Frequency of periodontal pathogens in equivalent peri-implant and periodontal clinical statuses

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ABSTRACT

Objectives: This study tested the hypotheses that there is: (1) higher bacterial frequency in peri-implantitis/periodontitis, followed by mucositis/gingivitis and peri-implant/periodontal health; (2) similar bacterial frequency between comparable peri-implant and periodontal clinical statuses.

Design of study: The presence of Porphyromonas gingivalis, Tannerella forsythia, Campylobacter rectus, Prevotella intermedia, Treponema denticola and Aggregatibacter actinomycetemcomitans was evaluated in peri-implant (n = 53) and periodontal (n = 53) health; mucositis (n = 50), gingivitis (n = 50), peri-implantitis (n = 50) and periodontitis (n = 50).

Results: The pattern of peri-implant bacterial frequency was not as expected (peri-implantitis > mucositis > health). Except for P. intermedia (p < 0.05), bacterial frequency was higher in peri-implantitis than health (p < 0.05). The frequency of P. gingivalis and red complex species were higher in peri-implantitis than mucositis (p < 0.05). In periodontal samples, T. forsythia and T. denticola showed the expected pattern of frequency (periodontitis > gingivitis > health). The frequencies of C. rectus and T. forsythia were higher in healthy teeth/gingivitis than healthy implants/mucositis, respectively (p < 0.05). The frequency of P. gingivalis and A. actinomycetemcomitans were similar between periodontitis and peri-implantitis (p > 0.05) while all other species occurrences were higher in periodontitis than peri-implantitis (p < 0.05). Conclusions: Bacterial frequency increased from peri-implant/periodontal health to peri-implantitis/periodontitis but not from mucositis/gingivitis to peri-implantitis/periodontitis. There was a trend towards higher bacterial frequency in teeth than implants.

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1. Introduction

Despite many crucial histological and structural differences between teeth and implants, their clinical similarities lead researchers to apply some general well accepted statements in periodontal field to implant dentistry. The inflammation restricted to soft tissues in early stages followed by bone loss and increased pocket depth could exemplify these similarities.
In addition, peri-implant and periodontal diseases share some risk factors such as age, tobacco use and levels of oral hygiene.\textsuperscript{1-4} The fact that risk factors for periodontal disease could also increase the risk of development of peri-implant disease confirms that both disorders share some etiopathogenic aspects. Moinaz et al.\textsuperscript{5} reported smoking, a recognized risk factor for periodontitis, as the most important risk factor for the development of mucositis. For peri-implant disease similar findings were also observed by Karbach et al.\textsuperscript{6} in a sample of 100 patients with single implants. Interestingly, periodontitis history per se may also be considered a risk factor for peri-implant disease.\textsuperscript{7} Schou et al.,\textsuperscript{8} in a systematic review, showed a significantly increased incidence of peri-implantitis and peri-implant bone loss in subjects with periodontitis associated tooth loss. Similarly, Safi et al.\textsuperscript{9} demonstrated in a meta-analysis study that periodontitis subjects showed a higher risk of implant failure and greater marginal bone loss than periodontally healthy subjects. This relation was recently reviewed by Donos et al.\textsuperscript{10}

Although periodontal diseases are multifactorial disorders, it is well established that subjects that harbour periodontal pathogens are more susceptible to gingivitis/periodontitis development.\textsuperscript{9} The microenvironment (i.e. sulcus/pockets) around teeth favours selective bacterial colonization and, the successive interactions among bacterial species ultimately contribute to the aggregation of microorganisms forming periodontopathogenic communities.\textsuperscript{10} The microorganisms considered to be periodontal pathogens may perpetuate the imbalance in the microbiota and the inflammatory response in periodontal tissues. Therefore, the presence of some key pathogenic species is well recognized to be related to the progression and severity of periodontal disease.\textsuperscript{11-13} Although present in smaller number in healthy periodontal sites, target periodontal species tend to increase as a healthy periodontal condition shift to a diseased periodontal status. This tendency was demonstrated in a well-known paper in which the authors compared the microbiota of healthy, gingivitis and initial periodontitis sites\textsuperscript{13} and confirmed by other investigations.\textsuperscript{14-16}

It has been suggested that bacteria which cause periodontal breakdown could migrate and colonize peri-implant sites.\textsuperscript{17} Quirynen et al.\textsuperscript{18} analysed the subgingival microbiota present in so-called “pristine pockets”, namely pockets created after insertion of transmucosal abutments in previously submerged dental implants. The authors demonstrated that periodontal pathogens were more frequently found when adjacent teeth also harboured them, showing that the development of subgingival plaque in implants is directly influenced by the supragingival environment. This plausible finding was corroborated by studies that observed that, even after the complete loss of teeth, some of these target species still remain in the oral cavity\textsuperscript{19,16} and, bacteria may be also detected in apparently healed alveolar bone.\textsuperscript{20} Therefore, not only teeth but also the oral soft tissues could act as important reservoirs of bacteria that can subsequent colonize the sulcus/pockets around dental implants. As observed in periodontal tissues, studies have suggested that the presence of periodontal pathogens could also lead to damage in the peri-implant tissues.\textsuperscript{21-24} However, it is not completely clear if there is a progressive increase in pathogens frequencies when different peri-implant statuses are compared; i.e. healthy peri-implant sites vs. mucositis vs. peri-implantitis. The pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Treponema dentica and Tannella forsythia were detected in Brazilians with healthy and diseased implants.\textsuperscript{25} In addition, little evidence arose from studies which concomitantly compared the microbiota of peri-implant and periodontal sites from healthy to diseased statuses.\textsuperscript{26,27}

Therefore, the first aim of this cross-sectional study is to verify if there is a tendency towards an increase in pathogen frequency from peri-implant health to established peri-implant diseases, as previously observed from healthy to diseased periodontal conditions. The second aim of the present study is to test if bacterial frequency is comparative between equivalent periodontal and peri-implant clinical statuses, i.e. healthy peri-implant vs. healthy periodontal sites, mucositis vs. gingivitis and, peri-implantitis vs. periodontitis.

2. Materials and methods

This research protocol was reviewed and approved by the Institutional Ethics Committees from University of Taubaté (2008/0098) and Guarulhos University (09/2005). After verbal and written explanations, individuals who agreed to participate signed an informed consent form. Participants received oral hygiene instructions and dental treatment according to their individual needs.

2.1. Study population

This convenience sample population was composed of subjects selected, from January 2006 to June 2010, according to six specific diagnoses: peri-implant (n = 53 subjects) or periodontal health (n = 53 subjects); peri-implant mucositis (n = 50 subjects) or gingivitis (n = 50 subjects); peri-implantitis (n = 50 subjects) or chronic periodontitis (n = 50 subjects).

Eligible subjects were screened from two Clinical Centres, Department of Dentistry of the University of Taubaté and Department of Periodontics of the University of Guarulhos, according to the following inclusion criteria: male or female; aged between 26 and 52 years; at least fifteen natural teeth; at least one single titanium implant (MKIII, Nobel Biocare) under function for at least one year (for the implant groups). In addition, some exclusion criteria were considered: smoking (current smokers and former smokers); alcohol abuse; diabetes mellitus; immunosuppressive systemic conditions; pregnancy and lactation; extensive fix or removable orthodontic or prosthetic appliances; local or systemic antibiotic therapy within 6 months prior to biofilm sampling; daily regular use of mouthwash two months prior to the study; any type of periodontal treatment in the past 12 months (for periodontal groups).

2.2. Clinical examination

Clinical parameters were measured by two trained and calibrated examiners at six sites per tooth or implant using
a manual periodontal probe (Hu-Friedy PCPUNC 15 Mfg Co. Inc., Chicago IL). After 7 days, periodontal examinations of 10 subjects were repeated showing intra and inter-examiners reproducibility scores higher than 0.85 (Kappa Test) for probing depth (PD) and clinical attachment level (CAL). Intra-class correlation tests showed scores higher than 0.90.

The following parameters were measured, as previously described,\textsuperscript{28} at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) per implant:

1) Bleeding on probing (BOP): presence (1) or absence (0) of bleeding within 15 seconds after gentle probing;
2) Suppuration (SUP): presence (1) or absence (0) of spontaneous suppuration or suppuration after probing;
3) PD: distance between the mucosal margin and the bottom of the peri-implant sulcus/pocket;
4) CAL: distance between the base of the abutment and the bottom of the peri-implant sulcus/pocket;
5) Mucosal marginal bleeding (MB): bleeding recorded by running a probe along the soft tissue margin, and
6) Peri-implant bone loss: the height of the alveolar bone crest around each implant was determined by intraoral radiographic examinations using the long-cone technique (the images were digitized and evaluated using the software ImageJ).

Based on these clinical and radiographic parameters, subjects were stratified in specific peri-implant or periodontal diagnostic groups (Table 1). If the same subject had healthy implants and implants with mucositis or peri-implantitis, he/she was included in only one group based on the worst diagnosis as follows: peri-implantitis, mucositis or health.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Clinical and radiographic parameters</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peri-implant health</td>
<td>Presence of PD $\leq$ 5 mm without MB/BOP/SUP and radiographic bone loss; whole mouth mean CAL $&lt; 1.5$ mm; no sites showing CAL $&gt; 2$ mm; absence of gingival inflammation and alveolar bone loss</td>
<td>Maximo et al.\textsuperscript{28} Cortelli et al.\textsuperscript{19}</td>
</tr>
<tr>
<td>Periodontal health</td>
<td>Whole mouth mean CAL $&lt; 1.5$ mm; no sites showing CAL $&gt; 2$ mm; absence of gingival inflammation and alveolar bone loss</td>
<td>Maximo et al.\textsuperscript{28}</td>
</tr>
<tr>
<td>Peri-implant mucositis</td>
<td>MB and/or BOP without radiographic bone loss or presence of radiographic bone loss $&lt; 3$ threads</td>
<td>Maximo et al.\textsuperscript{28}</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>PD $&lt; 4$ mm, gingival redness and bleeding in more than 25% of sites</td>
<td>Lopez et al.\textsuperscript{29}</td>
</tr>
<tr>
<td>Peri-implantitis</td>
<td>Presence of PD $\geq 5$ mm with BOP and/or SUP and radiographic bone loss (bone level $\geq 3$ threads)</td>
<td>Maximo et al.\textsuperscript{28}</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>At least four teeth with one or more sites with PD $\geq 4$ mm and CAL $\geq 3$ mm</td>
<td>Lopez et al.\textsuperscript{29}</td>
</tr>
</tbody>
</table>

Subgingival biofilm samples were obtained from two non-contiguous periodontal sites distributed in two different quadrants for the periodontal health, gingivitis and periodontitis groups. Submucosal biofilm samples were collected from one or two peri-implant sites for peri-implant health, mucositis and peri-implantitis groups. If the subject had more than one diseased implant with the same diagnosis, two sites from different implants within the same clinical diagnosis per subject were chosen for biofilm sampling. For healthy groups, mesial sites with no MB/GI, BOP or SUP and presenting PD $\leq 3$ mm in first molars (upper right and lower left) or implants were sampled. For gingivitis and mucositis groups, the presence of BOP and/or GI/MB was used as the criterion for sampling sites selection. For periodontitis and peri-implantitis groups, sites with the deepest PD ($\geq 5$ mm) presenting BOP were selected for biofilm sampling. If two or more sites presented similar PD values, the most anterior site was chosen. No periodontal sites presenting furcation involvement was selected for biofilm sampling.

2.4. Microbiological analysis

Microbiological examinations were conducted as previously described.\textsuperscript{19} Each selected implant/tooth site was isolated with sterile cotton rolls and the supragingival biofilm was
removed with sterile curettes. A sterilized #30 paper point (Tanari, Tanarimau Industrial Ltda., Manacapuru, Brazil) was carefully inserted into the depth of the sulcus/pocket and kept in position for 60 s. The pooled subgingival samples were stored at −80 °C in microtubes containing 1 ml of reduced Ringer’s solution until processing.

Prior to microbial analysis, polymerase chain reaction (PCR) was carried out using unspecific “Universal primers” (16S rRNA) to detect bacterial DNA in the samples. Subsequently, the presence of Campylobacter rectus, P. gingivalis, T. forsythia, P. intermedia, T. denticola and A. actinomycetemcomitans was established using specific primers [P. gingivalis, sense: 5′-AGGCCAGCTGTCCGCACTGGG-3′, and antisense: 5′-ACTGT-TAGCAACTACCGATGT-3′ (product size: 404 bp); T. forsythia, sense: 5′-GCCTAGTAAACTGCGCCGG-3′, and antisense: 5′-TGCTTCAGTGTCAGTTATACCT-3′ (product size: 641 bp); C. rectus, sense: 5′-TTCGAGGGCTAAACTCTTTTTC-3′, and antisense: 5′-TTTTGTGAAGGTAAGGCGCC-3′, and antisense: 5′-TCACACCTCTGATCTCGCTCTTCCG-3′, (product size: 578 bp); T. denticola, sense: 5′-TTAATACC-GAACTGCTGCAATTCACT-3′, and antisense: 5′-TCAAAGAAGCATCTCCCTCCTCTATCTA-3′ (product size 316 bp) and A. actinomycetemcomitans, sense: 5′-AACCCATCTTCTGAGTC-TTCTCTTCA-3′ and antisense: 5′-ATGCCAATCTTAGGTAAAT-3′ (product size: 550 bp)] under standard conditions.

The genomic DNA was extracted using PureLink™ Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. PCR was performed in a Mastercycler Gradient (Eppendorf®, Westbury, NY, USA) thermocycler as follows: one cycle 94 °C for 5 min, 35 cycles 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and a final cycle of 72 °C for 5 min. The following annealing temperatures were applied: P. gingivalis and T. forsythia 57 °C; T. denticola 56 °C; C. rectus, P. intermedia and A. actinomycetemcomitans 55 °C. After electrophoresis in 1.5% agarose gel, the DNA fragments were stained with SYBR Safe (Invitrogen, Carlsbad, CA, USA) and visualized by UV illumination. The PCR amplificates were compared with both positive and negative controls. A molecular weight marker (Ladder 100, Invitrogen) was added in each set. To ensure PCR reproducibility, 20% of the samples were re-amplified.

2.5. Statistical analysis

To determine the degree of similarity among implant and periodontal groups, clinical parameters were compared using ANOVA (analysis of variance) and Student’s t-test. Subsequently, an additional analysis was performed to confirm or reject the hypothesis that there was a higher bacterial frequency in peri-implantitis/periodontitis followed by mucositis/gingivitis and healthy peri-implant/periodontal sites. Therefore, frequency of target bacterial species observed in each specific clinical implant status was compared to each other using Chi-square test. Similarly, the bacterial frequencies among periodontal clinical statuses were submitted to this same statistical analysis.

A third analysis was performed to confirm if there was similar bacterial frequency when equivalent periodontal and peri-implant clinical statuses were compared. Therefore, bacterial frequency between peri-implant and periodontal sites was compared using Chi-square test within each clinical status (peri-implantitis vs. periodontitis, mucositis vs. gingivitis and peri-implant vs. periodontal health).

The frequency of the red complex species was determined as the simultaneous presence of P. gingivalis, T. forsythia and T. denticola.

Differences were considered statistically significant when \( p < 0.05 \). Statistical analysis was performed using the software Bioestat 5.0 and SPSS 11.0. Ouvir.

3. Results

A total of 306 subjects (38.33 ± 13.19 years old) participated in the present study. Out of 153 subjects that composed implant groups, 10 (6.53%) subjects had one installed implant, 135 (88.23%) had two and only 8 (5.22%) subjects had three or more implants under function. Table 2 shows the demographic data of the study population and mean values of clinical parameters for all implant and periodontal groups at sampled site level. In the diseased sites, a mean proximal peri-implant loss of \( 4.2 ± 1.2 \) mm and a mean proximal periodontal bone loss of \( 4.9 ± 0.8 \) mm were observed.

The comparative frequency of target bacterial species among peri-implant or periodontal clinical statuses is

### Table 2 – Demographic data of the study population and mean values of measured clinical parameters for all implant and periodontal groups at sampled tooth/implant level.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Peri-implant health (n = 53)</th>
<th>Periodontal health (n = 53)</th>
<th>Mucositis (n = 50)</th>
<th>Gingivitis (n = 50)</th>
<th>Peri-implantitis (n = 50)</th>
<th>Periodontitis (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.34 ± 9.39</td>
<td>25.01 ± 5.96</td>
<td>40.40 ± 9.97</td>
<td>32.80 ± 11.58</td>
<td>40.30 ± 9.41</td>
<td>52.44/11.37</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>18/35</td>
<td>17/36</td>
<td>17/33</td>
<td>15/35</td>
<td>16/34</td>
<td>13/37</td>
</tr>
<tr>
<td>Time of loading (years)</td>
<td>2.1 ± 1.2</td>
<td>2.10 ± 0.21</td>
<td>3.6 ± 1.8</td>
<td>3.18 ± 0.25</td>
<td>6.06 ± 0.73</td>
<td>5.78 ± 0.61</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>2.76 ± 0.78</td>
<td>1.05 ± 0.17b</td>
<td>3.05 ± 0.95b</td>
<td>1.96 ± 0.20b</td>
<td>5.26 ± 1.50</td>
<td>5.06 ± 0.91</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>3.02 ± 1.07a</td>
<td>0.96 ± 0.03a</td>
<td>0.96 ± 0.03a</td>
<td>0.89 ± 0.11a</td>
<td>0.84 ± 0.13a</td>
<td>0.91 ± 0.09a</td>
</tr>
<tr>
<td>BoP (0/1)</td>
<td>0</td>
<td>0</td>
<td>0.45 ± 0.09a</td>
<td>0.39 ± 0.12a</td>
<td>0.84 ± 0.19b</td>
<td>0.97 ± 0.02b</td>
</tr>
<tr>
<td>SUP (0/1)</td>
<td>0</td>
<td>0</td>
<td>0.22 ± 0.26b</td>
<td>0.22 ± 0.26b</td>
<td>0.05 ± 0.11b</td>
<td>0.05 ± 0.11b</td>
</tr>
</tbody>
</table>

Different lower-case letters within lines indicate differences among groups (ANOVA and Student’s t-tests; \( p < 0.05 \)). M: male; F: female; PD: probing depth; CAL: clinical attachment level; MB: mucosal bleeding; GB: gingival bleeding; BoP: bleeding on probing; SUP: supputation; NM: not measured.
Table 3 – Frequency of bacterial species in peri-implant and periodontal subgingival biofilm samples from healthy or diseased sites.

<table>
<thead>
<tr>
<th>Bacterial frequency (%)</th>
<th>Healthy</th>
<th>Mucositis</th>
<th>Peri-implantitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Implants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>12.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. rectus</em></td>
<td>44.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>22.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. forsythia</em></td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Red complex species (P. gingivalis, T. forsythia and T. denticola)</strong></td>
<td>8.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacterial prevalence (%)</th>
<th>Healthy</th>
<th>Gingivitis</th>
<th>Periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Teeth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. rectus</em></td>
<td>98.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>12.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. forsythia</em></td>
<td>32.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>12.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Red complex species (P. gingivalis, T. forsythia and T. denticola)</strong></td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different lower-case letters indicate statistically significant differences within the lines revealing, therefore, different frequencies among groups (peri-implant health vs. mucositis vs. peri-implantitis or periodontal health vs. gingivitis vs. periodontitis). Chi-square test ($p < 0.05$).

described in Table 3. The pattern of bacterial frequency observed was not as expected, i.e. peri-implantitis > mucositis > health. Except for *P. intermedia*, which did not differ among implant groups ($p > 0.05$), the additional bacterial species showed higher frequency in peri-implantitis than healthy implant sites ($p < 0.05$). However, when bacterial frequencies between peri-implantitis and mucositis were compared, similarities ($p > 0.05$; for *C. rectus*, *A. actinomycetemcomitans*, *T. forsythia* and *T. denticola*) were more evident than differences ($p < 0.05$; for *P. gingivalis* and simultaneous presence of red complex species).

Considering periodontal samples, a higher frequency of *P. intermedia*, *P. gingivalis*, *T. forsythia*, *T. denticola*, *A. actinomycetemcomitans* and simultaneous presence of red complex species was observed in periodontitis group when compared to gingivitis and health ($p < 0.05$). Contrary to peri-implant findings (peri-implantitis vs. mucositis) the periodontal bacterial frequency pattern was different between periodontitis and gingivitis. Except for *C. rectus* ($p > 0.05$), the other bacteria frequencies were significantly lower in gingivitis than periodontitis ($p < 0.05$). Finally, *T. forsythia* and *T. denticola* showed the expected pattern of frequency, i.e. periodontitis > gingivitis > health ($p < 0.05$).

A second analysis was performed by comparing the frequency of each bacterial species between similar periodontal and peri-implant clinical status (healthy peri-implant vs. healthy periodontal sites, mucositis vs. gingivitis and peri-implantitis vs. periodontitis; Figs. 1–3, respectively). An overall tendency towards higher frequency of bacteria was observed for periodontal sites, especially in periodontitis ones. The frequencies of *C. rectus* and *T. forsythia* were higher in periodontal health and gingivitis when compared to peri-implant health and mucositis, respectively (Figs. 1 and 2, $p < 0.05$). On the contrary, when the supportive tissues were involved, dissimilarities were more evident between implants and teeth. The frequencies of *P. gingivalis* and *A. actinomycetemcomitans* were similar between periodontitis and peri-implantitis ($p > 0.05$) while the frequencies of all other bacterial species and red complex species were higher in periodontitis than peri-implantitis (Fig. 3, $p < 0.05$).

4. Discussion

The disequilibrium between host-compatible and pathogenic microorganisms of the oral cavity plays an important role in the ethiopathogenesis of several oral diseases including periodontitis. Periodontitis is one of the major causes of tooth loss in adults and dental implants have been successfully used to replace lost teeth. It was demonstrated that even after tooth loss, key periodontal pathogens remain colonizing oral cavity and that periodontitis history was positively
correlated to peri-implantitis and peri-implant bone loss.\(^7,8,28\) Therefore, one plausible explanation for the relationship between periodontal and peri-implant diseases is associated with the microbial component.\(^9,24\) In fact, clinically, similar microenvironments including sulcus/pockets are presented around dental implants and teeth, which could favour similar bacteria colonization. Although studies have shown that the subgingival microbiota associated with health and disease is similar around implants and teeth,\(^32\) the occurrences of key periodontal species according to different peri-implant and periodontal clinical conditions and their direct comparisons still need further evaluation.

Therefore, the present study firstly aimed to verify if the frequencies of target periodontal species would increase progressively throughout health, reversible (mucositis and gingivitis) and irreversible (periodontitis and peri-implantitis) established peri-implant and periodontal diseases. For peri-implant sites, overall, the results showed that the majority of the bacterial frequencies were higher in peri-implantitis than in healthy implants, as demonstrated by previous studies.\(^21,22\) However, the results of the present study did not show clear differences between peri-implantitis and mucositis and, the hypothesis that the bacterial frequencies would increase gradually from healthy to mucositis and peri-implantitis was rejected. Maybe, the overlapping profile of microbial frequencies between mucositis and peri-implantitis indicates that, similarly to what happens in gingivitis,\(^33\) mucositis, as an intermediate reversible stage, could progress to peri-implantitis in susceptible subjects or even be a self-limiting disease in resistant subjects. Renvert et al.\(^34\) did not observed marked differences in the proportions of 40 bacteria species and total bacterial load in relation to different peri-implant status. Maximo et al.\(^23\) using chequerboard hybridization technique, showed that T. forsythia counts were higher in peri-implantitis than peri-implant health and mucositis. In addition, although not statistically significant, P. gingivalis was the species found at the highest levels in the peri-implantitis when compared to the other clinical conditions. In support of our results, the authors found higher proportion of red complex species in the submucosal area around peri-implantitis, followed by mucositis and by the healthy implants. In the present study, as previously shown,\(^19,12\) microbial differences among healthy and diseased periodontal clinical statuses were evident. Although the expected pattern of progressive increased frequency of detection from health to periodontitis was observed for T. forsythia and T. denticola, it should be mentioned that differences in frequencies of all other species were not observed between gingivitis and health. Macuch and Tanner\(^35\) also found similar bacterial frequency between gingivitis and health. Also, in a previous study by our group with children showing high levels of plaque, some important pathogens (P. gingivalis and T. forsythia) did not differ among three different levels of gingival bleeding.\(^36\) Together, these results suggest that the loss of alveolar bone and soft tissues more than the presence of gingival inflammation may be related to an increased occurrence of some pathogens around periodontal tissues.

The second hypothesis of the present study was to test if bacterial frequencies were comparative in equivalent periodontal and peri-implant status (i.e. healthy peri-implant sites vs. healthy periodontal sites, mucositis vs. gingivitis and peri-implantitis vs. periodontitis), evaluating sites matched for clinical parameters within each clinical condition. The results showed that only C. rectus and T. forsythia presented significantly higher frequencies of detection in periodontal healthy sites than in peri-implant healthy ones as well as in gingivitis than in mucositis. These findings indicate that similarities in bacteria frequencies were evident between periodontal and peri-implant sites when health or a reversible inflammation process were present. In support of our results, Gerber et al.\(^37\) compared the microbiota at predominantly healthy tooth and implant sites and found only minor microbial differences between groups. Aoki et al.\(^38\) studying the sources of peri-implant colonization by periodontal...
pathogens, observed similar detection rates of selected species in subgingival samples from adjacent periodontal sites and newly formed peri-implant sulci. Nowzari et al.26 showed higher frequency of detection and higher levels of periodontal pathogens around clinically healthy periodontal than clinically healthy peri-implant sites, but the difference was not statistically significant. However, it is important to note that the authors used culture methods and evaluated a small sample size (only 11 subjects). Recently, Heuer et al.27, using broad-range PCR techniques, demonstrated that the microbial diversity of the microbiota surrounding gingivitis (19 different bacteria genera) was significantly higher than the diversity of the microbiota associated to mucositis (6 different bacteria genera). Vered et al.39 reported significantly higher numbers of aerobic and anaerobic oral bacteria in samples collected from teeth than those collected from implants within the same mouth. However, no systematic characterization of the clinical statuses of the teeth and implants was described.

In this study, dissimilarities in bacteria occurrences between peri-implantitis and periodontitis were more evident, since higher frequencies of T. forsythia, C. rectus, P. intermedia, T. denticola and red complex species were observed in periodontal than in peri-implant sites. Based on our findings, it is clear that the peri-implant microbial composition shifts towards a higher proportion of periodontal pathogens during peri-implantitis formation. However, our findings also suggest that, although periodontitis and peri-implantitis may harbour the same type of bacteria species as previous reported, the rate of pathogens occurrence around peri-implantitis seems to be lower than periodontitis. Interestingly, previous studies compared the microbiota of teeth and soft intra-oral sites (cheek and/or tongue) and demonstrated that teeth were more permissive sites to harbour pathogens in the oral cavity.16,19 Together, these data confirm the hypothesis that different surfaces and ecological niches of the oral cavity, including dental implants, present a particular influence on the microbiota composition. Structural differences and properties of surfaces, that could affect the bacterial adhesion, could be one of the possible explanations for the microbial differences between dental and implant surfaces. In addition, microbiota composition may be also a consequence of the characteristics of the mucosal or gingival tissues and, the inflammatory reactions in tissues. These microbial differences between teeth and implants, even though minor, may have several implications including differences in disease progression and inflammatory processes as well as in therapeutic strategies. It seems that the development of periodontitis and peri-implantitis lesions follows a similar succession of events. However, peri-implantitis can be expected to progress quickly because of absence of a healthy connective tissue fibre compartmentwalling off the lesion from the alveolar bone.52 Such observations regarding peri-implantitis progression and biofilm composition support the notion that peri-implant tissues do not have the same potential to deal with pathogenic microbiota as periodontal tissues.

In summary, bacterial frequency tended to be higher in peri-implantitis and periodontitis sites than in healthy peri-implant and periodontal sites. However, the first hypothesis was not totally confirmed since a progressive increase in the frequencies of pathogens from health to gingivitis/mucositis and to periodontitis/peri-implantitis was not observed for all species. Considering the second hypothesis, an overall trend towards higher frequency of pathogens was observed in periodontal than peri-implant sites, especially when periodontitis was compared to peri-implantitis condition. Therefore, diseased implants may have an implant-specific bacterial microbiota that is not totally similar to that of the diseased teeth and the clinical implications of these findings should be further evaluated. Finally, other species of bacteria not searched in the present study may be involved in peri-implant disease pathogenesis which might have lead to these somewhat not expected results.

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Competing interests

There is no conflict of interest related to the submitted manuscript.

Ethical approval

This research protocol was reviewed and approved by the Institutional Ethics Committees from University of Taubaté (2008/0098) and Guarulhos University (09/2005).

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