         | Albania                                           | 83  | 36.37 (31.53)| 1.33§       | −7.85  | 10.52   | 0.775   |
| PII (%) | Periodontal status group                        |     |           |               |        |         |
|         | Health/gingivitis                               | 55  | 35.36 (23.52)| −33.21*     | −44.48 | −21.95  | <0.001  |
|         | Periodontitis I–II                              | 58  | 68.58 (27.35)| −14.66†     | −25.68 | −3.64   | 0.005   |
|         | Periodontitis III–IV                            | 60  | 83.25 (23.15)| −47.88‡     | −59.05 | −36.71  | <0.001  |
|         | Country                                           |     |           |               |        |         |
|         | Spain                                             | 90  | 64.21 (24.97)| 2.29§       | −7.41  | 12.00   | 0.641   |
|         | Albania                                           | 83  | 61.91 (37.74)| 2.29§       | −7.41  | 12.00   | 0.641   |
|         | Periodontal status by country                    |     |           |               |        |         |
|         | Health /gingivitis                               | 30  | 48.84 (20.88)| 29.65§      | 19.97  | 39.34   | <0.001  |
|         | Albania                                           | 25  | 19.18 (14.79)| 29.65§      | 19.97  | 39.34   | <0.001  |
|         | Periodontitis I–II                              | 30  | 66.76 (57.24)| −3.77§      | −18.40 | 10.85   | 0.607   |
|         | Albania                                           | 28  | 70.53 (30.39)| −3.77§      | −18.40 | 10.85   | 0.607   |
|         | Periodontitis III–IV                            | 30  | 77.02 (21.31)| −12.48§     | −24.06 | −0.83   | 0.036   |
|         | Albania                                           | 30  | 89.47 (23.58)| −12.48§     | −24.06 | −0.83   | 0.036   |

PD, probing depth; CAL, clinical attachment loss; BoP, bleeding on probing; PII, plaque index; n, sample size; SD, standard deviation; CI, confidence interval

* One-way ANOVA test
Table 2 (continued)

| | Health/gingivitis (n = 25) | Periodontitis I–II (n = 28) | Periodontitis III–IV (n = 30) | Differences among groups p value | Health/gingivitis versus periodontitis p value |
|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | Mean (SD) | p value | p value |
| **Aggregatibacter actinomycetemcomitans** | | | | | |
| Counts | 0.19 (0.96) | 0 | 0.46 (1.42) | 0.208 <sup>a</sup> | 0.831 <sup>c</sup> |
| Proportions | 0.14 (0.74) | 0 | 0.00 (0.01) | 0.215 <sup>a</sup> | 0.851 <sup>c</sup> |
| Frequency<sup>b</sup> | 1 (4.0) | 0 (0.0) | 3 (10.0) | 0.270 <sup>c</sup> | 1.000 <sup>d</sup> |
| **Porphyromonas gingivalis** | | | | | |
| Counts | 2.98 (2.36) | 4.42 (2.28) | 4.68 (2.50) | 0.005 | 0.002 |
| Proportions | 8.19 (18.49) | 12.75 (17.34) | 15.18 (23.08) | 0.053 | 0.016 |
| Frequency | 17 (68.0) | 23 (82.1) | 24 (80.0) | 0.314 | 0.195 |
| **Prevotella intermedia** | | | | | |
| Counts | 1.99 (2.17) | 3.30 (2.30) | 3.84 (2.31) | 0.005 | 0.002 |
| Proportions | 5.68 (18.31) | 1.91 (4.40) | 2.79 (7.48) | 0.055 | 0.020 |
| Frequency | 13 (52.0) | 20 (71.4) | 23 (76.7) | 0.057 | 0.048 |
| **Tannerella forsythia** | | | | | |
| Counts | 0.68 (1.61) | 1.73 (2.38) | 1.05 (2.16) | 0.168 | 0.155 |
| Proportions | 0.07 (0.26) | 0.32 (0.46) | 0.51 (1.58) | 0.113 | 0.097 |
| Frequency<sup>c</sup> | 3 (12.0) | 10 (35.7) | 6 (20.0) | 0.533 | 0.121 |
| **Parvimonas micra** | | | | | |
| Counts | 0.74 (1.74) | 0 | 0.66 (1.73) | 0.106 | 0.237 |
| Proportions | 0.30 (0.64) | 0 | 0.33 (1.10) | 0.062 | 0.094 |
| Frequency | 5 (20.0) | 0 (0.0) | 4 (13.3) | 0.505 | 0.120 |
| **Fusobacterium nucleatum** | | | | | |
| Counts | 3.83 (1.52) | 4.17 (1.35) | 4.11 (1.84) | 0.114 | 0.053 |
| Proportions | 1.65 (2.00) | 1.17 (1.46) | 2.38 (4.18) | 0.487 | 0.313 |
| Frequency | 23 (92.0) | 26 (92.9) | 26 (86.7) | 0.489 | 1.000 |
| **Campylobacter rectus** | | | | | |
| Counts | 0 | 0.27 (1.01) | 0.16 (0.91) | 0.393 | 0.250 |
| Proportions | 0 | 0.00 (0.03) | 0.02 (0.14) | 0.393 | 0.250 |
| Frequency | 0 (0.0) | 2.7 (1.7) | 1 (3.3) | 0.555 | 0.550 |
| **Eikenella corrodens** | | | | | |
| Counts | 0.55 (1.31) | 1.41 (2.12) | 2.16 (2.41) | 0.020 | 0.014 |
| Proportions | 0.07 (0.17) | 0.21 (0.51) | 2.65 (12.75) | 0.065 | 0.043 |
| Frequency | 4 (16.0) | 9 (32.1) | 14 (46.7) | 0.016 | 0.035 |
| **Capnocytophaga spp.** | | | | | |
| Counts | 0.32 (1.10) | 0.12 (0.65) | 0.94 (1.93) | 0.091 | 0.550 |
| Proportions | 0.03 (0.11) | 0.02 (0.10) | 0.27 (0.93) | 0.106 | 0.526 |
| Frequency | 2 (8.0) | 1 (3.6) | 6 (20.0) | 0.135 | 0.717 |
| **Actinomyces odontolyticus** | | | | | |
| Counts | 0.37 (1.30) | 0 | 0 | 0.095 | 0.030 |
| Proportions | 0.63 (2.19) | 0 | 0 | 0.096 | 0.030 |
| Frequency | 2 (8.0) | 0 (0.0) | 0 (0.0) | 0.063 | 0.088 |

<sup>a</sup> Kruskal–Wallis test  
<sup>b</sup> Chi-square with Bonferroni correction  
<sup>c</sup> Mann–Whitney test  
<sup>d</sup> Chi-square  
<sup>e</sup> Number and percentage of positive samples  
n, sample size; SD, standard deviation
samples from Albanian subjects presented less frequently \( P. \) gingivalis (82.1\% versus 96.7\%, \( p = 0.097 \)) and \( P. \) intermedia (71.4\% versus 90.0\%, \( p = 0.071 \)). Similar findings were observed for \( T. \) forsythia in stages III–IV periodontitis (20.0\% versus 43.3\%, \( p = 0.052 \)). Conversely \( A. \) actinomycetemcomitans (10.0\% versus 0\%), and \( E. \) corrodens (76.7\% versus 23.3\%, \( p = 0.058 \)) were present more frequently in the Albanian population, in stages III–IV periodontitis.

Table 9 depicts the counts and frequencies of detection of target pathogens by clinical categories. Lower counts, proportions, and frequencies of detection of \( P. \) gingivalis and \( P. \) intermedia were detected in the periodontal health/gingivitis group, compared with stages I–II (\( p \leq 0.05 \)) or stages III–IV periodontitis (\( p \leq 0.05 \)) groups. Similarly, \( T. \) forsythia was detected in lower counts, proportions, and frequencies of detection in the periodontal health/gingivitis group, compared with stages I–II periodontitis (\( p \leq 0.05 \)); \( F. \) nucleatum was detected in lower counts in periodontal health/gingivitis group when compared with stages I–II (\( p = 0.012 \)) or stages III–IV periodontitis (\( p = 0.001 \)); and \( E. \) corrodens was detected in lower counts in periodontal health/gingivitis when compared with stages III–IV periodontitis (\( p = 0.028 \)). For \( P. \) micra, statistically significant higher frequencies of detection were observed in the periodontal health/gingivitis group when compared with stages I–II periodontitis (\( p = 0.045 \)).

**Discussion**

The present study evaluated two similar populations in terms of age, gender and smoking habits, but from two countries with different environments (Albania and Spain). These subjects have provided microbiological samples processed by anaerobic culturing in a single laboratory using the same microbiological diagnostic technology. These recruited subjects were present in similar numbers in the three diagnostic categories, with minimal differences in the clinical parameters (deeper mean PD in Spanish subjects within the stages III–IV periodontitis category, and lower plaque index levels in Albanian subjects in the periodontal health/gingivitis group but higher in the stage III–IV periodontitis). The analysis of the subgingival cultivable bacteria of these two distinct populations has shown that Albanian subjects presented higher anaerobic counts, especially in periodontal health/gingivitis subjects, and lower proportions of \( F. \) nucleatum in periodontal health/gingivitis and stages I–II periodontitis.

The fact that all samples were processed by the same microbiological laboratory may reduce the differences due to sample processing reported in previous studies with similar objectives [6, 8, 11, 13, 14]. Also, the fact that the clinical parameters within the pre-established clinical categories were based on the diagnostic criteria of the new classification of periodontal diseases [19, 20] may have reduced the likely differences in the subgingival...
Table 5  Counts, proportions and frequencies of detection of target species by country, in periodontal health/gingivitis subjects

| Periodontal health/gingivitis | Country | Counts<sup>a</sup> | Proportions<sup>a</sup> | Frequency<sup>b</sup> |
|------------------------------|---------|--------------------|-----------------------|---------------------|
|                              |         | n | Mean<sup>c</sup> (SD) | Median (IQR) | p value | n | Mean (SD) | Median (IQR) | p value | n (%) | p value |
| Aggregatibacter actinomycetemcomitans | Spain  | 30 | 0 | 0 | 0.273 | 30 | 0 | 0 | 0.273 | 0 (0.0) | 0.455 |
|                               | Albania | 25 | 0.19 (0.96) | 0.00 (0.00) | 25 | 0.14 (0.74) | 0.00 (0.00) | 1 (40) | 0.455 |
| Porphyromonas gingivalis      | Spain  | 30 | 2.25 (2.39) | 1.55 (4.13) | 0.276 | 30 | 6.74 (14.35) | 0.14 (3.87) | 0.574 | 15 (50.0) | 0.178 |
|                               | Albania | 25 | 2.98 (2.36) | 3.77 (45.4) | 25 | 8.19 (18.49) | 0.12 (40.6) | 17 (68.0) | 0.178 |
| Prevotella intermedia         | Spain  | 30 | 1.95 (2.12) | 1.23 (3.79) | 0.943 | 30 | 2.80 (8.97) | 0.03 (0.57) | 0.760 | 15 (50.0) | 0.883 |
|                               | Albania | 25 | 1.99 (2.17) | 2.00 (3.87) | 25 | 5.68 (18.31) | 0.00 (0.31) | 13 (52.0) | 0.883 |
| Tannerella forsythia          | Spain  | 30 | 0.72 (1.65) | 0.00 (0.00) | 0.906 | 30 | 0.48 (2.10) | 0.00 (0.00) | 0.563 | 5 (16.7) | 0.715 |
|                               | Albania | 25 | 0.68 (1.61) | 0.00 (0.00) | 25 | 0.07 (0.26) | 0.00 (0.00) | 3 (12.0) | 0.715 |
| Parvimonas micra             | Spain  | 30 | 0.45 (1.40) | 0.00 (0.00) | 0.511 | 30 | 1.44 (6.91) | 0.00 (0.00) | 0.393 | 3 (10.0) | 0.446 |
|                               | Albania | 25 | 0.74 (1.74) | 0.00 (0.00) | 25 | 0.30 (0.46) | 0.00 (0.00) | 5 (20.0) | 0.446 |
| Fusobacterium nucleatum      | Spain  | 30 | 3.76 (4.14) | 41.1 (100) | 0.584 | 30 | 4.55 (5.57) | 2.60 (5.22) | 0.022 | 27 (90.0) | 1.000 |
|                               | Albania | 25 | 3.53 (1.51) | 43.0 (105) | 25 | 1.65 (5.00) | 1.09 (1.70) | 23 (92.0) | 1.000 |
| Campylobacter rectus         | Spain  | 30 | 0.38 (1.17) | 0.00 (0.00) | 0.107 | 30 | 0.13 (0.52) | 0.00 (0.00) | 0.107 | 3 (10.0) | 0.242 |
|                               | Albania | 25 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 (0.0) | 0.07 |
| Eikenella corrodens          | Spain  | 30 | 0.67 (1.39) | 0.00 (0.00) | 0.753 | 30 | 0.05 (0.14) | 0.00 (0.00) | 0.821 | 6 (20.0) | 0.741 |
|                               | Albania | 25 | 0.55 (1.31) | 0.00 (0.00) | 25 | 0.07 (0.17) | 0.00 (0.00) | 4 (16.0) | 0.741 |
| Capnocytophaga spp.          | Spain  | 30 | 0.76 (1.56) | 0.00 (0.00) | 0.215 | 30 | 0.35 (1.06) | 0.00 (0.00) | 0.164 | 6 (20.0) | 0.269 |
|                               | Albania | 25 | 0.32 (1.10) | 0.00 (0.00) | 25 | 0.03 (0.11) | 0.00 (0.00) | 2 (8.0) | 0.269 |
| Actinomyces odontolyticus    | Spain  | 30 | 0 | 0 | 0.118 | 30 | 0 | 0 | 0.118 | 0 (0.0) | 0.202 |
|                               | Albania | 25 | 0.37 (1.30) | 0.00 (0.00) | 25 | 0.63 (2.19) | 0.00 (0.00) | 2 (8.0) | 0.202 |

<sup>a</sup> Mann–Whitney test  
<sup>b</sup> Chi-square test  
<sup>c</sup> Mean of log-transformed counts  

n, sample size; SD, standard deviation; IQR, interquartile range; n (%), number and percentage of positive samples
Table 6  Counts, proportions and frequencies of detection of target species by country, in stages I–II periodontitis

| Periodontitis stages I–II | Country       | Counts<sup>a</sup> | Proportions<sup>a</sup> | Frequency<sup>b</sup> |
|---------------------------|---------------|--------------------|------------------------|----------------------|
|                           |               | n, sample size; SD, standard deviation; IQR, interquartile range; n (%), number and percentage of positive samples |                       |                       |
|                           |               | n | Mean<sup>c</sup> (SD) | Median (IQR) | p value | n | Mean (SD) | Median (IQR) | p value | n (%) | p value |
| **Aggregatibacter actinomycetemcomitans** | Spain | 30 | 0 | 0 | 1.000 | 30 | 0 | 0 | 1.000 | 0 (0.0) | 1.000 |
|                           | Albania | 28 | 0 | 0 |            | 28 | 0 | 0 |            | 0 (0.0) |            |
| **Porphyromonas gingivalis** | Spain | 30 | 5.17 (1.31) | 5.22 (1.02) | 0.431 | 30 | 15.96 (19.24) | 7.53 (18.83) | 0.171 | 29 (96.7) | 0.097 |
|                           | Albania | 28 | 4.42 (2.28) | 5.03 (2.03) |            | 28 | 12.75 (17.34) | 2.02 (21.52) |            | 23 (82.1) |            |
| **Prevotella intermedia** | Spain | 30 | 4.03 (1.61) | 4.32 (1.70) | 0.532 | 30 | 1.98 (3.70) | 0.83 (1.49) | 0.190 | 27 (90.0) | 0.071 |
|                           | Albania | 28 | 3.30 (2.30) | 4.00 (5.36) |            | 28 | 1.91 (4.40) | 0.26 (1.52) |            | 20 (71.4) |            |
| **Tannerella forsythia** | Spain | 30 | 1.58 (2.28) | 0.00 (4.38) | 0.748 | 30 | 0.31 (0.54) | 0.00 (0.67) | 0.826 | 10 (33.3) | 0.849 |
|                           | Albania | 28 | 1.73 (2.38) | 0.00 (4.78) |            | 28 | 0.32 (0.46) | 0.00 (0.68) |            | 10 (35.7) |            |
| **Parvimonas micra** | Spain | 30 | 0.17 (0.93) | 0.00 (0.00) | 0.334 | 30 | 0.48 (2.64) | 0.00 (0.00) | 0.334 | 1 (3.3) | 1.000 |
|                           | Albania | 28 | 0 | 0 |          | 28 | 0 | 0 |          | 0 (0.0) |            |
| **Fusobacterium nucleatum** | Spain | 30 | 4.16 (1.96) | 4.84 (1.16) | 0.233 | 30 | 2.93 (3.35) | 2.16 (3.61) | 0.034 | 25 (83.3) | 0.425 |
|                           | Albania | 28 | 4.17 (1.35) | 4.65 (1.43) |            | 28 | 1.17 (1.46) | 0.53 (1.57) |            | 26 (92.9) |            |
| **Campylobacter rectus** | Spain | 30 | 0.50 (1.55) | 0.00 (0.00) | 0.632 | 30 | 0.94 (4.54) | 0.00 (0.00) | 0.655 | 3 (10.0) | 1.000 |
|                           | Albania | 28 | 0.27 (1.01) | 0.00 (0.00) |            | 28 | 0.00 (0.03) | 0.00 (0.00) |            | 2 (7.1) |            |
| **Eikenella corrodens** | Spain | 30 | 0.99 (1.85) | 0.00 (0.83) | 0.429 | 30 | 0.20 (0.55) | 0.00 (0.00) | 0.508 | 7 (23.3) | 0.453 |
|                           | Albania | 28 | 1.41 (2.12) | 0.00 (3.83) |            | 28 | 0.21 (0.51) | 0.00 (0.29) |            | 9 (32.1) |            |
| **Capnocytophaga spp.** | Spain | 30 | 0.75 (1.74) | 0.00 (0.00) | 0.090 | 30 | 0.12 (0.36) | 0.00 (0.00) | 0.102 | 5 (16.7) | 0.196 |
|                           | Albania | 28 | 0.12 (0.65) | 0.00 (0.00) |            | 28 | 0.02 (0.10) | 0.00 (0.00) |            | 1 (3.6) |            |
| **Actinomyces odontolyticus** | Spain | 30 | 0.12 (0.67) | 0.00 (0.00) | 0.334 | 30 | 0.00 (0.00) | 0.00 (0.00) | 0.334 | 1 (3.3) | 1.000 |
|                           | Albania | 28 | 0 | 0 |            | 28 | 0 | 0 |            | 0 (0.0) |            |

<sup>a</sup> Mann–Whitney test  
<sup>b</sup> Chi-square test  
<sup>c</sup> Mean of log-transformed counts
### Table 7  Counts, proportions and frequencies of detection of target species by country, in stages III–IV periodontitis

| Country                  | Countsa | Proportionsa | Frequencyb |
|--------------------------|---------|--------------|------------|
|                          | n       | Mean (SD)    | Median (IQR)  | p value       | n Mean (SD)    | Median (IQR)  | p value       | n (%) | p value |
| Aggregatibacter actinomycemcomitans |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 0            | 0          | 0.078        | 30            | 0            | 0.078        | 0      | 0.237   |
| Albania                  | 30      | 0.46 (1.42)  | 0.00 (0.00) |            | 30            | 0.00 (0.01)  | 0.00 (0.00)  | 3      | 0.100   |
| Porphyromonas gingivalis |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 5.28 (1.93)  | 5.74 (1.39) | 0.559        | 30            | 18.86 (20.39)| 11.04 (31.23)| 0.173  | 0.472   |
| Albania                  | 30      | 4.68 (2.50)  | 5.50 (2.10) |            | 30            | 15.18 (23.08)| 5.04 (16.55) | 24     | 0.800   |
| Prevotella intermedia    |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 4.50 (1.97)  | 5.00 (1.67) | 0.285        | 30            | 6.79 (12.08)| 2.02 (5.57)  | 0.038  | 0.317   |
| Albania                  | 30      | 3.84 (2.31)  | 4.84 (3.80) |            | 30            | 2.79 (7.48)  | 0.43 (2.30)  | 23     | 0.767   |
| Tannerella forsythia     |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 2.11 (2.49)  | 0.00 (4.68) | 0.099        | 30            | 0.65 (1.54)  | 0.00 (0.067)| 0.096  | 0.052   |
| Albania                  | 30      | 1.05 (2.16)  | 0.00 (0.00) |            | 30            | 0.51 (1.58)  | 0.00 (0.00)  | 6      | 0.200   |
| Parvimonas micra         |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 0.32 (1.24)  | 0.00 (0.00) | 0.402        | 30            | 0.08 (0.37)  | 0.00 (0.00)  | 0.379  | 0.671   |
| Albania                  | 30      | 0.66 (1.73)  | 0.00 (0.00) |            | 30            | 0.33 (1.10)  | 0.00 (0.00)  | 4      | 0.133   |
| Fusobacterium nucleatum  |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 4.29 (1.74)  | 4.95 (1.33) | 0.593        | 30            | 2.19 (2.52)  | 1.24 (2.69)  | 0.329  | 0.100   |
| Albania                  | 30      | 4.11 (1.84)  | 4.84 (1.44) |            | 30            | 2.38 (4.18)  | 0.84 (2.82)  | 26     | 0.867   |
| Campylobacter rectus     |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 0.64 (1.67)  | 0.00 (0.00) | 0.175        | 30            | 0.21 (0.60)  | 0.00 (0.00)  | 0.147  | 0.353   |
| Albania                  | 30      | 0.16 (0.91)  | 0.00 (0.00) |            | 30            | 0.02 (0.14)  | 0.00 (0.00)  | 1      | 0.333   |
| Eikenella corrodens      |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 1.07 (2.00)  | 0.00 (1.00) | 0.065        | 30            | 0.29 (1.02)  | 0.00 (0.02)  | 0.092  | 0.058   |
| Albania                  | 30      | 2.16 (2.41)  | 0.00 (0.00) |            | 30            | 2.65 (2.75)  | 0.00 (0.36)  | 16     | 0.767   |
| Capnocytophaga spp.      |         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 1.38 (2.18)  | 0.00 (4.00) | 0.408        | 30            | 0.36 (1.00)  | 0.00 (0.28)  | 0.398  | 0.371   |
| Albania                  | 30      | 0.94 (1.93)  | 0.00 (0.00) |            | 30            | 0.27 (0.93)  | 0.00 (0.00)  | 6      | 0.200   |
| Actinomyces odontolyticus|         |              |            |              |              |              |              |       |         |
| Spain                    | 30      | 0            | 0          | 1.000        | 30            | 0            | 0          | 1.000  | 0.000   |
| Albania                  | 30      | 0            | 0          | 0            | 30            | 0            | 0          | 0      | 0.000   |

\(n\), sample size; SD, standard deviation; IQR, interquartile range; \(n\ (%), number and percentage of positive samples

\(a\) Mann–Whitney test

\(b\) Chi-square test

\(c\) Mean of log-transformed counts
bacteria due to the differences in periodontal status. Although the Spanish patients showed deeper mean PDs and lower PII levels in stages III–IV periodontitis, and Albanian patients presented a lower PI in the periodontal health/gingivitis group, these differences may be anecdotal and with a clear lack of clinical significance, except in stages III–IV periodontitis.

In the present study, when evaluating the microbiological profile of the Albanian population, the bacterial species with the highest frequency of detection in periodontitis were \( P. \) gingivalis, \( P. \) intermedia, \( F. \) nucleatum and \( E. \) corrodens. There is no available information from Albania to compare with the findings. If they are compared with findings from other geographical locations (Spain, Morocco, Colombia, Chile), \( P. \) gingivalis, \( P. \) intermedia and \( F. \) nucleatum are consistently three of the most frequently detected bacterial species in subgingival samples in patients with periodontitis by means of culture techniques [8, 11, 13, 14]. \( E. \) corrodens has also shown higher frequencies in periodontitis than in periodontally healthy subjects [26]. However, in the Albanian population studied, only \( P. \) intermedia and \( E. \) corrodens showed a statistically significantly higher prevalence in periodontitis than in subjects without periodontitis, which may suggest that \( P. \) gingivalis and \( F. \) nucleatum are also frequently present in subjects without periodontitis in Albania.

When the Albanian subjects were compared to a similar Spanish population, two main differences were identified, namely total anaerobic counts and the proportions of specific target species. For the differences in total anaerobic counts, statistically significant differences were detected in the whole sample, which corresponds to higher total counts in Albanian patients than in Spanish patients in the group of subjects with periodontal health or gingivitis. This finding is consistent with a previous study observing microbiological differences in subjects according to race/ethnicity, family income or education, as well as smoking, diet and health habits, or access to dental care [27]. While smoking is not a differentiating variable in the present study, socio-economic and/or socio-demographic differences might have influenced the results.

For differences in the proportions of specific target species, significantly lower proportions of \( F. \) nucleatum, in periodontal health/gingivitis and in stages I–II periodontitis, were detected in Albanian samples. This finding is in line with a study showing that subjects with low socio-economic status and low levels of oral diseases (caries and/or periodontitis) have lower amounts of certain members of the \( Fusobacterium \) genus [28]. It is unclear whether these differences may reflect that dysbiotic biofilms in Albanian patients were not clearly associated with specific pathogens, while the corresponding dysbiotic biofilms in Spanish subjects were associated with specific pathogens. Previous studies with populations in Spain have highlighted the possible relevant role of \( P. \) gingivalis [8, 13, 14], what may support the importance of

| Table 8 | Comparison of total anaerobic counts and proportions of selected species by adjusting for confounding factors |
|---------|--------------------------------------------------|
|         | Country | n  | Mean* (SD) | p value^ | Adjusted mean† (95% CI) | Adjusted p value^ |
| Total anaerobic counts | Health/gingivitis | Spain | 30 | 5.77 (0.60) | 0.001 | 5.62 (5.42–5.82) | 0.000 |
| | | Albania | 25 | 6.29 (0.43) | | 6.46 (6.24–6.69) |
| Proportions | | | | | | |
| | F. nucleatum | Health/gingivitis | Spain | 30 | 4.55 (5.57) | 0.022 | 0.40 (0.20–0.60) | 0.040 |
| | | | Albania | 25 | 1.65 (2.00) | | 0.05 (-0.17–0.27) |
| | | Periodontitis I–II | Spain | 30 | 2.93 (3.35) | 0.034 | 0.25 (0.05–0.46) | 0.004 |
| | | | Albania | 28 | 1.17 (1.46) | | -0.18 (-0.39–0.02) |
| | P. intermedia | Periodontitis III–IV | Spain | 30 | 6.79 (12.08) | 0.038 | 0.34 (0.44–0.63) | 0.078 |
| | | | Albania | 30 | 2.79 (7.48) | | -0.06 (-0.03–0.22) |

* Mean of log-transformed counts  
^ Student t test  
† Adjusted mean of log-transformed counts  
^ ANCOVA  
* Mann–Whitney test  
^ Adjusted mean of log-transformed proportions; ANCOVA model: (a) plaque index and probing depth were entered in the model as co-variates; and (b) to achieve the homogeneity of variances, the proportions of \( F. \) nucleatum and \( P. \) intermedia were log-transformed.
### Table 9  Counts, proportions and frequencies of detection of target bacterial species by periodontal status

| Country                        | Health/gingivitis | Periodontitis I–II | Periodontitis III–IV | Periodontitis II–IV | Periodontitis III–IV |
|--------------------------------|-------------------|-------------------|---------------------|--------------------|----------------------|
| **Counts**                     | **Counts**        | **Counts**        | **Counts**          | **Counts**         | **Counts**           |
| n                              | Meanc (SD)        | Median (IQR)      | p value             | n                  | Meanc (SD)          | Median (IQR) | p value |
| Aggregatibacter actinomycetemcomitans | 55 0.08 (0.65) | 0.00 (0.00)       | 0.191               | 55 0.06 (0.50) | 0.00 (0.00)         | 0.194         | 1 (1.8) 0.243 |
| Porphyromonas gingivalis       | Health/gingivitis | 55 2.58 (2.38) | 3.47 (4.34) | 0.000*             | 55 7.40 (16.02) | 0.12 (3.51) | 0.000* 32 (58.2) 0.000* |
|                                | Periodontitis I–II | 58 0.23 (1.02) | 0.00 (0.00) | 60 0.00 (0.00) | 0.00 (0.00) | 3 (5.0) |
|                                | Periodontitis III–IV | 60 0.23 (1.02) | 0.00 (0.00) | 60 0.00 (0.00) | 0.00 (0.00) | 3 (5.0) |
| Prevotella intermedia          | Health/gingivitis | 55 3.68 (1.99) | 4.30 (1.75) | 0.220†             | 58 1.95 (4.02) | 0.63 (1.34) | 0.744‡ 47 (81.0) 1.000‡ |
|                                | Periodontitis I–II | 58 4.17 (2.16) | 4.92 (1.57) | 60 4.79 (10.17) | 0.87 (3.11) | 0.000‡ 49 (81.7) 0.000‡ |
|                                | Periodontitis III–IV | 60 4.17 (2.16) | 4.92 (1.57) | 60 4.79 (10.17) | 0.87 (3.11) | 0.000‡ 49 (81.7) 0.000‡ |
| Porvimonas micra               | Health/gingivitis | 55 0.70 (1.62) | 0.00 (0.00) | 55 0.29 (1.56) | 0.00 (0.00) | 0.048* 8 (14.5) 0.042* |
|                                | Periodontitis I–II | 58 1.65 (2.31) | 0.00 (4.63) | 58 0.31 (0.50) | 0.00 (0.62) | 1.000† 20 (34.5) 1.000† |
|                                | Periodontitis III–IV | 60 1.58 (2.37) | 0.00 (4.48) | 60 0.58 (1.50) | 0.00 (0.51) | 0.099‡ 19 (31.7) 0.000‡ |
| Fusobacterium nucleatum        | Health/gingivitis | 55 3.80 (1.45) | 4.30 (1.00) | 0.012*             | 55 3.23 (4.53) | 1.74 (3.38) | 0.241 50 (90.9) 0.666 |
|                                | Periodontitis I–II | 58 4.16 (1.68) | 4.69 (1.09) | 58 2.08 (2.74) | 1.12 (3.05) | 51 (87.9) |
|                                | Periodontitis III–IV | 60 4.20 (1.78) | 4.90 (1.30) | 60 2.28 (3.43) | 1.08 (2.59) | 53 (88.3) |
| Campylobacter rectus           | Health/gingivitis | 55 0.20 (0.88) | 0.00 (0.00) | 55 0.07 (0.88) | 0.00 (0.00) | 0.787 3 (5.5) 0.566 |
|                                | Periodontitis I–II | 58 0.39 (1.31) | 0.00 (0.00) | 58 0.49 (3.27) | 0.00 (0.00) | 5 (8.3) |
|                                | Periodontitis III–IV | 60 4.40 (1.35) | 0.00 (0.00) | 60 0.12 (0.44) | 0.00 (0.00) | 5 (8.3) |
| Eikenella corrodens            | Health/gingivitis | 55 0.62 (1.34) | 0.00 (2.25) | 0.386*             | 55 0.06 (0.15) | 0.00 (0.00) | 0.089 10 (18.2) 0.128 |
|                                | Periodontitis I–II | 58 1.19 (1.98) | 0.00 (3.38) | 58 0.20 (0.53) | 0.00 (0.02) | 16 (27.6) |
|                                | Periodontitis III–IV | 60 1.61 (2.26) | 0.00 (4.00) | 60 0.47 (9.04) | 0.00 (2.2) | 21 (35.0) |
| Capnocytophaga spp.            | Health/gingivitis | 55 0.56 (1.38) | 0.00 (0.00) | 55 0.20 (0.80) | 0.00 (0.00) | 0.097 8 (14.5) 0.125 |
|                                | Periodontitis I–II | 58 0.45 (1.33) | 0.00 (2.70) | 58 0.07 (0.27) | 0.00 (0.00) | 6 (10.3) |
|                                | Periodontitis III–IV | 60 1.16 (2.05) | 0.00 (2.70) | 60 0.31 (0.96) | 0.00 (0.09) | 15 (25.0) |
| Actinomyces odontolyticus      | Health/gingivitis | 55 0.17 (0.88) | 0.00 (0.00) | 55 0.28 (1.49) | 0.00 (0.00) | 0.326 2 (3.6) 0.137 |
|                                | Periodontitis I–II | 58 0.06 (0.48) | 0.00 (0.00) | 58 0.00 (0.00) | 0.00 (0.00) | 1 (1.7) |
|                                | Periodontitis III–IV | 60 0.00 (0.00) | 0.00 (0.00) | 60 0.00 (0.00) | 0.00 (0.00) | 0 (0.0) |

n, sample size; SD, standard deviation; IQR, interquartile range; n (%), number and percentage of positive samples

* Kruskal–Wallis test

† Chi-square with Bonferroni correction

‡ Mean of log-transformed counts

§ Periodontal health/gingivitis group versus periodontitis I–II group

® Periodontitis I–II group versus periodontitis III–IV group

‖ Periodontal health/gingivitis group versus periodontitis III–IV group
this pathogen as a key-stone pathogen responsible of the 
bacterial dysbiosis concept [29].

Although not statistically significant, other microbiolog-
ical differences also showed clear tendencies in terms of 
frequencies of detection, depicting higher prevalence of 
A. actinomycetemcomitans and E. corrodens in Alba-
nia, and higher prevalence of P. gingivalis, P. intermedia 
and T. forsythia in Spain. These findings support previ-
ous reports comparing the subgingival cultivable bacte-
ria of Colombian and Spanish patients [14], that suggests 
that dysbiotic biofilms could be associated with larger 
amounts of microorganisms in Albanian subjects, while 
in Spain the impact of key pathogens may be more re-
levant. In addition, the role of A. actinomycetemcomitans 
in promoting dysbiosis of in a limited number of Alba-
nian patients should also be considered, which is consist-
ent with the results of different studies on Eastern Europe 
populations [30–32], as compared with lower levels in 
Spain [8, 12–14].

When evaluating the microbiological findings within 
the different periodontal categories, differences were 
observed in terms of total anaerobic counts, and in 
counts, proportions and frequencies of detection of tar-
get bacterial species, including the most relevant perio-
dontal pathogens, P. gingivalis, T. forsythia, P. intermedia 
and F. nucleatum, what is in agreement with previous 
studies using other classification systems [6, 8, 31] or 
with studies using the same 2018 classification [14, 32]. 
Whether these differences are causal or secondary to dif-
fences in PDs cannot be explored in a cross-sectional 
study [33].

The present study used culture techniques for the 
identification of the cultivable bacteria associated with 
periodontitis. While Next Generation Sequencing (NGS) 
approaches are currently frequently used, an initial char-
acterization of a previously not tested population (as 
Albanian subjects) may benefit from a simpler approach. 
However, the value of culture techniques should not be 
underestimated, alone or in combination with other 
approaches, since it has been considered that it is impor-
tant to have parallel culture libraries [34, 35], that ben-
efits from the improvement of microbiological culturing, 
e.g. with the introduction of more competent anaerobic 
handling and incubation procedures, so culture is rein-
vented every day [36]. Thus, many other researchers 
still believe that cultivation continues to be an interest-
ing alternative for microbiological testing [37] and its use 
allows for appropriate comparisons with previous studies 
that have also used culture techniques.

The results of the present study should be interpreted 
with caution due to the clear limitations of the micro-
biological methodology used, e.g. the microbiological 
samples from Albania were sent by courier to Spain, and 
although the same standardised approach to sampling 
was followed in both centres [38], and the time inter-
val between sampling and plating was the same for both 
countries (24–36 h), it cannot be discarded that the ideal 
transport conditions might not have been maintained for 
some samples, which could have impacted on the viabili-
ty of some microorganisms [24]. Another limitation is 
associated with the sampling strategy, since only the four 
deepest sites were sampled in each patient, which may 
underestimate detection frequencies [39]; however, this 
strategy was validated in the early nineties [40, 41] and 
it has been extensively used in periodontal microbiol-
ogy. Moreover, the relatively small sample size without 
providing information about other possible sources of 
bias (as differences in socio-economic status) may have 
limited the opportunity to find significant differences. 
Finally, culture techniques are not able to provide a thor-
ough research of the subgingival microbiota, thus further 
in-depth analysis, e.g. using NGS approaches, would be 
necessary to have a more comprehensive picture of the 
whole microbiota of the Albanian population, including 
non-culturable bacterial species.

Conclusions
Within the limitations of the present study, it can be con-
cluded that the microbiological profile of the subgingival 
cultivable bacteria in periodontitis and non-periodontitis 
patients has demonstrated statistically significant dif-
f erences between Albanian and Spanish patients, with 
higher total anaerobic counts in Albania and higher pro-
portions of cultivable bacteria of F. nucleatum in Spain.

Abbreviations
UCM: University Complutense of Madrid; PD: Probing depth; CAL: Clinical 
attachment loss; BoP: Bleeding on probing; PlI: Plaque index; RBL: Radiogra-
phical bone loss; RTF: Reduced transport fluid; PBS: Phosphate buffer saline; SD: 
Standard deviation; IQR: Interquartile range; CFU: Colony-forming units; Cl:
Confidence interval; n: Sample size; n (%): Number and percentage of positive 
samples.

Supplementary Information
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Additional file 1. Presumptive identification of bacterial species in 
culture.

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Availability of data and materials
The datasets generated and analyzed during the current study are not publicly available due to data protection regulations and ethical concerns but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The authors declare that the study has been approved by the Clinical Research Ethical Committee of Hospital Clinico San Carlos in Madrid, Spain (reference number 18/128-E) and by the Ethics Committee of Albanian University (reference number 197) and considers all aspects of the Helsinki Declaration regarding experimentation involving human. Informed consents were obtained from all included participants.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1. ETEP (Etiology and Therapy of Periodontal and Peri-Implant Diseases) Research Group, School of Dentistry, University Complutense of Madrid (UCM), Madrid, Spain. 2. Dental Clinic, School of Dentistry, Albanian University, Tirana, Albania.

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