

Randomized, blind clinical controlled trial to evaluate the primary cause of periodontal disease, the predominant percentage of bacteria, and the total bacterial load before and after non-surgical therapy

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ABSTRACT

Objectives: To evaluate the main cause of periodontal disease, bacterial predominance, and total bacterial load before and after. **Materials and methods:** Microbiological testing: consists of taking the crevicular fluid colonized by bacteria and containing epithelial cells of the individual under study by sterile paper cone of diameter 60/80 in the periodontal or peri-implant pocket for at least 30 seconds (so that it is soaked in crevicular fluid possibly free of blood) is placed back inside the tube; the procedure is repeated to have a minimum of two to a maximum of four paper cones. The test was performed on 420 patients ages 30-60 years 236 women 184 men pockets from 4-11mm chronic moderate-severe periodontitis for a total sampling of 498 in 78 patients a second sampling was performed after treatment. **Results:** The study pointed out that the main cause of periodontal disease are parafunctions (Bruxism, clenching, spoiled habits, atypical swallowing, oral breathing), dental misalignment, precontacts and incongruous prosthetic artifacts. Thus as "direct and triggering" local etiologic factors and no longer "indirect and predisposing" as has always been claimed. Secondarily by bacteria.

Keywords Periodontal disease, inflammation, plaque, tartar, bacteria, microbiological analysis, parafunctions.

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INTRODUCTION

Periodontal disease

The term periodontal disease (periodontitis) refers to a group of inflammatory, infectious pathologies clinically characterized by the pathological involvement of all tissue

components of the periodontium (gingiva, periodontal ligament, alveolar bone, and root cementum) (1-3). "Direct and triggering" local etiological factors: Bacterial (dental plaque, calculus, materia alba, food debris). "Indirect and aggravating" local factors: Functional (occlusal trauma, parafunctions, oral habits, mouth breathing, atypical swallowing, hypofunction) (Fig. 1-4). "Indirect and predis-

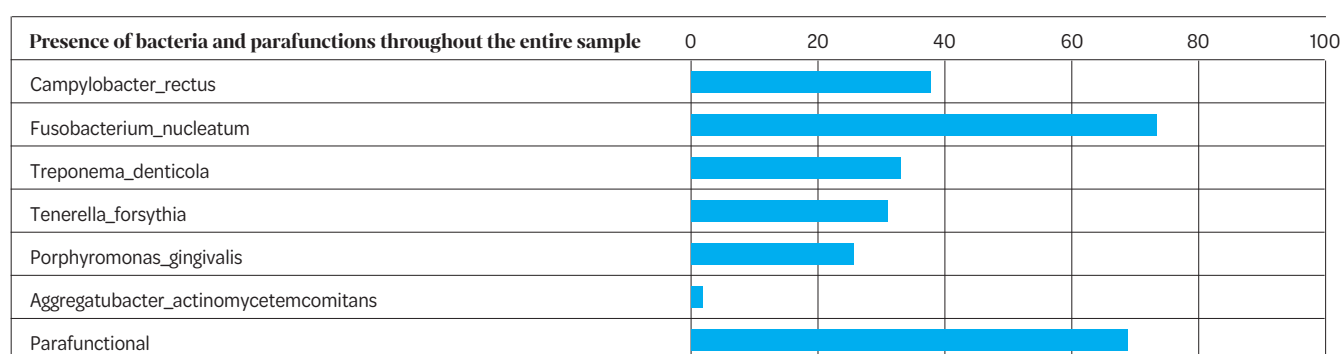


Fig. 1

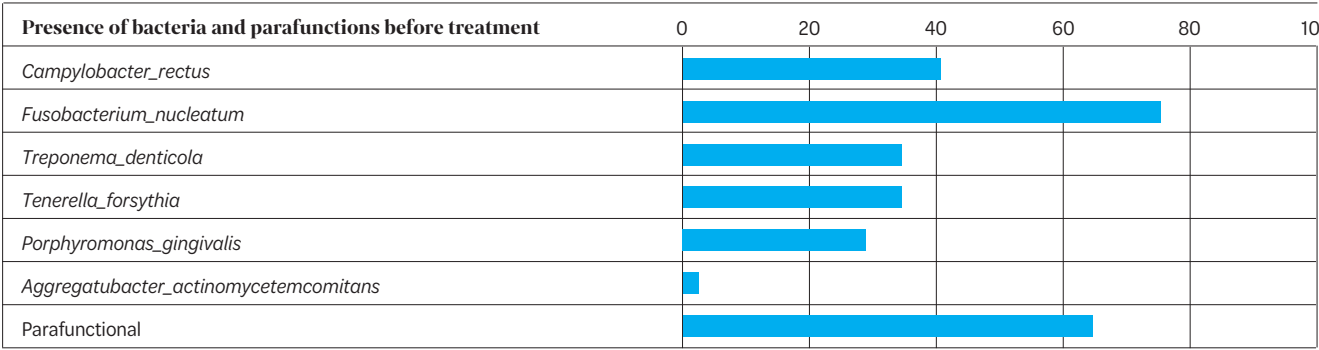


Fig. 2

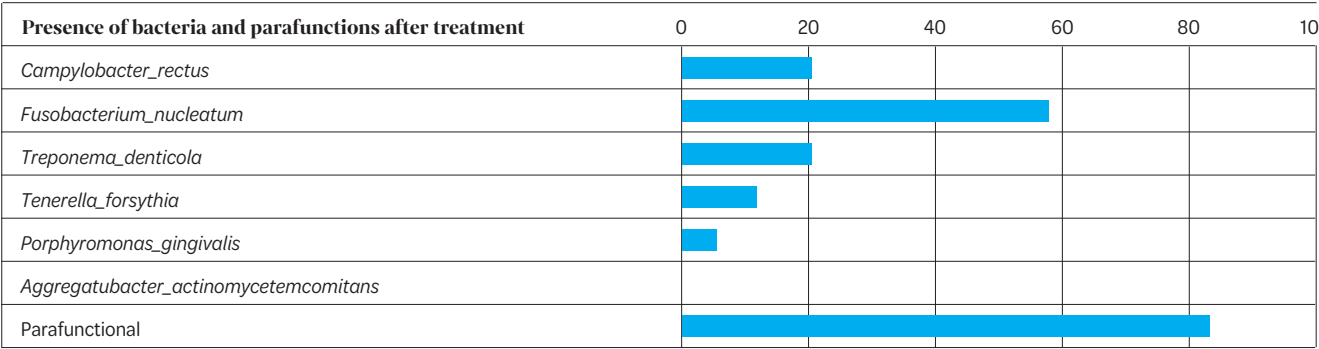


Fig. 3

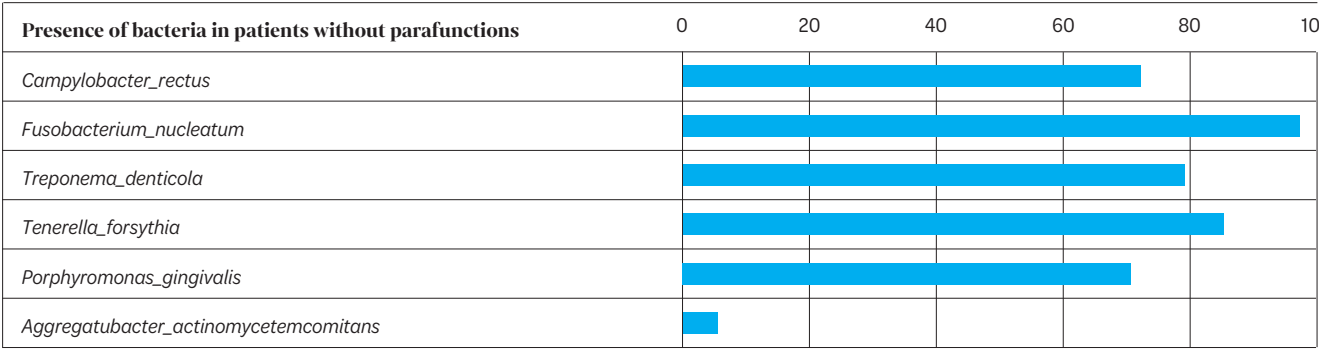


Fig. 4

posing" factors: Mechanical (improper oral hygiene, food impaction). Anatomical (tooth malposition, tooth shape, shape of periodontal tissues). "Indirect, predisposing, and aggravating" factors: Iatrogenic (overhanging restorations, defective prosthetic margins, oversized prosthetic crowns, and orthodontic appliances) (4-5). The onset and progression of periodontal disease are commonly attributed to pathogenic bacteria of the oral microbiota, mainly belonging to the red/orange complexes:

- *Aggregatibacter actinomycetemcomitans*
- *Porphyromonas gingivalis*
- *Tannerella forsythia*
- *Treponema denticola*
- *Fusobacterium nucleatum*
- *Campylobacter rectus*

Other factors include oral habits, anatomical and iatrogenic factors, as well as genetic and hereditary factors. The aim of this study is to evaluate the primary cause of periodontal disease, the predominant percentage of bacteria, and the total bacterial load before and after non-surgical therapy (6-7).

MATERIALS AND METHODS

Microbiological testing: This consists of sampling crevicular fluid colonized by bacteria and containing epithelial cells of the examined subject using a sterile paper point (size 60/80) placed into the periodontal or peri-implant pocket for at least 30 seconds (so it becomes imbued with crevicular fluid, preferably blood-free). The point is

Fig. 5 Distribution of bacterial load before treatment

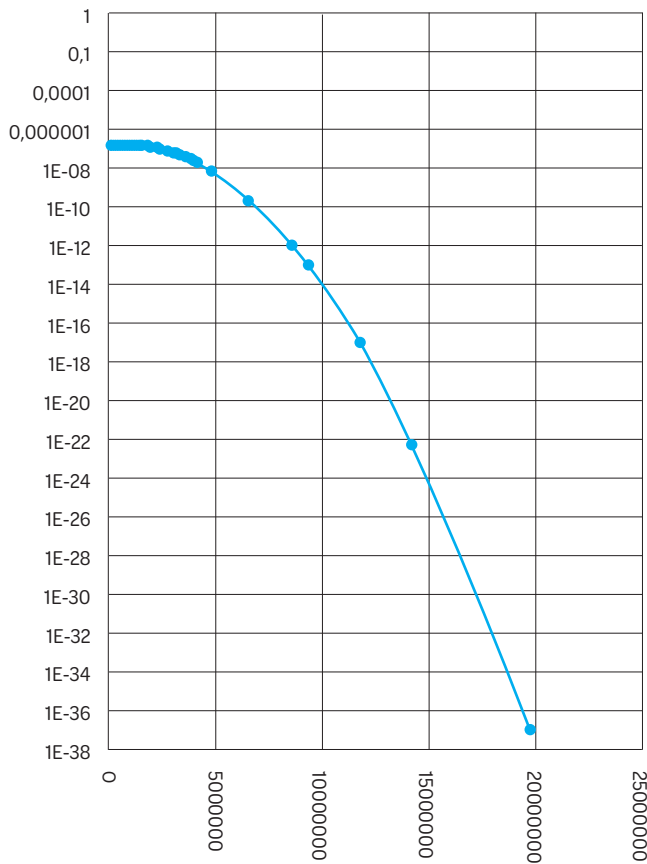
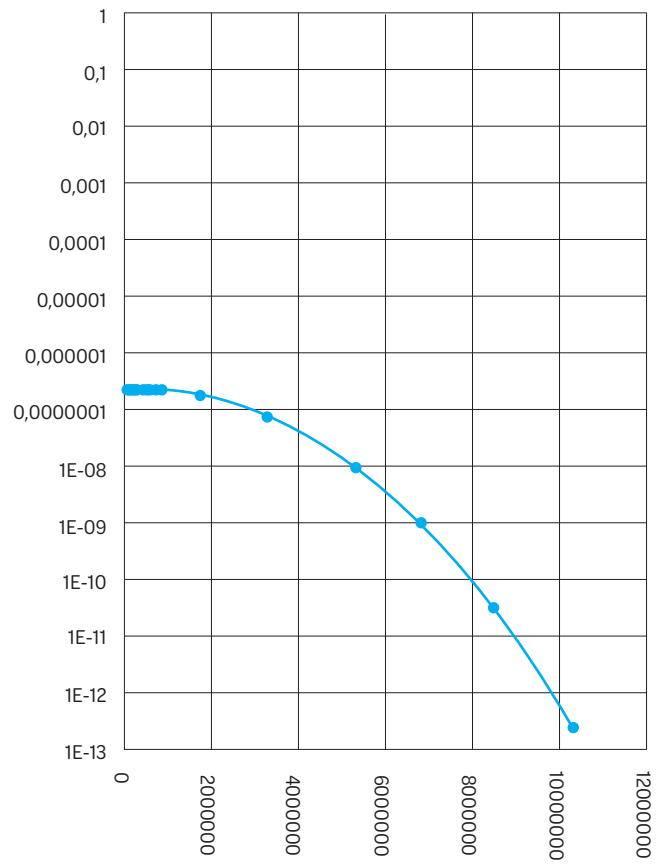


Fig. 6 Distribution of bacterial load after treatment

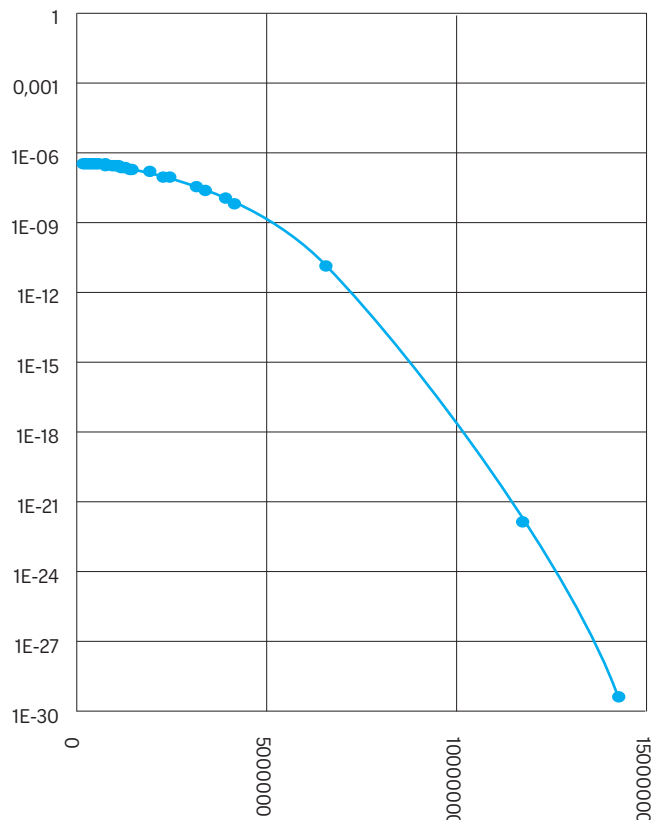


then placed inside a test tube; the procedure is repeated to obtain a minimum of two to a maximum of four paper points. The test was performed on 420 patients aged 30-60 years (236 females, 184 males) presenting with 4-11 mm pockets and generalized moderate to severe chronic periodontitis, for a total of 498 samples. In 78 patients, a second sampling was performed after treatment(6-9). The patients had not received antibiotics or periodontal treatment during the last 6 months, and had no systemic diseases. The methodology differs primarily in patient selection, focusing on specific characteristics (age, smokers of 10-20 cigarettes/day, moderate-severe periodontitis, 4-11 mm periodontal pockets) that are more representative of the general population. The following parameters were evaluated: Plaque Index (PI), Bleeding on Probing (BoP), Probing Pocket Depth (PPD), Mobility (M), and Gingival Recession (REC). All patients were provided with information regarding the study and signed an informed consent form (10-14).

Clinical Procedures

Microbiological testing was utilized for the study. The mesial area of the maxillary right first molar (1.6) and the mesial area of the mandibular left first molar (3.6) were selected. A group of 78 patients underwent a second sampling after treatment. (Scaling and root planing) (Fig. 5-7).

Fig. 7 Normal distribution of bacterial load in patients with parafunctional habits before treatment



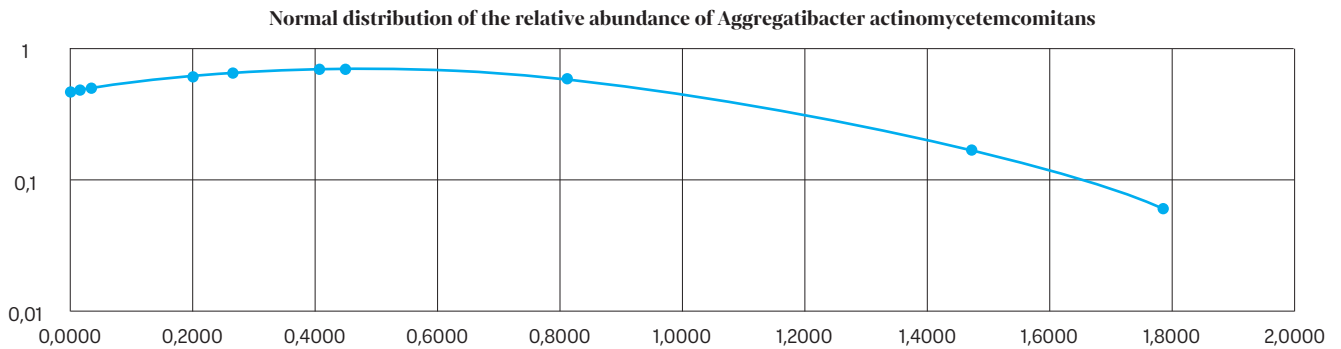


Fig. 8

Microbiological Analysis Results

The following sample was analyzed: 498 samples from a total of 420 patients. In 78 patients, a second sampling was performed after treatment, corresponding to 15.7% of the total samples and 18.6% of the patients. The bacterium *Aggregatibacter actinomycetemcomitans* is present in 2.2% of the samples (11 out of 420 cases) with values ranging from 0.0006% to 1.7875% of the total bacterial load, with a mean value of 0.4995% before treatment (Fig. 8).

The bacterium *Porphyromonas gingivalis* is present in 25.5% of the samples (127 out of 420 cases) with values ranging from 0.0003% to 48.5433%, with a mean value of 2.7917% of the total bacterial load. In patients measured

before treatment, the mean percentage value is 2.8689%, which drops to 0.4178% after treatment (Fig. 9).

The bacterium *Tannerella forsythia* is present in 31.3% of the samples (156 out of 420 cases) with values ranging from 0.0004% to 15.1197%, with a mean percentage value of 1.1961% of the total bacterial load. In patients measured before treatment, the mean percentage value is 1.2331%, and the mean value after treatment is 0.5912% (Fig. 10).

The bacterium *Treponema denticola* is present in 32.7% of the samples (163 out of 420 cases) with values ranging from 0.0010% to 52.4201%, with a mean percentage value of 1.5862% of the total bacterial load. In patients measured before treatment, the mean percentage value is 1.6668%, and the mean value after treatment is 0.846% (Fig. 11).

The bacterium *Fusobacterium nucleatum* is present in

Fig. 9 Normal distribution of the relative abundance of *Porphyromonas gingivalis*

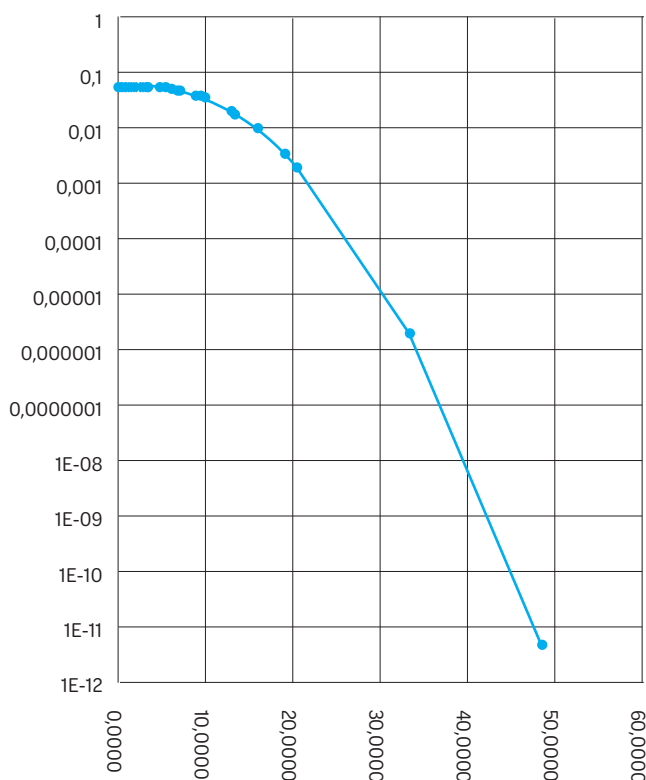


Fig. 10 Normal distribution of the relative abundance of *Tannerella forsythia*

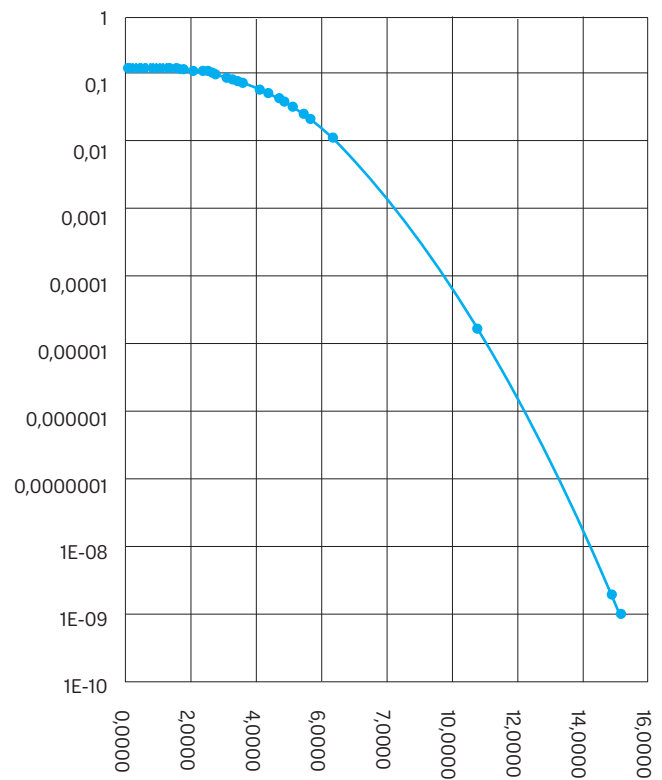


Fig. 11 Normal distribution of the relative abundance of *Treponema denticola*

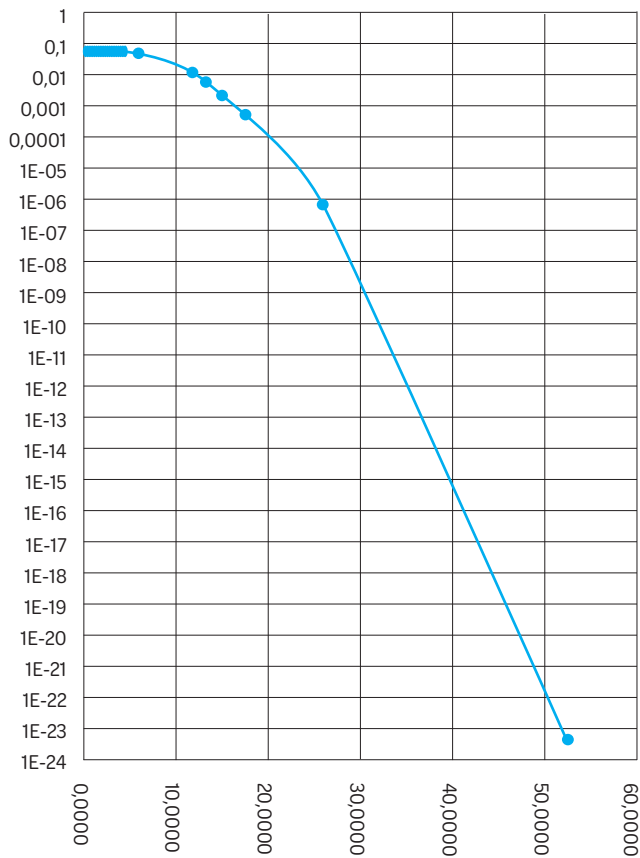


Fig. 12 Normal distribution of the relative abundance of *Fusobacterium nucleatum*

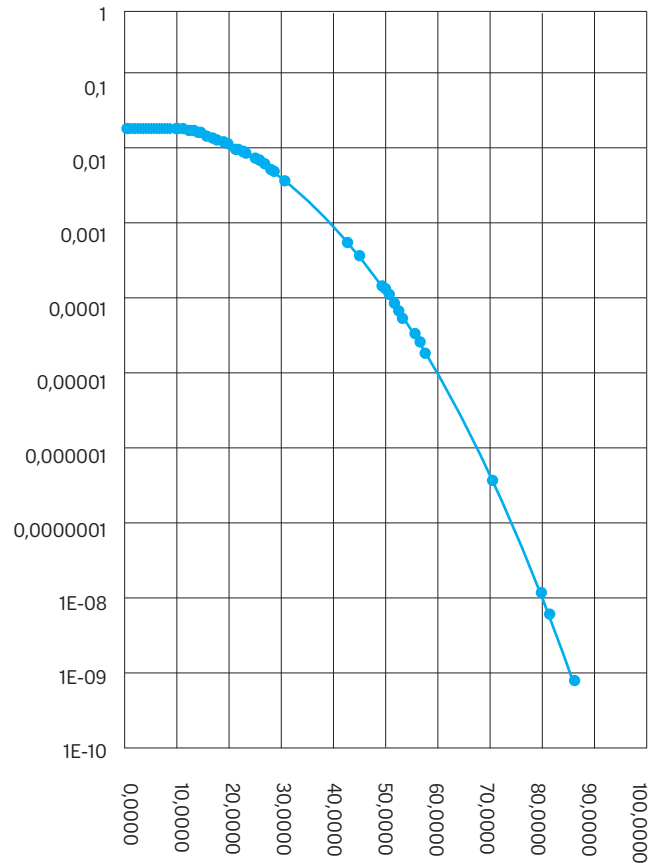


Fig. 13 Normal distribution of the relative abundance of *Campylobacter rectus*

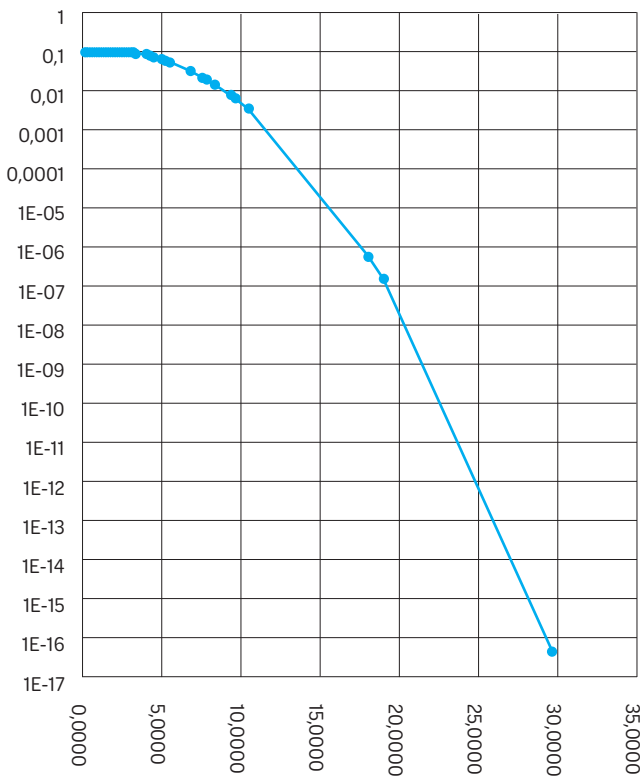
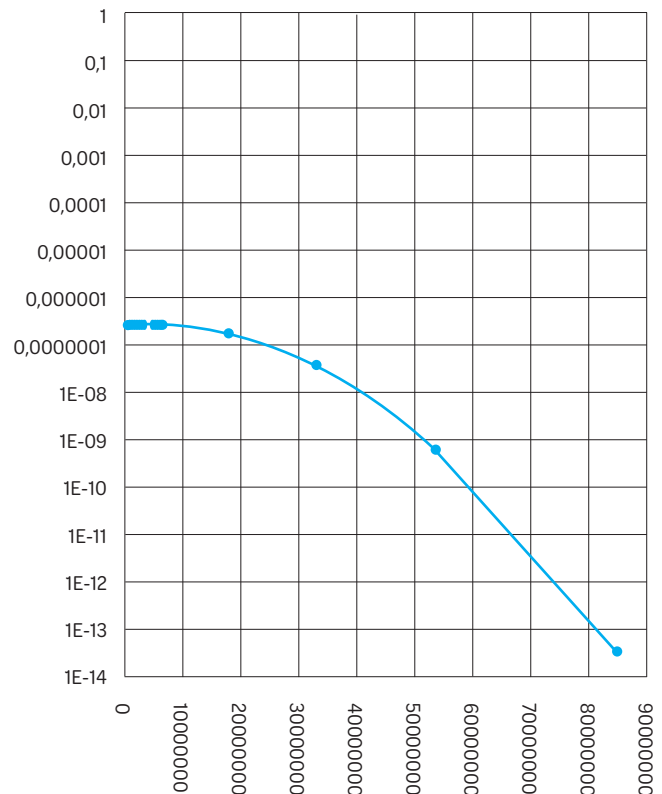


Fig. 14 Normal distribution of bacterial load in patients with parafunctions after treatment



Before or After Treatment	Patient ID	Treatment	Sex	Parafunctions	Aggregatibacter actinomycetemcomitans	Porphyromonas gingivalis	Tenerella forsythia	Treponema denticola	Fusobacterium nucleatum	Campylobacter rectus
After	1	Yes	M	Bruxism	0	0	0	0	1928	2662
Before	2	Yes	F	Premature Contact	0	0	0	1554	1412	0
Before	3	Yes	F	Clenching	0	0	0	1554	1412	0
-	4	Yes	F	Clenching	0	0	0	0	187	0
-	5	Yes	F	Premature Contact	0	0	0	2076	35883	1611
-	6	Yes	F	Clenching	0	0	0	0	2475	0
-	7	Yes	F	Premature Contact	0	0	0	0	0	564
-	8	Yes	M	Bruxism	0	0	0	0	613	580
-	9	Yes	F	Bruxism	0	0	0	0	103	0
-	10	Yes		Premature Contact	0	0	0	0	0	0
-	11	Yes	F	Clenching	0	0	71	0	28331	0
-	12	Yes	F	Bruxism	0	0	0	56	0	0
-	13	Yes	F	Clenching	0	0	0	0	0	0
-	14	Yes	M	Bruxism	0	0	0	0	4688	0
-	15	Yes	F	Premature Contact	0	0	0	0	716	493
Before	16	Yes	M	Premature Contact	0	151	0	0	1529	1009
-	17	Yes	M	Bruxism	0	0	0	0	1952	279
-	18	Yes	F	Premature Contact	0	0	0	0	5698	159
-	19	Yes	F	Bruxism	0	0	0	0	58	0
-	20	Yes	F	Bruxism	0	0	0	0	1949	0
-	21	Yes	F	Bruxism	0	0	0	92	0	0
-	22	Yes	F	Bruxism	0	0	0	0	1114	0
Before	23	Yes		Premature Contact	0	0	0	0	572	965
-	24	Yes	F	Premature Contact	0	85	237	0	281	568
-	25	Yes	M	Bruxism	0	0	0	0	0	0
-	26	Yes	F	Bruxism	0	0	0	0	8985	4526
-	27	Yes	M	Premature Contact	0	0	0	0	0	0
-	28	Yes	F	Clenching	0	0	0	0	154	0
-	29	Yes	F	Premature Contact	0	0	0	0	0	0
-	30	Yes	F	Clenching	0	0	0	0	0	0
Before	31	Yes	F	Clenching	0	0	0	0	0	0
Before	32	Yes	M	Clenching	0	0	0	0	2115	10660
-	33	Yes	M	Bruxism	0	0	0	0	1136	426
Before	34	Yes	F	Bruxism	0	0	0	0	0	0
-	35	Yes	F	Clenching	0	0	0	0	0	0
-	36	Yes	M	Clenching	0	0	0	0	1383	92
-	37	Yes	F	Clenching	0	0	0	0	4346	2505
Before	38	Yes	F	Premature Contact	0	0	0	0	1910	144
Before	39	Yes	F	Premature Contact	0	0	0	212	101371	15915
-	40	Yes	M	Premature Contact	0	0	0	0	0	0
-	41	Yes	M	Bruxism	0	0	0	0	0	0

Tab. 1A Presence of bacteria in patients with parafunctions

Total Bacterial Load	Aggregatibacter actinomycetemcomitans (Relative Abundance)	Porphyromonas gingivalis (Relative Abundance)	Tenerella forsythia (Relative Abundance)	Treponema denticola (Relative Abundance)	Fusobacterium nucleatum (Relative Abundance)	Campylobacter rectus (Relative Abundance)
3277397	0,0000	0,0000	0,0000	0,0000	0,0588	0,0812
95789	0,0000	0,0000	0,0000	1,6223	1,4741	0,0000
95789	0,0000	0,0000	0,0000	1,6223	1,4741	0,0000
72376	0,0000	0,0000	0,0000	0,0000	0,2584	0,0000
519970	0,0000	0,0000	0,0000	0,3993	6,9010	0,03098
174417	0,0000	0,0000	0,0000	0,0000	1,4190	0,0000
32061	0,0000	0,0000	0,0000	0,0000	0,0000	1,7591
274734	0,0000	0,0000	0,0000	0,0000	0,2231	0,2111
118676	0,0000	0,0000	0,0000	0,0000	0,0868	0,0000
2435	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
143417	0,0000	0,0000	0,0495	0,0000	19,7543	0,0000
120143	0,0000	0,0000	0,0000	0,0466	0,0000	0,0000
128673	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
415385	0,0000	0,0000	0,0000	0,0000	1,1286	0,0000
6531	0,0000	0,0000	0,0000	0,0000	10,9631	7,5486
101658	0,0000	0,1485	0,0000	0,0000	1,5041	0,9925
133606	0,0000	0,0000	0,0000	0,0000	1,4610	0,2088
203460	0,0000	0,0000	0,0000	0,0000	2,8006	0,0781
45088	0,0000	0,0000	0,0000	0,0000	0,1286	0,0000
441213	0,0000	0,0000	0,0000	0,0000	0,4417	0,0000
1362868	0,0000	0,0000	0,0000	0,0068	0,0000	0,0000
90735	0,0000	0,0000	0,0000	0,0000	1,2278	0,0000
135111	0,0000	0,0000	0,0000	0,0000	0,4234	0,7142
40704	0,0000	0,2088	0,5823	0,0000	0,6903	1,3954
38099	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
3332463	0,0000	0,0000	0,0000	0,0000	0,2696	0,1358
80914	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
320538	0,0000	0,0000	0,0000	0,0000	0,0480	0,0000
31681	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
62790	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
99420	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
14225762	0,0000	0,0000	0,0000	0,0000	0,0149	0,0749
29388	0,0000	0,0000	0,0000	0,0000	3,8655	1,4496
4101536	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
4101536	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
87633	0,0000	0,0000	0,0000	0,0000	1,5782	0,1050
64383	0,0000	0,0000	0,0000	0,0000	6,7502	3,8908
694984	0,0000	0,0000	0,0000	0,0000	0,2748	0,0207
191938	0,0000	0,0000	0,0000	0,1105	52,8145	8,2917
77556	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000
142997	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000

Tab. 1B Presence of bacteria in patients with parafunctions



Fig. 15 Patient.



Fig. 16 Dental Arch.



Fig. 17 Panoramic Radiograph.

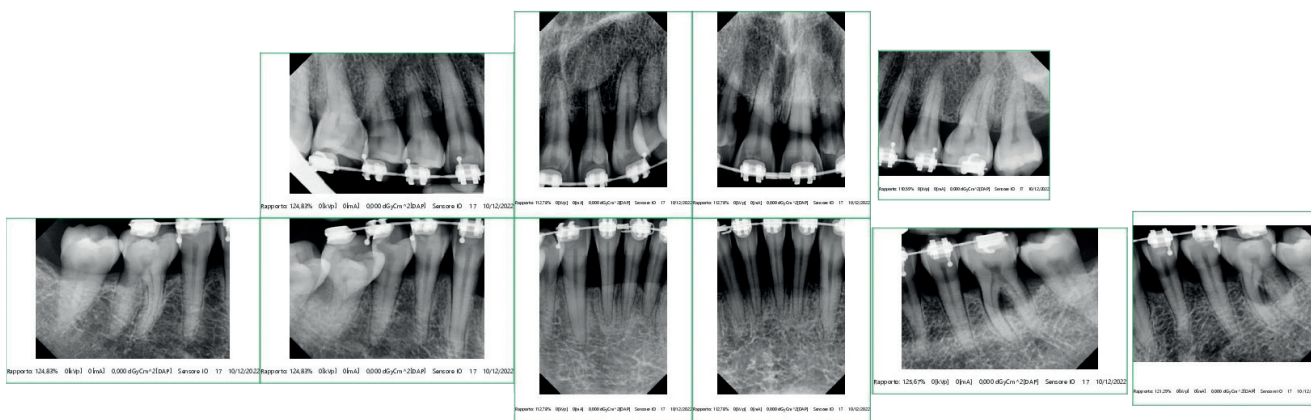


Fig. 18 Full-Mouth Intraoral Radiographic Series.

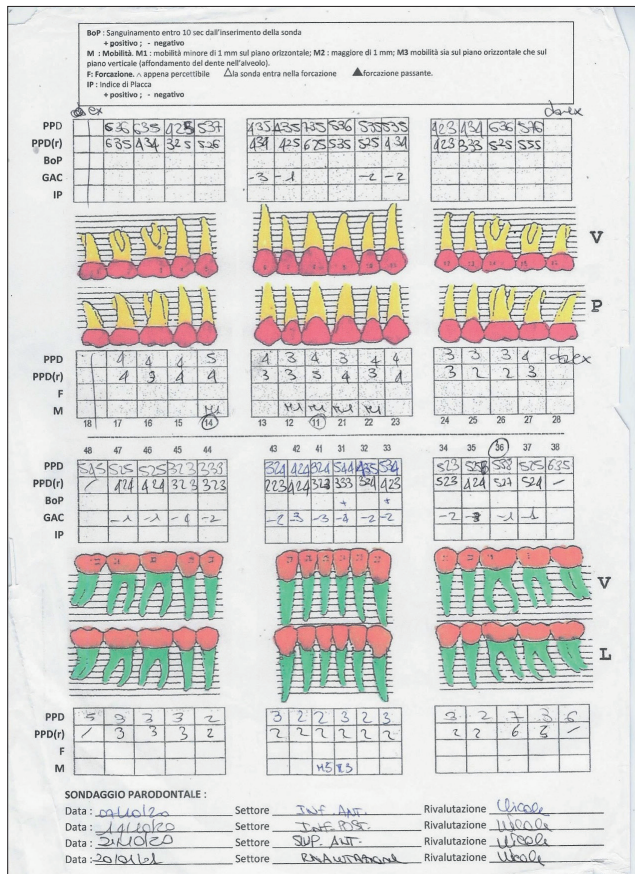


Fig. 19 Periodontal Chart.

72.5% of the samples (361 out of 420 cases) with values ranging from 0.0035% to 86.3505%, with a mean value of 6.4628% of the total bacterial load. In patients measured before treatment, the mean percentage value is 7.0148%, and the mean value after treatment is 2.5865% (Fig. 12). The bacterium *Campylobacter rectus* is present in 37.5% of the samples (187 out of 420 cases) with values ranging from 0.0006% to 29.5476%, with a mean value of 1.5387% of the total bacterial load. In patients measured before treatment, the mean percentage value is 1.4527%, which rises to 2.4589% after treatment (Fig. 13).

DISCUSSION

The graphs highlighted that after treatment (Scaling and root planing), there is a drastic reduction (elimination of some bacteria) of bacteria and the total bacterial load. Before treatment, the presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* is very low in percentage, and in many cases, they are absent (Fig. 14, 15-15; Tab. 1), conversely we have a very high percentage of the bacterium *Fusobacterium nucleatum*, which is also present in the intestine and causes colorectal cancer (studies have shown a correlation between the bacterium in the oral cavity and colorectal cancer). In Figure 14, parafunctions account for 70% (bruxism, clenching, atypical swallowing,

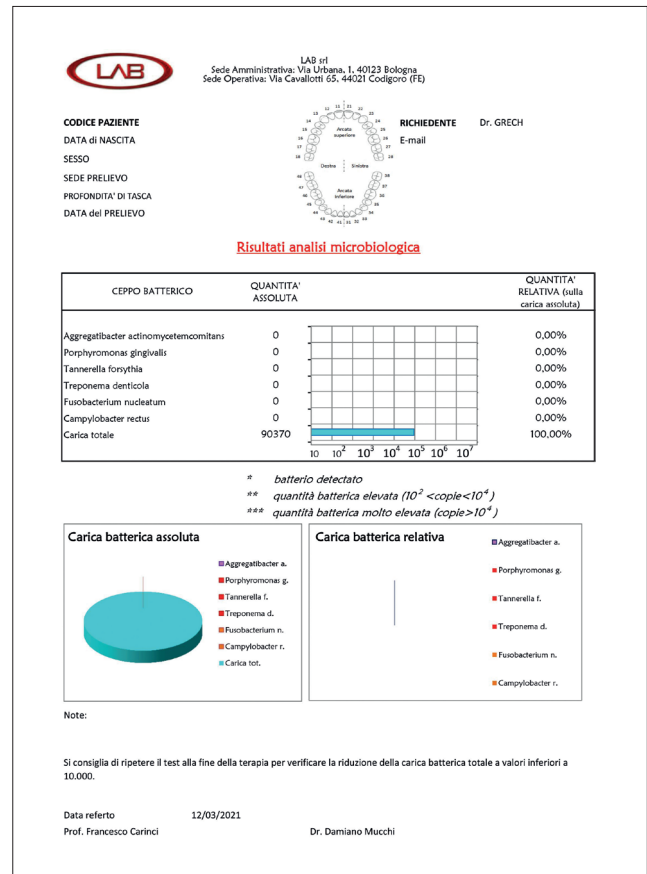


Fig. 20 Microbiological Test.

premature contacts, oral habits, dental misalignment, mouth breathing, and inadequate prosthetic restorations), presenting a high bacterial load. In some cases, primary bacteria are absent, while in other cases, there is an almost constant presence of *Fusobacterium nucleatum*. Out of 420 patients, 263 present with parafunctions, whereas in 157, periodontal disease is attributed to bacteria.

Among these 263 patients:

- 60 have no bacteria
- 86 are free of 5 bacteria
- 112 are free of the first 4 bacteria
- 2 have only *Aggregatibacter actinomycetemcomitans*
- 13 have only *Porphyromonas gingivalis*
- 173 have *Fusobacterium nucleatum*

RESULTS

The study highlighted that the primary causes of periodontal disease are parafunctions (Bruxism, clenching, oral habits, atypical swallowing, mouth breathing), dental misalignment, premature contacts, and inadequate prosthetic restorations. Therefore, these act as "direct and triggering" local etiological factors, rather than "indirect and predisposing" as historically maintained. Secondly by bacteria. Before treatment, the presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*,

Tannerella forsythia is extremely low or absent in many cases, whereas Fusobacterium nucleatum is found in very high percentages, present also in the intestine and causes colorectal cancer (studies have demonstrated a correlation between the bacteria in the oral cavity and colorectal cancer). After treatment (Scaling and root planing), there is a drastic reduction (elimination of some bacteria) of bacteria and the total bacterial load. Out of 420 patients, 263 present with parafunctions; in 157, periodontal disease is attributed to bacteria.

Among these 263 patients (Fig. 20):

- 60 have no bacteria
- 86 are free of 5 bacteria
- 112 are free of the first 4 bacteria
- 2 have only Aggregatibacter actinomycetemcomitans
- 13 have only Porphyromonas gingivalis
- 173 have Fusobacterium nucleatum

Out of 78 patients who underwent a second sampling after treatment, 40% (31 patients) still constantly presented with Fusobacterium nucleatum.

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