# Evaluation of oral changes and modification of the oral microbiome in patients admitted to the Intensive Care Unit

Avaliação das alterações bucais e modificação do microbioma bucal em pacientes internados na Unidade de Terapia Intensiva

Evaluación de cambios bucales y modificación del microbioma bucal en pacientes ingresados en la Unidad de Cuidados Intensivos

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**Abstract**
Introduction: Chlorhexidine oral decontamination protocols are the usual care offered to patients in Intensive Care Units (ICU). The admission evaluation performed by dentists is absent from the routine, leading the medical team to overlook oral diseases, as they are rarely the main cause of hospitalization and the common absence of pain or other symptoms. Healthy or not, the oral cavity has an extensive reservoir of pathogens and can spread systemic infections and modify or worsen the general health of hospitalized patients. Methods: We conducted a prospective longitudinal study in the general ICU of Hospital Sírio Libanês, in São Paulo. Oral health was assessed at admission, three, and seven's day of hospitalization by clinically (bedside oral examination (BOE), plaque and mucosa index (MPS), presence of infectious foci) and microbiologically indexes(identification of microorganisms in saliva and biofilm).Results: Sixty patients were initially included, with 70% of oral care dependency, and 39.47% were admitted and remained with oral infectious foci throughout the study. 38 patientes clinically followed presented improvement in BOE and MPS indices. With no statistically significant differences between saliva and biofilm during the hospital stay, 27 patients had a prevalence of Candida spp, ESKAPE (E. faecalis, S.aureus, K.pneumoniae, A.baumannii, P.mirabilis, and Enterobacter spp.) and pathogens associated with nosocomial pneumonia (S.aureus, K.pneumoniae, P.aeruginosa, E.cloacae, P.mirabilis, Streptococcus spp, E.coli, H.influenza, and S.pneumoniae). Conclusion: In addition to nursing care, oral assessment at hospital admission and dental follow-up can align patients' oral hygiene protocols with their real systemic needs.
**Keywords:** Intensive unit care; Oral care; Critical care.

**Resumo**
Introdução: Os protocolos de descontaminação oral com clorexidina são os cuidados usuais oferecidos aos pacientes em Unidades de Terapia Intensiva (UTI). A avaliação de internação realizada pelos dentistas está ausente da rotina, levando a equipe médica a negligenciar as doenças bucais, pois raramente são a principal causa de internação e a ausência comum de dor ou outros sintomas. Saudável ou não, a cavidade oral possui um extenso reservatório de patógenos, podendo disseminar infecções sistêmicas e modificar ou piorar a saúde geral dos pacientes hospitalizados. Métodos: Foi realizado um estudo longitudinal prospectivo na UTI geral do Hospital Sírio Libanês, em São Paulo. A saúde bucal foi avaliada na admissão, três e sete dias de internação por índices clínicos (exame bucal à beira do leito (BOE), índice de placa e mucosa (MPS), presença de focos infecciosos) e microbiologicamente (identificação de microrganismos na saliva e biofilme). Resultados: Inicialmente foram incluídos 60 pacientes, com 70% de dependência de higiene bucal, e 39,47% foram internados e permaneceram com focos infecciosos bucais durante todo o estudo. 38 pacientes acompanhados clinicamente apresentaram melhora nos índices BOE e MPS. Sem diferenças estatisticamente significativas entre saliva e biofilme durante a internação, 27 pacientes tiveram prevalência de Candida spp, ESKAPE (E. faecalis, S.aureus, K.pneumoniae, A.baumannii, P.mirabilis e Enterobacter spp.) e patógenos associados à pneumonia nosocomial (S.aureus, K.pneumoniae, P.aeruginosa, E.cloacae, P.mirabilis, Streptococcus spp, E.coli, H.influenza e S.pneumoniae). Conclusão: Além dos cuidados de enfermagem, a avaliação bucal na admissão hospitalar e o acompanhamento odontológico podem alinhar os protocolos de higiene bucal do paciente às suas reais necessidades sistêmicas.
**Palavras-chave:** Unidade de terapia intensiva; Higiene bucal; Cuidados intensivos.

**Resumen**
Introducción: Los protocolos de descontaminación oral con clorhexidina son los cuidados habituales que se ofrecen a los pacientes en las Unidades de Cuidados Intensivos (UCI). La evaluación de ingreso realizada por los odontólogos está ausente de la rutina, lo que lleva al equipo médico a pasar por alto las enfermedades bucales, ya que rara vez son la principal causa de hospitalización y la ausencia común de dolor u otros síntomas. Saludable o no, la cavidad oral tiene un reservorio extenso de patógenos y puede propagar infecciones sistémicas y modificar o empeorar la salud general de los pacientes hospitalizados. Métodos: Realizamos un estudio longitudinal prospectivo en la UTI general del Hospital Sírio Libanês, en São Paulo. La salud bucal se evaluó al ingreso, tres y siete días de hospitalización mediante índices clínicos (examen oral de cabecera (BOE), índice de placa y mucosa (MPS), presencia de focos infecciosos) y microbiológicos (identificación de microorganismos en saliva y biopelícula). Resultados: Inicialmente se incluyeron 60 pacientes, con un 70% de dependencia del cuidado bucal, y un 39,47% ingresaron y permanecieron con focos infecciosos bucales durante todo el estudio. 38 pacientes seguidos clínicamente presentaron mejoría en los índices BOE y MPS. Sin diferencias estadísticamente significativas entre la saliva y el biofilm durante la estancia hospitalaria, 27 pacientes presentaron prevalencia de Candida spp, ESKAPE (E. faecalis, S.aureus, K.pneumoniae, A.baumannii, P.mirabilis y Enterobacter spp.) y patógenos asociados con neumonía nosocomial (S.aureus, K.pneumoniae, P.aeruginosa, E.cloacae, P.mirabilis, Streptococcus spp, E.coli, H.influenza y S.pneumoniae). Conclusión: Además de los cuidados de enfermería, la evaluación bucal al ingreso hospitalario y el seguimiento odontológico pueden alinear los protocolos de higiene bucal de los pacientes con sus necesidades sistémicas reales.
**Palabras clave:** Unidad de cuidados intensivos; Cuidado bucal; Cuidado crítico.

## 1. Introduction

Studies show the negative impact of hospitalization in the oral cavity (Sachdev et al., 2013; Terezakis et al., 2011). Accumulation of oral biofilm, hyposalivation, and candidiasis is the most common affections (Cruz et al., 2014; Needleman et al., 2012; Silva et al., 2014). In cancer patients, oral mucositis and candidiasis are closely linked to treatments for the underlying disease(Silva et al., 2014). When not previously treated, these oral complications can lead to severe oral pain, interruption, and suspension of systemic treatment, increasing the length of stay and hospital costs (Bezinelli et al., 2014).

Dental infections mainly consist of tooth decay and periodontal disease (gingivitis and periodontitis) with common implications such as tooth loss, orofacial pain, and, in some cases, systemic implications (Hajishengallis, 2014; Kumar, 2017; Li et al., 2000). These oral diseases can extend beyond natural barriers and result in potentially fatal complications, such as infections of the deep fascial spaces of the head and neck (Bali et al., 2015; Gilway & Brown, 2016; Lyle et al., 2011; Segura-Sampedro et al., 2016; Tosun et al., 2005).

Periodontal infection can also be associated with a variety of systemic disorders, including fever of unknown origin, bacterial spread to heart valves and prosthetic devices, premature birth of low birth weight children, and increased risk of coronary heart disease and cerebrovascular events (Bali et al., 2015; Hajishengallis, 2014; Nagpal et al., 2015).

Despite these scientifically established premises, the bidirectional relationship between oral diseases and systemic diseases and their treatments is disregarded, and the only oral care for patients offered in the ICU are oral hygiene protocols with chlorhexidine, procedures included in prevention bundles of ventilator-associated pneumonia (VAP) (Deschepper et al., 2018).

In a review of the construction of this paradigm, Parreco et al. (Parreco et al., 2020) ) described that, in the last two decades, more than 20 studies had evaluated the efficacy of oral chlorhexidine in reducing VAP rates. However, most of the first tests include patients undergoing cardiac surgery, with a short stay in the ICU and little opportunity to develop VAP. Three initial reports and limitations coincided with the widespread adoption of guidelines and packages to prevent VAP, which eventually included oral hygiene with an antiseptic agent. A controlled observational study showed the relationship between the use of oral chlorhexidine and mortality(Deschepper et al., 2018) and authors who contraindicate its indiscriminate use and that there is a need for more research on systemic outcomes associated with oral hygiene in the ICU (Bouadma et al., 2018; Dale et al., 2019; Klompas et al., 2014; Papazian et al., 2020).

Thus, this protocol is far from being the gold standard for oral care integrated into patients' systemic conditions. We reinforce the importance of epidemiological studies to understand oral characteristics, systemic outcomes, and actual needs for dental care during hospitalization.

In this context, our study evaluated the clinical and microbiological oral conditions of patients admitted to the ICU and their evolution over a week of hospitalization.

## 2. Methodology

This single-center study was approved, and a waiver for informed consent was granted, by the Hospital Sírio Libanês Institute of Research Ethics Committee (approval number HSL 2016-24). Informed written consent was obtained from each patient or the answerable for.

This was a prospective observational study of a convenience sample of consecutive 60 patients admitted in the general ICU of Sírio Libanês Hospital, Brazil, with a 7-day hospital stay forecast, reported on admission by the patient's doctor. The unit has 30 beds distributed in 3 integrated aisles and offers intensive care to medical and surgical patients. It is managed by doctor-led multidisciplinary teams and intensivists.

The systemic disease and its severity was evaluated in admission by Simplified Acute Physiology Score (SAPS3), Charlson index, Sequential Organ Failure Assessment (SOFA). Length of stay in the ICU and total hospital length of stay. This data was obtained through reports from the Epimed program, which houses and manages information for critically ill patients.

Patients enrolled in the study were prospectively followed up three times during hospitalization: at the first 24hours (D1), 72 hours before (D4), and a week (D8).

The initial oral conditions were characterization by the permanent decayed, lost, and filled teeth index (DMFT). With the aim of include patients with dental prostheses and a specific record of oral mucosa changes in the study, we herein as oral alterations the bedside oral care exam (BOE), mucosal plaque score (MPS), hipossalivação, mucositis, tongue index plaque (TPI). The presence of oral inflammations and infectious foci (exposed cavities, gingival inflammation with dental calculus and/or others periodontal involvement) were noted too.

Simultaneously to clinical evaluations, samples of saliva and biofilm were collected. Saliva was obtained from the oral floor, unstimulated, with a sterile Pasteur plastic pipette. The dental biofilm sample was carefully collected with a dental

curette from the supra-gingival surface of the lower incisors teeth, e, in the absence of these, from the surfaces of the same place, but in prosthesis. Samples were stored in a plastic tube (Eppendorf, Germany) and frozen at -80º C. All pipettes and tubes were properly sterilized by the hospital's materials sterilization center. Adult patients with any number or condition of teeth and / or patients with dental prostheses were considered eligible for the study. The inclusion criterion was considered the estimated hospital stay longer than seven days registered at the time of admission to the ICU. Totally edentulous patients without any kind of dental prosthesis were excluded. The examinations were performed in the interval between oral hygiene and taking care not to change the daily routine of the patient and the multidisciplinary care team.

**Oral microorganisms identification**

The microbiological identification technique used was MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) Biotyper. It was used to identify/classify microorganisms as it is suitable for high productivity and rapid microbial identification at low costs, being an alternative to the conventional biochemical tests (Croxatto et al., 2012)

MALDI-TOF BioTyper was validated by comparison with the "gold standart" 16S rDNA sequential analysis(Timperio et al., 2017). Many researches demonstrated its usefulness with high sensitivity and high productivity, time of analysis, and easy of use (Alves et al., 2016; Antezack et al., 2020; Wang et al., 2015).

**Bacteria Isolation**

Bacteria were isolated from saliva and oral biofilm using Brain Heart Infusion (BHI) agar 37 gL$^{-1}$. Samples were grown at 37º C, during approximately 3 days. In order to purify the bacteria morphologically and, obtaining single colonies this process was repeated as many times as necessary.

**MALDI-TOF Biotyper Analysis**

In tube extraction protocol were used since prior test showed that the higher scores were observed for the in tube extraction (11). The extraction protocol is described next.

Bacterial cells were transferred to an extraction tube (Eppendorf, Germany) containing 100 µL of ultra purified water. After, 300 µL of ethanol was added and the sample was centrifuged during 2 min at 17900×g. The supernatant was discharged, and residual ethanol removed, The pellet was suspended in 30 µL of 70% formic acid and vortexed. Then 30 µL of Acetonitrile was added, vortexed and centrifuged. The α-cyano-4-hydroxycinnamic acid matrix was prepared as a saturated solution in 50% acetonitrile and 2.5% trifluoroacetic acid. Subsequently, 0.6 µL of sample extract was spotted onto a steel target plate and air dry. Then, 0.6 µL of matrix solution was added and left to air dry.

Mass Spectrometry analyses were carried out by an UltrafleXtreme MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) operating in the linear positive ion mode. Mass spectra were acquired in a mass range from 2 to 20 kDa with ions formed by irradiation of smartbeam using a frequency of 2,000 Hz, PIE 100ns, 7kV lens. The voltage for the first and second ion sources was 25kV and 23kV, respectively. Microorganisms were identified by means of the Biotyper 3.1 database. The identification cut off values higher than 2 and 1.7 were used for species and genus identification, respectively. All sample collection and processing processes were previously tested, standardized, and validated (Alves et al., 2016)

**Statistical Analysis**

Continuous and semi-continuous data were initially compared with the Gauss curve using the K-S distance test (Kolmogorov-Smirnov). After being determined as non-parametric, they were represented by median and 25-75 percentile.

Categorical data were represented by absolute (n) and relative (%) frequency. Two-time analysis for the same sample was performed using the Wilcoxon test. The IBM SPSS 20.0 statistics program was used.

## 3. Results

Of the 60 patients initially included and had the first examination performed on D1, nine left the hospital before D4 (eight discharged and one death). From D4 to D8, 13 patients were discharged home (Table 1).

Despite the loss to follow-up in 22 patients, the remaining 38 did not present significant differences in their clinical characteristics when compared to the 60, and in this group, 11 presented samples that were not viable for various reasons. Therefore, the microbiological study was completed with 27 patients (Table 1).

The average length of stay in the ICU was 3 (2-7) days, and the total length of stay was 22 (11-33) days. Patients discharged from the ICU and admitted to other hospital units were followed up (Table 1).

The dental conditions of the patients presented two contradictions of results to be observed: although with a high rate of missing teeth [21 (6-32)] and despite being partially or edentulous, all patients were rehabilitated with a dental prosthesis - 21 (78 .8%) in the upper jaw and 15 (56.6%) in the lower jaw) (Table 1). However, 8 (30%) patients had to replace the prosthesis among the rehabilitated due to fracture, inadequacy, or excessive wear.

**Oral changes during hospitalization**

During hospitalization, there was an improvement in the BOE index through a significant decrease in severe dysfunction score from 17 (63%) to 4 (15%) (Table 2). Hyposalivation, Tpi, and MPS also showed clinical improvement during hospitalization when compared to D1.

**Table 1 -** Characteristics of the 60 baseline and 38 clinically followed up patients*.

| Characteristics | 60 | 38 |
|---|---|---|
| Median age - n(p25-75) | 75.3(67-85) | 76(67-85) |
| Gender – male - n(%) | 35(58.3) | 18(47.4) |
| Admission type name - n(%) | | |
|     Neurological Diseases | 16(26.7) | 10(26.32) |
|     Sepsis | 21(35) | 15(39.4) |
|     Respiratory failure | 8(13.3) | 8(21) |
|     Others[1] | 15(25) | 5(13.1) |
| Gravity Scores - median(p25-p75) | | |
|     SAPS3 | 51.5 (41.5-58) | 52(43.5-58) |
|     SOFA | 3(1-6) | 3.5(1-8) |
|     Length of ICU stay | 3(1-5) | 3(2-7) |
|     Length of hospital stay | 11(7-22.5) | 18.2(11-29.7) |
| Nutritional status - n(%) | | |
|     Oral | 38(63.3) | 19(50) |
|     Non oral[2] | 22(35.7) | 19(50) |
| Type of ventilation - n(%) | | |
|     Self-ventilation | 32(53.3) | 14(36.8) |
|     Face mask oxygen | 17(28.3) | 14(36.8) |
|     Invasive ventilation[3] | 11(17.4) | 10(26.3) |
| Medications during the study - n(%) | | |
|     Antibiotics | 43(71.7) | 31(81.58) |
|     Corticoides | 20(30) | 20(52.63) |
| Dental characteristics | | |
|     DMFT (median, p25-75) | 23.5(16-32) | 23.5(16-32) |
|     D (decayed) | 0(0-0) | 0(0-0) |
|     M (missed) | 20(20-32) | 20(20-32) |
|     F (filled) | 0(0-5.5) | 0(0-5.5) |
| Oral Prosthesis use - n(%) | | |
|     upper jaw | 46(76.7) | 27(61.1) |
|     lower jaw | 33(55) | 20(52.63) |

*p <0.005 between the groups. [1] postoperative monitoring, changes in the digestive system, cardiovascular and hematological [2] include nasogastric and parenteral feed. [3] include oral endotrachal tube and tracheostomy. Source: Authors.

Among the 15 patients on D1 with oral infectious foci, 12 remained the same throughout the hospital stay (8 with several foci plus periodontal diseases; three with periodontal diseases and one prosthesis fracture). During hospitalization, dental problems were resolved by identifying the problem and activating the dental team: on admission 3 D1 patients - one patient with intubated and tracheostomized orthodontic appliance, one patient with tongue interposition due to trauma/self-injury and oral candidiasis (Table 2). All other lesions presented by the patients were oral candidiasis.

The vulnerability of patients concerning oral care was high, with 19 (70%) patients dependent on oral hygiene and 17 (63%) to 19 (70%) patients included in the oral hygiene protocol with chlorhexidine.

**Table 2 -** Oral clinical changes during hospitalization, 38 patients*

| Oral characteristics | Day of hospitalization | | |
|---|---|---|---|
| | D1 n(%) | D4 n(%) | D8 n(%) |
| **Oral Comorbities** | | | |
| Hipossalivation | 29 (76.3) | 22(57.9) | 19(50)* |
| Tpi | 30(78.9) | 17(44.7)* | 17(44.7)* |
| Oral foci | 15(39,47) | 15(39,47) | 15(39,47) |
| Oral lesions | 3(7,89) | 9(23,68)* | 8(21,05)* |
| **Bedside Oral Exam** | | | |
| Normal | 3(7.9) | 5(13.1) | 11(28.9)* |
| Moderate dysfunction | 16(42.1) | 23(60.5) | 21(55.2) |
| Severe dysfunction | 19(50) | 10(26.3)* | 6(15.8) *† |
| **MPS** | | | |
| Acceptable | 14(36.8) | 21(55.2)* | 24(63.1)* |
| Unacceptable | 20(52.6) | 14(36.9) | 11(28.9)* |
| Poor | 4(10.5) | 3(7.9) | 3(7.9) |
| **Routine oral care** | | | |
| Chlorhexidine protocol | 26(68.4) | 25(65.7) | 24(63.1) |

*: $p < 0.05$ vs D1 †: $p < 0.05$ vs D4 - Wilcoxon test. BOE- Bedside oral exam. MPS – mucosal plaque index. Tpi - tongue plaque index. Source: Authors.

**Microbiome Results**

In saliva, we found ten unique microorganisms and in biofilm four (Table 3). In addition, we identified 26 common microorganisms at both locations.

The clinical and oral diversity of the patient was reflected in the microbiological findings: ten microorganisms were found once in the patients' saliva*: A. baumannii, A.* pascens, *K. pneumoniae, N. macacae, Novosphingobium resinovorum, S.mitis* e S.*parasanguis, L.paracasei, paralimentarius* e *vialimentarius*). The biofilm showed four unique microorganisms: *B.safenis, C.albicans_africana, P.oryzuhabitante*s e *P.aeruginosa*) (Table 3).

**Table 3 -** Frequency of microrganisms found throughout the study, according to saliva and biofilm, 27 patients.

| | Biofilm | | | Saliva | | |
|---|---|---|---|---|---|---|
| | D1 | D4 | D8 | D1 | D4 | D8 |
| *Candida albicans* | 15 | 14 | 7 | 8 | 9 | 9 |
| *Candida glabrata* | 2 | 0 | 0 | 1 | 2 | 1 |
| *Candida orthopsilosis* | 1 | 0 | 0 | 0 | 1 | 0 |
| *Candida parapsilosis* | 1 | 1 | 1 | 2 | 1 | 1 |
| *Corynebacterium striatum* | 1 | 1 | 0 | 1 | 1 | 0 |
| *Enteroccocus faecalis* | 5 | 3 | 4 | 2 | 2 | 2 |
| *Escherichia coli* | 0 | 2 | 3 | 1 | 0 | 0 |
| *Neisseria flavescens* | 1 | 0 | 0 | 2 | 0 | 0 |
| *Neisseria meningitidis* | 0 | 0 | 2 | 1 | 0 | 1 |
| *Neisseria perflava* | 0 | 0 | 1 | 1 | 0 | 0 |
| *Neisseria polysaccharea* | 1 | 0 | 0 | 1 | 0 | 0 |
| *Proteus mirabilis* | 1 | 0 | 2 | 0 | 0 | 1 |
| *Rothia dentocariosa* | 1 | 2 | 0 | 4 | 3 | 1 |
| *Serratia marcenscens* | 0 | 0 | 1 | 0 | 0 | 1 |
| *Staphylococcus aureus* | 2 | 1 | 1 | 3 | 1 | 2 |
| *Staphylococcus capitis* | 0 | 0 | 1 | 1 | 0 | 1 |
| *Staphylococcus condimenti* | 0 | 1 | 0 | 0 | 0 | 1 |
| *Staphylococcus epidermidis* | 3 | 2 | 4 | 5 | 10 | 8 |
| *Staphylococcus haemolyticus* | 1 | 0 | 1 | 1 | 1 | 2 |
| *Staphylococcus hominis* | 1 | 0 | 1 | 2 | 2 | 1 |
| *Stenotrophomonas_maltophilia* | 0 | 0 | 1 | 0 | 0 | 3 |
| *Streptococcus oralis* | 2 | 1 | 1 | 4 | 1 | 2 |
| *Streptococcus pneumoniae* | 2 | 1 | 0 | 2 | 1 | 2 |
| *Trichosporan asahii* | 1 | 1 | 1 | 1 | 0 | 0 |
| *Acinetobacter baumannii* | - | - | - | 0 | 1 | 0 |
| *Artherobacter pascens* | - | - | - | 1 | 0 | 0 |
| *Enterobacter cloacae* | - | - | - | 1 | 0 | 0 |
| *Klebsiela pneumoniae* | - | - | - | 0 | 0 | 1 |
| *Lactobacillus paracasei spp* | - | - | - | 0 | 0 | 1 |
| *Lactobacillus paralimentarius* | - | - | - | 1 | 0 | 0 |
| *Lactobacillus vini* | - | - | - | 0 | 0 | 1 |
| *Micrococcus luteus* | - | - | - | 1 | 1 | 1 |
| *Neisseria macacae* | - | - | - | 0 | 1 | 0 |
| *Novosphingobium resinovorum* | - | - | - | 1 | 0 | 0 |
| *Streptococcus mitis* | - | - | - | 1 | 0 | 0 |
| *Streptococcus parasanguinis* | - | - | - | 0 | 1 | 0 |
| *Bacillus safensis* | 1 | 0 | 0 | - | - | - |
| *Candida albicans_africana* | 0 | 0 | 2 | - | - | - |
| *Enterobacter cloacae* | 1 | 0 | 1 | - | - | - |
| *Pseudomonas aeruginosa* | 1 | 0 | 1 | - | - | - |
| *Pseudomonas oryzuhabitans* | 1 | 0 | 0 | - | - | - |

Source: Authors.

*Staphylococcus epidermidis* t was the second most found microorganism. Distributions and changes during hospitalization were different in saliva [D1 (18%), D4 (37%), D8 (30%] of the biofilm [D1 (11%), D4 (7%), D8 (15%)] (Table 3) There was no statistically significant difference between the findings, whether compared by collection day or medium (saliva/biofilm).

Due to the wide variation of microorganisms found, we grouped the most frequent ones into three groups, which, in saliva and biofilm, were the same: *Candida spp* (*C.glabrata*, *C.orthopsilosis*, *C.albicans_africana*), ESKAPE(*E. faecalis*, *S.aureus*, *K.pneumoniae*, *A.baumannii*, *P.mirabilis* e *Enterobacter spp.*) and HAP(*S.aureus*, *K.pneumoniae*, *P.aeruginosa*, *E.cloacae*, *P.mirabilis*, *Streptococcus spp*, *E. coli*, *H. influenzaeee* and *S.pneumoniae*) (Graph 1).

**Graph 1 -** Frequency of microrganisms in 27 patients according to the most three prevalent group in saliva and biofilm, during hospitalization: *Candida spp*, ESKAPE and HAP (error bars: 95%). - *Candida spp* (*C.glabrata*, *C.orthopsilosis*, *C.albicans_africana*), ESKAPE(*E. faecalis*, *S.aureus*, *K.pneumoniae*, *A.baumannii*, *P.mirabilis* e *Enterobacter spp.*) *e* patógenos associados à pneumonia nosocomial - hospital-acquired pneumonia(HAP: *S.aureus*, *K.pneumoniae*, *P.aeruginosa*, *E.cloacae*, *P.mirabilis*, *Streptococcus spp*, *E. coli*, *H. influenzae* e *S.pneumoniae*).
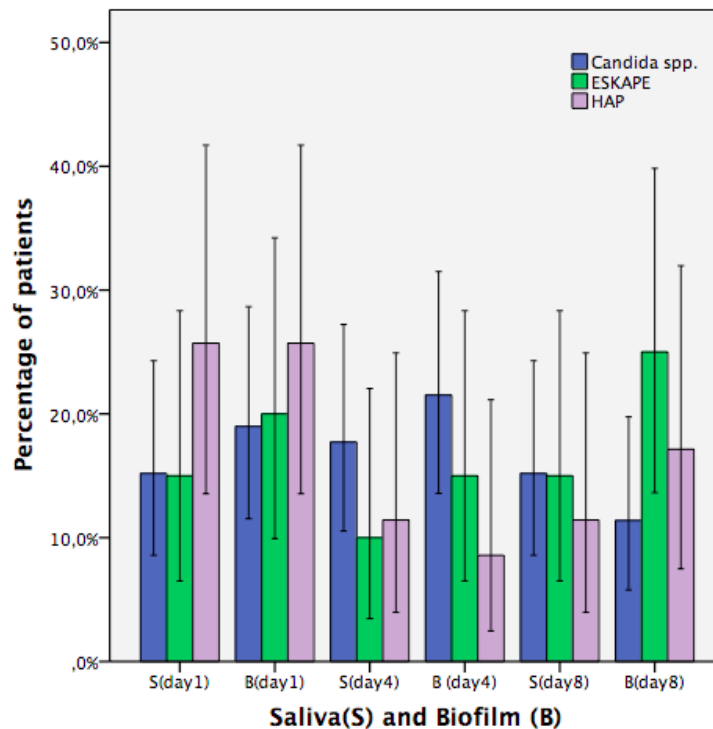


Source: Authors.

## 4. Discussion

Our main finding was the observation that patients admitted to our ICU had their oral hygiene indexes improved during hospitalization due to well-established protocols by the hospital's nursing staff. In addition, the dental team was called when nurses identify oral complications. However, this model does not consider patients' clinical and oral microbiological conditions: 39% of patients were hospitalized and remained with oral infectious foci during hospitalization. In addition, 70% had candida in biofilm and 35% in saliva, and a considerable percentage of PAH (30%).

**Weakeness and strenghts**

We understand that this was the first study that described oral conditions in our general ICU hospital, and these results will be helpful for further research, on a larger scale, with the possibility of avoiding sample size calculation bias.

The strength of our study was the measurement of oral alterations with adequately tested and validated indices, regardless of the presence of a specific group or number of teeth.

Historically, periodontal disease is considered the central disseminator of oral pathogens associated with the VAP outcome. However, this disease only affects teeth and their supporting tissues. Thus, its measurement indices exclude patients who do not have specific numbers or teeth, such as older adults with dentures. Thus, contradictorily, although the elderly stay longer in the ICU when they are mechanically ventilated, they are included in oral hygiene to prevent VAP, without their oral characteristics having been considered in the established oral care protocols.

To solve this issue, our study was groundbreaking. We included all patients with dentures and dentate using the BOE bedside oral examination and the MPS mucosa and plaque index, both validated and published in the literature.

Prendergast et al, in 2013 published a study where a multidisciplinary health care team used a PDCA (Plan, Do, Check and Act = Plan, Do, Check and Act) to build a cost-effective guide to oral hygiene in the ICU, the from the BOE.

**Socioeconomic determinants of oral conditions and care**

As in the world population, the elderly in our study had significant tooth loss. In contrast, despite the good socioeconomic conditions benefiting these patients with the replacement of lost teeth with prostheses and sophisticated dental implants, it was a population with oral problems such as poorly adapted dentures, periodontal diseases, and chronic caries.

Several studies have shown poor oral conditions of totally dependent older adults, reinforcing the importance of training in oral care for the medical team and caregivers of frail, immunosuppressed patients who do not verbalize pain or other oral symptoms(Arpin et al., 2008; Baumgartner et al., 2015; Berkey & Scannapieco, 2013; Preston et al., 2006; Scannapieco & Shay, 2014).

**Oral conditions associated to sistemic outcomes**

The improvement in our clinical oral indices throughout hospitalization was due to the well-structured nursing team. However, we noted the inconsistency between the patients' oral condition and the clinical outcome associated with the oral care protocol.

In our ICU, oral decontamination with chlorhexidine is another standard of recommendation. This protocol is based on the PAV outcome, and our rates are five episodes/year. In addition, the use of chlorhexidine was formulated based on periodontal disease, which excludes the oral characteristics of our patients: elderly people with prostheses, the predominant study population.

We corroborate with Vogelarers et al. and Klompas et al., who show the importance of the associated systemic outcome and the reassessment of oral care instead of the widespread and indiscriminate use of oral chlorhexidine(Klompas et al., 2014; Vogelaers et al., 2017).

With these results, we suggest that future studies include patients with sepsis, a profile of 39.4% of the study population, among the significant causes of admissions to our service. It is a disease with high morbidity, mortality, and prevalence in the world population. A premise in treating sepsis is infectious foci, which must be diagnosed, located, and treated (Azevedo et al., 2018). In contrast, 50% of the patients with sepsis in our study were not included in the criteria of the decontamination protocols, and 40% of them had infectious oral foci.

Bellissimo et al. They showed the benefits of the dentist in the routine of the ICU team. They achieved a reduction in lower respiratory tract injuries when, in addition to oral hygiene care, there was the removal of oral infectious foci (Bellissimo-Rodrigues et al., 2014).

**The puzzle of oral microbiome, oral care and systemic outcomes in critical patients**

Historically, the identification of microorganisms by MALDI-TOF Biotyper has been shown to be a good option for use in saliva and biofilm. With a larger sample, our results were similar to Singer et al.,(Sachdev et al., 2013) with 30% of patients with PAH. In addition to these, we also find other microorganisms that are not common to the oral cavity, ESKAPE, and *Candida spp*.

We shed significant light on the role of C. Albicans informing resident bacterial communities and driving oral mucosal dysbiosis. Likewise, *Candida spp*. and *Pseudomonas* were among the most common pathogens recovered from endotracheal tube biofilm and tracheal secretions in patients with VAP(Krause et al., 2016).  El-Azizi et al. reported that *C. Albicans* biofilms might contain other organisms, including P. *aeruginosa*, within the endotracheal tube, may be associated with dental prostheses and were found more frequently in biofilms(Krause et al., 2016).

In addition, the high frequency of Candida spp may be a transient manifestation due to systemic factors specific to the hospital or systemic condition of the patient (immunosuppression, diabetes, smoking, diabetes, HIV, and use of antibiotics)(Singh et al., 2014). ). On the other hand, it may represent colonization with the risk of candidiasis. In this case, the oral manifestation can be the first sign/symptom.

**Saliva e biofilm**

Also associated with the high frequency of C. Albicans may be the increased rates of hyposalivation of patients in our study. Older adults often present decreased salivary flow as a cause of dehydration, lousy posture, orotracheal tube, medications, or diseases. Hyposalivation can lead to tender points in the mucosa, lack of retention, and contribute to mucosal lesions. Some salivary parameters, such as salivary flow and pH, are related to each other, and a reduction in salivary flow generates a significant decline in oral defense systems, which can cause caries and inflammation of the oral mucosa.

The non-shedding characteristics of oral bacteria uniquely define the tooth surface compared to the natural shedding surface of the oral mucosa. Consequently, when teeth are not cleaned, the tooth surface can harbor pathogenic organisms that lead to the development of thick biofilms and an increased microbial load of pathogens (Marsh et al., 2016).

The same happens with dental prostheses but with a different microbiome from teeth. Our most frequent finding Candida spp is strongly associated with local causes, such as the use of dentures, poor oral hygiene, oral infectious foci, and antibiotics. These data corroborate the recommendation for early diagnosis of oral candidiasis and a hygiene protocol for dental prostheses (Bianchi et al., 2016; Garcia-Cuesta et al., 2014).

## 5. Final Considerations

Patients admitted to our ICU had their oral hygiene indexes improved during hospitalization due to well-established protocols by the hospital's nursing staff. In addition, the dental team is called when nurses identify oral complications. However, this model does not consider patients' clinical and oral microbiological conditions: 39% of patients were hospitalized and remained with oral infectious foci during hospitalization. In addition, 70% had candida in biofilm and 35% in saliva, and a considerable percentage of HAP (30%).

Epidemiological surveys of oral health in ICUs can describe the oral health conditions on admission, the patient's oral self-care and its association with the treatment of the underlying disease, and the clinical evolution throughout the hospital stay. These data can help define the systemic outcome associated with oral health conditions, optimize the workload of the nursing team, and cost-effectively structure dental services.

Based on our results and the references described above, we suggest new studies with care models centered on the patient's needs. We suggest studies that can help clarify the complexity of the following factors associated with oral care in the ICU: oral conditions on admission, underlying diseases, medications in use, use of antibiotics, and current types of oral care offered.

The multidisciplinary team plays an essential role in maintaining the oral hygiene of patients during hospitalization. Oral Care in the ICU must be reformulated based on the combination of oral conditions on admission and the bidirectional relationship between oral diseases and systemic diseases and their treatments. Oral evaluation from hospital admission and dental follow-up can benefit patients, individualizing and optimizing oral care during the hospital stay.

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