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ORIGINAL RESEARCH


# Periodontal and Microbiological Profile of Intensive Care Unit Inpatients

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## ABSTRACT

**Introduction:** The bidirectional relationship between the periodontal diseases and systemic diseases was attributed to the focal infection concept. The aims of this study were to assess the periodontal and microbiological profile of intensive care unit (ICU) inpatients submitted to orotracheal intubation, and classify them regarding gender, age group, ethnic, hospitalization reason and period, nosocomial infection occurrence, and death.

**Materials and methods:** Inpatients were assessed, distributed into toothed and toothless groups. The periodontal clinical condition was assessed 24 hours after the ICU admission through plaque index, gum index, probing depth, and clinical level of insertion. All microbiological samples were collected on the 6th day of admission. These samples were collected from different intraoral sites, depending on the group: In the toothed group, samples were collected from gingival sulcus and in the toothless group, from buccal mucosa and tongue. Identification for *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), and *Tannerella forsythia* (Tf) was accomplished and analyzed, using absolute quantification and specific primer pairs through an amplification system with probes.

**Results:** Forty subjects composed the sample: Gender characterized by 60% of male, 27.5% of all patients were older than 60, and 22.5% were hospitalized due to cerebrovascular accident. Regarding hospitalization period, 55% of patients were hospitalized for 6 days and 70% of them died during the period of hospitalization. Of inpatients, 40% presented periodontal disease and 100% presented dental biofilm on assessed sites. When assessing the microbiota, statistical significance was observed between Aa, Pg, and Tf, for both toothed and toothless group ($p < 0.0001$).

**Conclusion:** Large quantities of Aa were found in samples of toothless inpatients, a fact that suggests that the oral environment, even without teeth, presents favorable conditions for bacterial biofilm formation with a related pathogenic potential.

**Clinical significance:** The dental biofilm may comprise pulmonary pathogen colonies, promoting a perfect environment for their growth and development, facilitating the colonization of the lower airways, as well as colonization by bacteria originally from the oral cavity.

**Keywords:** Biofilm, Intensive care unit, Periodontal disease.

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## INTRODUCTION

Periodontal disease has a multifactor etiology, a primary infectious origin caused by dental biofilm that colonizes the sulcular regions between the tooth surface and peripheral gingiva, which may affect the tooth protection and support tissues. Some factors determine the appearance, gravity, and progression of this disease, such as genetic factors, socioeconomical conditions, and microbial composition.[1,2]

Although more than 700 species have been identified in the oral cavity, it is assumed that only a small number of these present any pathogenic potential.[3] Among the known species, the Gram-negative *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Tannerella forsythia* (Tf) are highlighted

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as related with the periodontal disease progression, and therefore, known as periodontopathogenic.[4]

The bidirectional relationship between the periodontal disease and systemic diseases was attributed to the focal infection concept, which is defined as microorganism dissemination and their products from chronically infected sites to other organs of the body.[5] Hence, it is possible to believe that periodontal disease is able to negatively affect the systemic health through dissemination of pathogenic microorganisms or from its subproducts in the blood stream, acting as a systemic metastatic infection.[6] The most frequent systemic changes studied are the metabolic control of diabetic patients, cardiovascular conditions, cerebrovascular accidents, pulmonary diseases, and premature birth associated with underweight babies.[7]

Hospitalized subjects, especially in intensive care units (ICU), present difficulties to have a proper oral hygiene due to the presence of an orotracheal tube and the unconscious state. Moreover, these patients remain with their mouths open, causing a mucous dehydration, decreased salivary flow and, consequently, dental biofilm increase.[8-10] The oral cavity suffers from a continuous colonization and a consequent microbiota unbalance, progressively becoming more aggressive, thus, compromising the integral health of the subject.[8]

The microorganisms present in the oral flora may reach the lower respiratory tract, resulting in contamination through aspiration of the oropharynx, leading to pneumonia.[11-13] The dental biofilm may be associated to respiratory infections, especially in patients under mechanical ventilation. These infections result from an elevated pathogen concentration in saliva, which may be aspired in large amounts, thus infecting the respiratory tract of subjects with an already compromised immune defense.[14,15]

Respiratory diseases are responsible for a significant morbidity and mortality portion for patients of all ages.[9] At specific conditions, such as the one faced by ICU patients, the dental biofilm may comprise pulmonary pathogen colonies, promoting a perfect environment for their growth and development, facilitating the colonization of the lower airways, as well as colonization by bacteria originally from the oral cavity.[9,10] These diseases are of particular interest to hospitals, especially in intubated patients, since more than 5% of all inpatients develop infections. Pneumonia represents 10 and 20% of these infections, becoming the most common cause of nosocomial infection.[10]

The identification of these oral microorganisms in samples of lower respiratory tract and dental bacteria biofilm can be performed in a practical and quick manner through molecular biology. Evidences that periodontal infections contribute to nosocomial pneumonia development are conflicting, presenting very different results.[13,16] Few studies have been investigating the presence of microorganisms, such as *A. actinomycetemcomitans, P. gingivalis,* and *T. forsythia*, known as periodontopathogens with a strong pathogenic power in both dentate and edentulous subjects.[2,10,26]

A detailed assessment of the periodontal condition through a periodontal clinical test is of great importance to establish a possible relationship between these values and the collected biofilm microorganisms. This study assessed the periodontal and microbiological profile of ICU patients as well as correlated the data with gender, age group, ethnic, length and reason for hospitalization, nosocomial infection occurrence, and death.

## MATERIALS AND METHODS

### Study Population

This prospective study was composed of patients from the ICU of the Hospital Geral Universitário de Cuiabá – Mato Grosso, Brazil, between January and December 2013. Inclusion criteria were determined by age, equal or above 18 years of age, at least 6 days hospitalization in the ICU with orotracheal intubation. Exclusion criteria were defined as patients younger than 18 years old, any limitation to mouth opening that impaired the proposed clinical examination, immunosuppressed patients (e.g., HIV+ or posttransplanted patients), or any impossibility to have clinical or microbial data collected. Patients were distributed in two groups based on their dentition: Dentate group or edentulous group. Additional demographic data and medical history have been assessed in patients' records. The patients or their legal guardians signed the informed consent and the study has been approved by the Ethics Committee of Universidade de Taubaté – UNITAU (number 444/2012).

### Periodontal and Microbiological Clinical Test

Periodontal clinical tests were performed 24 hours after inpatients ICU admission. Each clinical parameter was obtained by a single blind detector, previously calibrated according to the methodology described by Araujo et al.[17] The standard error of measurement (SEM) was applied to continuous variables (probing depth) and the Kappa test was used for categorical variables (plaque and gingival indexes). Thus, 10 tests were repeated in a 30-day interval and submitted to analysis. The detector considered calibrated upon SEM $\leq 0.8$ and $K > 0.8$ and $< 0.95$. The analyzed clinical measurements were: Plaque index (PI) and gingival bleeding index (GBI) through dichotomous assessments,[18] probing depth (PD), and clinical insertion level (CIL). Periodontal measurements were conducted in six sites per tooth (mesial-vestibular, vestibular,

distovestibular, mesial-lingual, lingual, distolingual) for all present teeth, except third molars, with the aid of a hand-operated periodontal probe (PCP-UNC 15, Hu-Friedy, Manufacturing Co. Inc., Chicago, IL).

All microbiological sampling were collected on the 6th day of ICU hospitalization. Samples from the dentate group were obtained from the intrasulcular mesial-vestibular sites of upper first molars, left lower molar, right upper central incisor, and right lower central incisor using a no. 30 autoclaved paper cone (Dentsply Maillefer, Switzerland). Whenever one of the proposed teeth was absent, samples were collected from the adjacent tooth. Each tooth was isolated with sterilized cotton rolls for sampling and the supragingival biofilm was removed with a sterilized cotton roll. Each paper cone was inserted in the most apical portion of the periodontal sulcus and held in position for 60 seconds.[19] Then, paper cones from each subject were placed on a single Eppendorf (Bio-Rad®, Hercules, CA, USA) type microtube containing 1.0 mL Ringer solution, kept at –20°C temperature until their processing. Microbial sampling for the edentulous group was obtained from patients' tongue and cheek through tongue collectors (Alça, São Paulo, Brazil) and a swab (Absorve, Jiangsu Suyun Medical Materials Co. Ltd., Jiangsu, China) was used to obtain biofilm samples from buccal mucosa and alveolar ridge and stored and processed in the exact same way as toothed group samples.

## Deoxyribonucleic Acid Extraction and Quantification

To proceed with the microbiological assessment, the genomic deoxyribonucleic acid (DNA) extraction was conducted with the aid of PureLink™ Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) under manufacturer's instructions. The material was previously homogenized in a mechanical shaker for 60 seconds and 500 µL of the sample was centrifuged (3 minutes × 12,000 rpm). After supernatant removal, 80 µL of PureLink™ Genomic Digestion Buffer and 20 µL of Proteinase K was added into tubes containing the bacterial cell suspension (pellet) and each mini-tube was incubated at 55°C for 90 minutes. After these procedures, 20 µL of RNase A was added to lysate and this solution was agitated and incubated for 2 minutes at room temperature. Then, 200 µL PureLink™ Genomic Lysis/Binding Buffer and 200 µL ethanol (100%) were added and the mini-tube agitated for 5 seconds until a homogeneous solution was formed. After this process, the whole lysate (around 640 µL) was transferred to a column (PureLink™ Spin Column containing a silica membrane) coupled to a collection tube and this set was centrifuged at 12,000 rpm for 1 minute. Then, the membrane was washed twice with 500 µL Wash Buffer 1 (12,000 for 1 minute) and Wash Buffer 2 (12,000 rpm for 3 minutes). Finally, 100 µL PureLink™ Genomic Elution Buffer was used to elute the DNA sticked to the silica membrane.

The bacteria strains originated from the Fundação Oswaldo Cruz, Instituto Nacional de Controle de Qualidade em Saúde (Brazilian Institute for Health Quality Control) and were grown in the University of Cuiabá Microbiology Laboratory.

The primers employed were drawn in compliance with the specific and highly maintained sequence of the 16S gene of the bacterial ribosome DNA of each microorganism involved and synthesized by Invitrogen Tech-Line℠ (Life Technologies do Brasil Ltd., São Paulo – SP) (Table 1). The search for the desired target sequences was conducted through NCBI Nucleotide Search (http://www.ncbi.nlm.nih.gov/). The software Primer 3 (http://frodo.wi.mit.edu/) was used to design primers. Identification and absolute quantification of the bacteria *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia* in clinical samples were performed through real-time polymerase chain reaction (PCR) by StepOne™ (Applied Biosystems, Foster City, CA, USA), using specific paired primers by the amplification

**Table 1:** Description of the DNA primers and probes used for each microorganism of the study

| *Primer* | *Sequence (3'–5')* | *Reference* |
|---|---|---|
| Pg – TAMRA-VIC | CAACCATGCAGCACCTACATAGAA | Saito et al[36] |
| Reverse | ACCTTACCCGGGATTGAAATG | |
| Forward | ATGACTGATGGTGAAAACCGTCTTCCCTTC | |
| Probe | | |
| Aa – TAMRA-FAN | TTCATTCACGCGGCATGGC | Nonnenmacher et al[37] |
| Reverse | CAAGTGTGATTAGGTAGTTGGTGGG | |
| Forward | ATCGCTAGCTGGTCTGAGAGGATGGCC | |
| Probe | | |
| Tf – TAMRA-FAN | TTCGCCGGGTTATCCCTC | Yuen et al[38] |
| Reverse | AGCGATGGTAGCAATACCTGTC | |
| Forward | CACGGGTGAGTAACG | |
| Probe | | |

*Porphyromonas gingivalis* (Pg); *Aggregatibacter actinomycetemcomitans* (Aa); *Tannerella forsythia* (Tf)

probe system TAQMAN® (Applied Biosystems, Foster City, CA, USA). Specificity test was accomplished by the software NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Primer's concentrations, as well as optimal conditions for the amplification process (concentrations of reactant/determination of temperatures involved), were previously established for each primers set "Forward and Reverse" included in the study. Real-time polymerase chain reaction (PCR) was conducted using a 25 µL final volume solution containing a concentration of 0.5 µL for each primer, 0.5 µL of (200 nmol) Taqman probe, 12.5 µL Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 9 µL sterile water, and 2 µL DNA solution. The amplification reaction was conducted under the following steps: Initial denaturation temperature 95°C for 10 minutes and amplification by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. After quantitative PCR (qPCR), the dissociation curve (melting curve) was accomplished with temperatures between 60° and 95°C to determine the qPCR specificity.

All reactions were performed in MicroAmp optical 48-well plates and optical adhesives (Applied Biosystems, Foster City, CA, USA). The data were analyzed using the Step One™ (Applied Biosystems, Foster City, CA, USA) software. Initially, to perform the standard curve, DNA extracted from pure bacteria was used, where a reading in a spectrophotometer was collected (NanoDrop® ND-1000 UV–Vis, NanoDrop Technologies, Wilmington, USA) through absorbance assessment at 260 nm and relationship between the absorbance at 260/280 nm. An initial concentration between 0.5 and 0.6 µg/µL of each bacterial DNA was obtained, and then, eight serial dilutions ($10^1$–$10^8$) was performed to obtain the standard curve.

The standard curve (which presented the known concentration) was used to convert the cycle threshold (CT) scores obtained with the fluid samples in exact DNA concentration numbers. Once the quantification thresholds were determined, the clinical samples were processed with the respective standard curve (positive control) of each bacterial species, in triple batches, and the mean values were used to calculate the bacterial levels. The reactions were performed while respecting an amplification efficiency for the standard curve between 110 and 93% (slope = –3.10 to –3.50) and a correlation coefficient ($R^2$) ≥ 0.98%. To obtain a high efficiency degree for the real-time PCR trial, the standard curve slope must be around –3.30.[20] The negative control was performed by substituting DNA for the same quantity of sterile water, in order to check possible contaminations.

### Statistical Analysis

Clinical and microbiological data underwent specific statistical treatment through the Statistical Package for the Social Sciences (SPSS) software, version 15.0. To analyze the clinical and microbiological characteristics between the two groups (dentate and edentulous), non-parametric Mann-Whitney test was used to compare the two independent sample means. The analysis of variance (ANOVA) test was conducted while associating age, gender, ethnic, hospitalization period, occurrence of nosocomial pneumonia, and death for both groups. All hypothesis tests developed in this study have considered a significance of 5%.

## RESULTS

Seventy-eight patients were initially assessed, however, after the analysis of inclusion criteria, mainly due to deaths and patient discharge before the 6th day, the sample of this study was composed of a total of 40 subjects, i.e., 20 for the dentate group and 20 for the edentulous group. Age distribution was seen from 18 to 70 years, 24 males and 16 females represented gender. Medical history regarding the reason for ICU hospitalization is displayed in Table 2. The most frequent condition was cerebrovascular accident followed by cancer and traumatic brain injury (22.5, 15, and 12.5% respectively). Regarding diabetes mellitus, 60% of all subjects did not present this condition. However, 16 subjects (40%) were affected by this metabolic disorder, 10 (62.5%) were in the toothed group. The period of hospitalization of 22 subjects (55%) was 6 days in the ICU and 67.5% did not present nosocomial pneumonia. From the 40 subjects assessed, 28 (70%) died.

The microbiological data refer to the total bacterial load (TB) and periodontal pathogen quantification of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythia*. Significant statistical difference was detected between the

**Table 2:** Absolute and relative frequency of inpatients background condition

| *Illness* | *n* | *Percentage* |
|---|---|---|
| Cerebrovascular accident | 9 | 22.5 |
| Aneurysm | 2 | 5 |
| Cancer | 6 | 15 |
| Chronic obstructive pulmonary disease | 2 | 5 |
| Arterial hypertension | 1 | 2.5 |
| Hepatic insufficiency | 1 | 2.5 |
| Congestive cardiac insufficiency | 1 | 2.5 |
| Infarction | 2 | 5 |
| Pneumonia | 1 | 2.5 |
| Traumatic brain injury | 5 | 12.5 |
| Fire arm perforation | 2 | 5 |
| Anaphylactic shock | 1 | 2.5 |
| Cirrhosis | 1 | 2.5 |
| Seizure | 1 | 2.5 |
| Diabetes | 2 | 5 |
| Overdose | 1 | 2.5 |
| Septicemia | 2 | 5 |

bacteria and CT, in both dentate and edentulous groups (Table 3). On the microbiological analysis through qPCR, a statistically significant difference was determined for *T. forsythia* (p = 0.041) and *P. gingivalis* (p = 0.021). The *T. forsythia* mean was 870 for the dentate group and 224 for the group, and for *P. gingivalis* the mean was 36 for the dentate group against 2 for edentulous group (Table 4). A statistically significant effect of the *T. forsythia* species (p-value = 0.005) was detected. For males the *T. forsythia* mean was 785 against 173 for females (Table 5). A statistically significant difference in the group (dentate and edentulous) *P. gingivalis* (p = 0.043) has also been detected. The mean of *P. gingivalis* species for dentate group was larger than edentulous group (26 and 2). For *A. actinomycetemcomitans*, a statistically significant effect was detected regarding gender (p = 0.045) and the interaction between group and ethnic (p = 0.023). The *A. actinomycetemcomitans* mean in males was higher than in females. It was possible to observe, through Tukey's multiple comparisons, that the difference detected in the interaction between ethnic and group for *A. actinomycetemcomitans* lied into the dentate group, where Caucasians presented a statistically significant difference against non-Caucasian (p = 0.05).

Regarding periodontal analysis, 40% of all patients were diagnosed with periodontal disease, with a probing depth mean of 2.83 mm and clinical level of insertion of 2.67 mm. The plaque index was positive for all assessed sites, demonstrating the poor oral hygiene of these patients. The bleeding index was verified in 70% of analyzed sites, demonstrating that the inflammatory process was already set into periodontium.

**Table 3:** Mean and standard deviation of microorganism from toothed and toothless groups

| *Groups* | *Types* | *Average* | *Standard deviation* | *p-value* |
|---|---|---|---|---|
| Toothed | Aa | 13836.3 | 34235.4 | <0.0001 |
| | Pg | 35.9 | 99.1 | |
| | Tf | 870.5 | 936.2 | |
| | TB | 27706871.5 | 58954713.5 | |
| Toothless | Aa | 100225.83 | 220709.13 | <0.0001 |
| | Pg | 2.17 | 6.42 | |
| | Tf | 224.19 | 181.23 | |
| | TB | 132787151.6 | 225241736.2 | |

Total bacterial load (TB); *Aggregatibacter actinomycetemcomitans* (Aa)*; Porphyromonas gingivalis* (Pg)*; Tannerella forsythia* (Tf)

## DISCUSSION

The periodontal profile in this study assessed the first 24 hours of patient admission to the ICU and intended to verify the pre-admission gingival condition. The results

**Table 4:** Mean and standard deviation (SD) of microbiological assessment by qPCR from toothed and toothless groups

| | *TB* | | *Aa* | | *Tf* | | *Pg* | |
|---|---|---|---|---|---|---|---|---|
| *Groups* | *Average* | *SD* | *Average* | *SD* | *Average* | *SD* | *Average* | *SD* |
| Toothed | 27706872 | 58954714 | 13836 | 34235 | 870 | 936 | 36 | 99 |
| Toothless | 132787152 | 225241736 | 100226 | 220709 | 224 | 181 | 2 | 6 |
| Statistics (Mann-Whitney) | 297 | | 328 | | 398 | | 393 | |
| p-value (Mann-Whitney) | 0.261 | | 0.885 | | 0.041 | | 0.021 | |

Total bacterial load (TB); *Aggregatibacter actinomycetemcomitans* (Aa)*; Porphyromonas gingivalis* (Pg)*; Tannerella forsythia* (Tf)

**Table 5:** Assessment of variables interaction for microbiological assessment

| | *TB* | | *Aa* | | *Tf* | | *Pg* | |
|---|---|---|---|---|---|---|---|---|
| *Groups* | *f* | *p-value* | *f* | *p-value* | *f* | *p-value* | *f* | *p-value* |
| Group | 2.830 | 0.115 | 0.950 | 0.401 | 0.830 | 0.384 | 4.950 | 0.043 |
| Age group | 0.480 | 0.751 | 0.760 | 0.615 | 0.910 | 0.496 | 0.220 | 0.923 |
| Gender | 1.230 | 0.287 | 4.850 | 0.045 | 13.070 | 0.005 | 0.390 | 0.544 |
| Ethnic | 0.010 | 0.936 | 0.410 | 0.566 | 0.120 | 0.733 | 1.470 | 0.245 |
| Hospitalization period | 0.620 | 0.443 | 0.230 | 0.668 | 1.060 | 0.327 | 0.060 | 0.804 |
| Pneumonia | 2.790 | 0.117 | 1.540 | 0.303 | 5.660 | 0.039 | 1.240 | 0.284 |
| Leaving | 0.090 | 0.769 | 0.170 | 0.704 | 1.230 | 0.293 | 0.420 | 0.527 |
| Group*Age group | 2.050 | 0.142 | 1.410 | 0.406 | 1.620 | 0.245 | 0.680 | 0.619 |
| Group*Gender | 3.600 | 0.079 | 0.350 | 0.598 | 4.410 | 0.062 | 0.020 | 0.903 |
| Group*Ethnic | 0.330 | 0.577 | 6.480 | 0.023 | 0.070 | 0.798 | 0.520 | 0.483 |
| Group*Hospitalization period | 0.160 | 0.698 | 0.120 | 0.747 | 0.620 | 0.449 | 0.080 | 0.776 |
| Group*Pneumonia | 1.000 | 0.333 | 0.050 | 0.839 | 0.060 | 0.818 | 1.910 | 0.189 |
| Group*Leaving | 0.740 | 0.404 | 0.290 | 0.629 | 0.020 | 0.891 | 1.810 | 0.200 |
| p-value (ANOVA) | 0.161 | | 0.553 | | 0.038 | | 0.790 | |
| p-value (Shapiro-Wilk) | 0.045 | | 0.141 | | 0.985 | | 0.075 | |

Total bacterial load (TB); *Aggregatibacter actinomycetemcomitans* (Aa)*; Porphyromonas gingivalis* (Pg)*; Tannerella forsythia* (Tf)

demonstrated that the patients presented unfavorable periodontal conditions, including high levels of dental biofilm (100%) and gingival bleeding (70%). These results are in accordance with Jones et al[21] study, which described the pattern of dental biofilm accumulation in 137 adult patients under mechanical ventilation, 24 hours after orotracheal intubation during a 7-day period, evidencing that these subjects were already admitted to the ICU with periodontal disease. Those authors also observed that these preexisting problems might increase vulnerability to systemic conditions and complicate the oral hygiene management of the intubated patient.

According to Craven and Steger,[8] ICU inpatients in critical condition present oral hygiene difficulties due to orotracheal tube and impossibility of self-hygiene procedure. Thus, oral cavity suffers from continuous colonization, leading to an unbalance of microbiota, which becomes increasingly aggressive, compromising the subject's integral health. At the ICU where this study was conducted, there is a protocol to have oral hygiene performed once daily, performed by a swab with gauze and antiseptic solution rubbed on patients' teeth, which is performed by the nurse technician. However, this study did not monitor the efficacy of this procedure.

Although the oral microbiota is very diverse, studies correlate some interesting bacterial species as microbiological profile indicators. Analyzing the periodontal disease and its evolution, it was opted to include three bacterial species: *A. actinomycetemcomitans* is considered an important pathogen of the etiology of chronic periodontitis[22]; *P. gingivalis* and *T. forsythia* are bacteria part of the "red complex," highly associated with periodontal tissue destruction.[23]

The microbiological collection of this study was performed on the 6th day after ICU admission. According to Löe et al,[24] the 5th or 6th day after all oral hygiene measures have been ceased, the second bacterial proliferation phase of the dental biofilm occurs, which is when the microorganism proliferation becomes more intense. The poor oral care was intimately related with the dental biofilm and bacterial proliferation increases. During that study, a great initial sample loss was noted, mainly due to death and ICU discharge before the 6th day; a longer period for microbiological sampling would be an obstacle for the performance of this study, decreasing even more the sample number, mainly due to high death rate (70%).

The ICU from the present study is a state reference for neurology and neurosurgery, therefore, presenting patients with high mortality risk, which might explain the high death rate of this study. Most frequent neurological reasons to ICU admission were by cerebrovascular accident followed by cancer and traumatic brain injury.

It was possible to verify a substantial bacterial species decrease of the toothed group against toothless, with a statistical difference for *P. gingivalis* and *T. forsythia.* Such data is in agreement with previous studies conducted by Kishi et al[25] which assessed the microbiota of 165 senior Japanese patients living in the community (93 dentate and 72 edentulous) through PCR for 41 bacterial species and found periodontal pathogenic bacteria most frequently associated to dentate seniors than to edentulous ones. Among the four periodontal pathogenic species assessed, the *T. forsythia* was the most frequently detected, followed by *P. gingivalis*, *Treponema denticola*, and *P. intermedia*. They also suggested that there was a stable periodontal pathogens flow between the gingival sulcus and the tongue surface over time, in the presence of teeth.

The larger prevalence of *P. gingivalis* in dentate patients was also found by Faghri et al,[26] when they studied the *P. gingivalis* prevalence in 61 patients with chronic periodontitis without systemic dysfunctions, with a 40% prevalence in dentate patients. Wara-aswapati et al[4] had the same objective and demonstrated a 45% prevalence of the same bacterium while analyzing sulcular samples in periodontally healthy adults. In accordance with such findings, Lamell et al[27] stated that *P. gingivalis* was the main periodontal pathogen in adults, which corresponded to the periodontal profile of 40% of ICU patients with periodontal disease of the present study.

Edentulous individuals at the ICU, which were critically ill and under orotracheal intubation, presented a great *A. actinomycetemcomitans* prevalence. The loss of teeth, a synonym of gingival sulcus loss, may affect the oral microbiota resulting in a significant periodontal pathogen decrease in healthy subjects.[28] However, the results presented here demonstrated that subjects who were edentulous, critically ill, and submitted to orotracheal intubation presented a greater *A. actinomycetemcomitans* prevalence when compared to the dentate group. Such finding is in accordance with Fernandes et al[29] studies, which assessed the presence of periodontal pathogens in dentate and edentulous senior subjects with previous periodontitis history. The results demonstrated that *A. actinomycetemcomitans* presented a similar incidence in both groups, while other periodontium pathogens were more prevalent in the dentate group.

The analysis of microorganisms was conducted through a molecular tool, qPCR, which was chosen due to its larger sensitivity and accuracy, precision, reproducibility, accuracy, analysis speed, better quality control in processing and a lower contamination risk.[30]

Epidemiological studies in systemically healthy subjects demonstrated a larger prevalence of periodontal

disease in men, reflecting greater deficiency to oral hygiene in this population.[31-33] In this study, the quantity of periodontal pathogens *A. actinomycetemcomitans* and *T. forsythia* were significantly larger in male subjects than in female ones, demonstrating that ICU patients follow the same patterns of healthy subjects.

The present study demonstrated that 32.5% of patients have developed nosocomial pneumonia, similarly to Morrow et al[34] study, in which around 30% of mechanically ventilated patients were diagnosed with ventilation-associated nosocomial pneumonia (VAP). According to Heo et al,[13] the dental biofilm acts as an important reservoir for respiratory pathogens in mechanically ventilated patients. Munro et al[35] have also demonstrated the relationship between the oral health conditions and VAP development, but Lazarevic et al,[16] in a recent study, suggest that the correlation of the oral hygiene status and VAP is still divergent.

## CONCLUSION

This study elucidated that critically ill patients admitted to the ICU presented unfavorable periodontal conditions that existed prior to admission to the unit. On microbiological test, a larger presence of *T. forsythia* and *P. gingivalis* microorganisms was noted in the dentate group, while the edentulous group presented a larger quantity of *A. actinomycetemcomitans*, thus suggesting that, even when there in the absence of teeth, the oral environment works as a reservoir for formation and development of a biofilm with a great pathogenic potential.

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