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ORIGINAL ARTICLE

Oral and endotracheal tubes colonization by periodontal bacteria: a case–control ICU study

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Abstract Periodontal infection is a possible risk factor for respiratory disorders; however, no studies have assessed the colonization of periodontal pathogens in endotracheal tubes (ET). This case–control study analyzed whether periodontal pathogens are able to colonize ET of dentate and edentulous patients in intensive care units (ICU) and whether oral and ET periodontal pathogen profiles have any correlation between these patients. We selected 18 dentate and 18 edentulous patients from 78 eligible ICU patients. Oral clinical examination including probing depth, clinical attachment level, gingival index , and plaque index was performed by a single examiner, followed by oral and ET sampling and processing by quantitative polymerase chain reaction (total bacterial load, *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,* and *Tannerella forsythia*). Data were statistically analyzed by Mann–Whitney U, two-way analysis of variance ($p < 0.05$). Among dentate, there was no correlation between clinical parameters and ET bacterial levels. Both dentate and edentulous patients showed similar ET bacterial levels. Dentate patients showed no correlation between oral and ET bacterial levels, while edentulous patients showed positive correlations between oral and ET levels of *A. actinomycetemcomitans, P. gingivalis,* and *T. forsythia*. Periodontal pathogens can colonize ET and the oral cavity of ICU patients. Periodontal pathogen profiles tend to be similar between dentate and edentulous ICU patients. In ICU patients, oral cavity represents a source of ET contamination. Although accompanied by higher oral bacterial levels, teeth do not seem to influence ET bacterial profiles.

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Introduction

Periodontal medicine can be viewed as a broad term that defines a branch of periodontology that has emerged within the last two decades. This medical field focuses on a wealth of new data that establishes a strong relationship between periodontal health and systemic health or disease status [1] and establishes periodontal infection as a probable risk factor for systemic diseases, including respiratory disorders [2].

The oral cavity has two main types of surfaces for microbial colonization, nonshedding surfaces (teeth) and shedding surfaces (mucosa), which lead to high microbial diversity. The number of different bacterial species identified in the human oral cavity is close to 700 [3]. There is also scientific evidence that some oral bacterial species, are implicated in causing pneumonia and lung abscesses [4–6].

Several mechanisms could explain how oral bacteria participate in the pathogenesis of respiratory infections. Microorganisms can contaminate the lower airways by four possible routes: (1) aspiration of oropharyngeal content [7], (2) inhalation of infectious aerosols [8], (3) spread of infective agents from contiguous sites [9], and (4) hematogenous spread from extrapulmonary sites of infection [10].

Aspiration of oropharyngeal content is the most common route of lower airways infection, making the oral cavity a possibly important source of bacteria that causes infections of the lungs. Although it has been suggested

that dental plaque, a tooth-borne biofilm that initiates periodontal disease and dental caries, may influence the initiation and progression of pneumonia [11], the participation of periodontal pathogens in the process has not been established. Paju and Scannapieco [11] suggested that bacteria from oral biofilms could migrate into the respiratory tract.

Microbiological and epidemiological studies have long suggested a relationship between poor oral health and respiratory disease, especially in high-risk subjects. Oral hygiene tends to be poor among patients in intensive care units (ICU), leading to high amounts of dental plaque containing large numbers of potential respiratory pathogens [12]. A systematic review [13] concluded that oral colonization by respiratory pathogens, fostered by poor oral hygiene and periodontal diseases, appears to be associated with nosocomial pneumonia. In a case–control study [14], periodontitis was associated with increased rates of nosocomial pneumonia. Although bacterial examinations were not included, it is well known that periodontitis patients harbor high counts of periodontal pathogens, and therefore it seems reasonable that periodontal pathogens could migrate to lower airways through endotracheal tubes (ET). ET are placed through the highly colonized oropharynx and larynx into the normally sterile tracheobronchial tree, creating a direct passage from an external ventilator to the patient's lungs [15]. Biofilms related to other bacterial species have been found along the inner and outer surfaces of ET after being in place for 24 h [16, 17]. In this context and also considering that biofilms can be found in ET within a few hours after its insertion, a better understanding about biofilm composition is essential to improve preventative strategies.

It was hypothesized that, similarly to other bacterial species, oral periodontal pathogens would be able to colonize ET surfaces. Also, in the presence of teeth—the primary habitat of periodontal pathogens —ET would harbor higher bacterial levels. The aims of this case–control study nested to a cross-sectional study were to analyze whether periodontal pathogens are able to colonize ET of dentate and edentulous patients in the ICU and whether oral and ET periodontal pathogen profiles differ between these patients.

Methods

Sample size calculation

This study comprised a total of 78 eligible patients admitted to the ICU of the general hospital of Cuiabá-Brazil, from January 2013 to December 2013. By lottery, 18 dentate and 18 edentulous patients were selected and underwent clinical and microbiological examinations.

Initially, the sample size calculation determined 15 individuals per group [18]. Later, after the evaluation of mean bacterial counts, and considering a significance level of 5 %, power of 80 %, and a 15 % difference between groups, this sample size was adjusted to ~17 individuals per group. It is important to notice that in the present study the coefficient of bacterial counts variation was ~15 % which indicated a study outcome precision.

Data and personal information related to medical and dental histories of the patients were obtained from responses by individuals or their parents to our questionnaire. The surrogates of all included participants signed an informed consent, which was approved by the Institutional Committee on Research Involving Human Subjects (protocol 444/2012).

Study design

Seventy-eight eligible patients from the ICU who were older than 18 years were examined. Out of these, 36 were selected for the study and composed this convenience sample. We excluded patients who had a restricted mouth opening, less than 12 teeth in the mouth (for the dentate group), clinical signs and symptoms of delirium tremens, angular cheilitis, or equipment that reduced the performance of oral examination.

Clinical procedures

Two trained and calibrated researchers conducted all clinical measurements (ANP) and collected the microbial samples (AB). In dentate patients a periodontal examination was conducted using a manual periodontal probe (PCPUNC 15; Hu-Friedy Mfg. Co., Inc., Chicago, IL, USA) to register probing depth (PD), clinical attachment level (CAL), gingival index (GI) [19], and plaque index (PI) [20] in four periodontal sites per tooth. The intra-examiner agreement values for clinical measurements were high (PD, $\kappa = 0.88$; CAL, $\kappa = 0.82$). The diagnosis of periodontal status followed the criteria defined by the World Workshop in Clinical Periodontics [21]. For patients noted as edentulous by nurses, a clinical examination was conducted to confirm the clinical absence of teeth.

Microbiological procedures

Oral sampling

A pooled subgingival sample was collected from two molars and two incisors of dentate subjects. To obtain the sample, sterile paper points were inserted to the depth of the periodontal pocket after using sterile curettes to remove supragingival plaque. For subjects missing those teeth, microbial samples were obtained from canines and premolars. After being placed in the periodontal pocket for 60s, paper points were removed and transferred into an empty minitube. In addition, a pooled

microbial sample taken from the left side of the cheek plus the dorsum of the tongue was obtained from all subjects (dentate [18 pooled subgingival/cheek + tongue samples] and edentulous patients [18 pooled cheek + tongue samples]). These samples were taken from areas of approximately 1 cm², using a swab with reduced Ringer's solution and rotated six times. Each swab was transferred into a microtube also containing reduced Ringer's solution (1 mL).

Sampling of ET

One extubated ET (Well Lead Medical Company, Guangdong, Mainland, China). was obtained from each of the 36 patients who were intubated and mechanically ventilated in the ICU. The duration of intubation prior to ET collection was at least 5 days and a maximum of 7 days.

Collected extubated ET were placed in a sealed sterile bag and transferred to the microbiology laboratory for processing (undertaken within 1 h of ET collection). The biofilm was aseptically removed from the ET lumen using a sterile swab. We collected two biofilm samples, one from the oral cavity side (upper) and the other from the endotracheal side of the tubes (ventral). Each swab was transferred into a minitube containing reduced Ringer's solution (1 mL). The bacterial cells in the minitube were dispersed using a vortex mixer at the maximal setting for 1 min and then maintained at −80 °C. Levels of target periodontal pathogens were determined by quantitative PCR (qPCR), as previously described [22].

Quantitative real-time polymerase chain reaction

Genomic DNA (gDNA) was extracted and purified from the pellet using a commercial Genomic DNA Mini Kit according to manufacturer's specifications.

The quantification of the total number of bacterial cells and levels of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia* was carried out by qPCR using a TaqMan (Life Technology, Carlsbad, CA, USA) assay with a specific set of primers/probes in a real time PCR system following the manufacturer's instructions in 25 μl reactions. The qPCR conditions were: 50 °C for 2 minutes, 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds, and 60 °C for 1 minute. The primers/probes that were used were designed using Primer3 software online version (v. 0.4.0): *A. actinomycetemcomitans* (forward: CAA GTC TGA TTA GGT AGT TGG TGG G; reverse: TTC ATT CAC GCG GCA TGG C; probe: 6FAMATC GCT AGC TGG TCT GAG AGG ATG GCCTAMRA); *P. gingivalis* (forward: ACC TTA CCC GGG ATT GAA ATG; reverse: CAA CCA TGC AGC ACC TAC ATA GAA; probe: VICATG ACT GAT GGT GAA AAC CGT CTT CCC TTC TAMRA); *T. forsythia* (forward: AGC GAT GGT AGC AAT ACC TGT C; reverse: TTC GCC GGG TTA TCC CTC; probe: 6FAMCAC GGG TGA GTA ACGTAMRA); and Universal (forward: TGG AGC ATG TGG TTT AAT TCG A; reverse: TGC GGG ACT TAA CCC AAC A; probe: VICCAC GAG CTG ACG ACA AGC CAT GCATAMRA). NCBI Blast database was used to check primer/probe specificity.

The absolute quantification of the target organisms was determined by plotting the cycle threshold (Ct) value obtained from each clinical sample. Standard curves, using known concentrations of each bacterial strains' gDNA in 10-fold serial dilutions (10^2 – 10^7 cells), were used to convert cycle threshold values (CT) into the number of bacterial cells in the samples.

Statistical analysis

The Mann–Whitney test compared oral (pooled subgingival and/or cheek plus tongue) and tube (upper, ventral and both sides) bacterial levels between dentate and edentulous patients. Two-way analysis of variance (ANOVA) was used to verify the influence of age, gender, race, pneumonia, and release from the ICU as well interactions among these variables on oral and tube bacterial levels in dentate and edentulous patients. Pearson's correlation coefficient evaluated the correlation between oral and tube bacterial levels. We searched for a possible correlation between upper and ventral areas within each endotracheal tube, and correlations among bacterial levels, dentate or edentulous status, and the clinical parameters PD and CAL were verified. ANOVA was followed by Tukey's test for multiple comparisons. Dependent variables were transformed and residual values analyzed by the Shapiro–Wilk test, or the Q–Q plot showed acceptable levels of normality and a small range of variances. The role of dentate or edentulous status and pneumonia as risk factors for death was determined by calculating the odds ratio.

A significance level of 5 % ($p < 0.05$) was adopted.

Results

A total of 36 subjects (mean age 48.19 ± 18.50 years) were enrolled in this study. The demographic data and general systemic conditions of the study population are summarized in Table 1. Despite a numerically higher number of deceased patients among the dentate group, dentate and edentulous statuses were not related to death (OR = 1.000; confidence interval [CI] = 0.271 – 3.694; $p = 1.000$). Similarly, pneumonia was not related to death (OR = 0.801; CI = 0.216–2.964; $p = 0.739$).

Based on the measured periodontal clinical parameters, dentate subjects (Table 2) had a diagnosis of moderate or advanced chronic periodontitis. Although all three pathogens were detected in the subgingival samples of dentate patients, *A. actinomycetemcomitans* occurred in the highest number (Table 2).

Table 1 Demographic and general systemic conditions of the study population ($N = 36$)

Variable		Number of subjects	Percentage of subjects (%)
Oral status	Dentate	18	50
	Edentulous	18	50
Gender	Female	14	39
	Male	22	61
Age	<30 years	8	22
	30-40 years	4	11
	41-50 years	5	14
	51-60 years	9	25
	>60 years	10	28
Race	White	17	47
	Non-white	19	53
Ventilator-associated pneumonia (VAP)	No	25	69
	Yes	11	31
VAP outcome ($n = 11$)	Released	2	18
	Deceased	9	82
Sepsis	No	34	94
	Yes	2	06
ICU outcome	Released	10	27.77
	Deceased	26	72.22

We conducted a microbial analysis in both dentate and edentulous groups comparing the samples of the cheek and the dorsum of the tongue. This analysis was performed using a pooled cheek + tongue sample from each group. Mean comparative values of total bacteria load and levels of three pathogens are listed in Table 3. *P. gingivalis* and *T. forsythia* showed higher levels in nondental oral samples from dentate patients in comparison to edentulous ones.

Mean values of total bacteria load, *A. actinomycetemcomitans, P. gingivalis,* and *T. forsythia* from ET samples of dentate and edentulous patients are compared in Table 4. The data are shown as oral cavity side (upper), tracheal side (ventral), and both sides. All comparisons demonstrated statistically similar ET bacterial levels between dentate and edentulous patients indicating no direct influence by teeth on ET colonization by periodontal pathogens.

Demographic data showed no influence on total bacterial load, *T. forsythia* and *P. gingivalis* levels in tube samples (data not shown). Caucasian dentate patients showed higher levels of *A. actinomycetemcomitans* (51,838.00 ± 100,701.00) in ET in comparison to non-Caucasian (694.00 ± 1,232.00) (Tukey's test; p value = 0.023). In the edentulous group, mean values between Caucasian (3,509.00 ± 7,093.00) and non-Caucasian were similar (4,102.00 ± 7,139.00) (Tukey's test; p value = 0.77). Further, female patients showed higher levels of *A. actinomycetemcomitans* (19,267.00 ± 58,746.00) in ET when compared to male (1,038.00 ± 2,598.00) (Tukey's test; $p = 0.04$).

Table 2 Mean periodontal clinical and microbiological parameters evaluated in the dentate population

Measure	Clinical parameters			
	Probing depth (mm)	Clinical attachment level (mm)	Plaque index (0/1)	Gingival index(0/1)
Mean	2.82	2.76	0.96	0.91
SD	1.17	1.17	0.09	0.20
Mean bacterial levels				
	Total bacteria load	*Aggregatibacter actinomycetemcomitans*	*Tannerella forsythia*	*Porphyromonas gingivalis*
Number of bacteria	2,858,906.77 A	21,318.30 B	7,858.96 C	7,500.00 C

Different capital letters within the line indicate statistically significant differences (A > B > C) regarding number of bacteria (Mann–Whitney's test; $p < 0.05$)

Table 3 Mean values of oral total bacteria load, *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia* from cheek plus tongue samples of both dentate and edentulous groups

Group	Total bacterial load (number of bacteria)		Aggregatibacter actinomycetemcomitans (number of bacteria)		Tannerella forsythia (number of bacteria)		Porphyromonas gingivalis (number of bacteria)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cheek plus tongue samples(mean bacterial levels)								
Dentate	27,706,872.00	58,954,714.00	13,836.00	34,235.00	870.00	936	343.00	123.00
Edentulous	132,787,152.00	225,241,736.00	10,0226.00	220,709.00	224.00	181	26.00	14.00
p-value	0.261		0.885		0.031		0.021	

SD standard deviation

Statistically significant difference ($p < 0.05$); Mann–Whitney test

Table 5 shows the correlation results. By combining the data from dentate and edentulous patients, total bacterial load from oral samples were positively correlated with total bacterial load from both sides of tube samples. In addition, higher levels of *A. actinomycetemcomitans* in oral samples were accompanied by higher levels of *A. actinomycetemcomitans* in tube samples.

Among dentate patients, there was no correlation between oral and tube levels for a given bacterial species or for total bacterial load. However, dentate patients who showed higher total bacterial load in oral samples also showed higher levels of *T. forsythia* in the upper tube area. In addition, total bacterial load in the ventral tube area was positively correlated to *T. forsythia* levels. In the dentate group, there was no significant correlation between PD, CAL, and tube bacterial levels.

On the other hand, there were significant correlations between oral and tube samples for the same bacterial species among edentulous patients. Higher levels of *A. actinomycetemcomitans*, *T. forsythia,* and *P. gingivalis* in oral samples were correlated with higher levels in the tubes.

Table 4 Mean values of endotracheal tubes total bacteria load, *A. actinomycetemcomitans, P. gingivalis* and *T. forsythia* in both dentate and edentulous groups

Group	Endotracheal tubes (mean bacterial levels)					
	Oral side (upper sample)		Trachea side (ventral sample)		Both sides	
	Mean	SD	Mean	SD	Mean	SD
Total bacterial load (number of bacteria)						
Dentate	13,937.23	35,994.19	170,564.65	274,823.03	184,501.88	280,057.65
Edentulous	81,0381.73	1,650,503.93	775,569.43	1,892,783.55	1,585,951.15	3,206,879.19
p-value	0.275		0.716		0.304	
Aggregatibacter actinomycetemcomitans (number of bacteria)						
Dentate	20,583.00	65,101.00	34,125.00	81,045.00	54,708.00	137,148.00
Edentulous	3,773.00	6,907.00	135,332.00	524,163.00	139,104.00	52,3675.00
p-value	0.833		0.531		0.495	
Porphyromonas gingivalis (number of bacteria)						
Dentate	77.30	17.60	66.40	47.50	94.10	87.20
Edentulous	74.10	17.40	72.60	96.50	77.00	65.90
p-value	0.102		0.566		0.312	
Tannerella forsythia (number of bacteria)						
Dentate	168.00	165.00	140.00	158.00	308.00	286.00
Edentulous	113.00	150.00	170.00	213.00	283.00	296.00
p-value	0.366		0.886		0.787	

SD standard deviation

Mann-Whitney's test ($p < 0.05$)

Table 5 Correlation between bacterial levels (total bacterial load and levels of target periodontal pathogens) in oral and endotracheal tube samples from dentate and edentulous patients. Endotracheal tube samples are shown as oral side (isolate bacterial levels from the side of the tube in contact with oral tissues), tracheal side (isolate bacterial levels from the ventral side of the tube) and both tube sides (combined bacterial levels)

Measure			Dentate				Edentulous				Total population (dentate + edentulous)			
Endotracheal tube		Coefficient, P value	Total bacterial load	Oral *A.a*	Oral T.f	Oral *P.g*	Total bacterial load	Oral *A.a*	Oral T.f	Oral *P.g*	Total bacterial load	Oral *A.a*	Oral T.f	Oral *P.g*
Oral side	Total bacterial load	R	−0.096	−0.148	−0.168	−0.036	0.408	−0.234	−0.204	−0.176	0.457	−0.121	−0.182	−0.091
		p value	0.705	0.557	0.505	0.887	0.093	0.350	0.417	0.485	**0.005***	0.483	0.287	0.599
	A.a	R	−0.115	0.002	0.020	0.027	−0.139	0.018	0.367	−0.177	−0.098	0.570	0.105	0.069
		p value	0.649	0.995	0.937	0.915	0.583	0.943	0.134	0.482	0.571	0.001*	0.542	0.691
	T.f	R	0.494	0.102	−0.222	−0.222	−0.155	−0.238	0.100	0.611	−0.063	−0.187	−0.053	−0.089
		p value	**0.037***	0.686	0.376	0.376	0.539	0.341	0.693	0.482	0.716	0.276	0.757	0.605
	P.g	R	0.135	0.106	−0.278	−0.224	−0.321	−0.272	0.447	0.384	−0.247	−0.225	−0.017	−0.080
		p value	0.594	0.675	0.264	0.371	0.194	0.275	0.063	0.116	0.146	0.187	0.924	0.641
			Dentate				Edentulous				Total population (dentate + edentulous)			
Traqueal side	Total bacterial load	R	0.092	0.468	0.104	0.242	0.329	−0.008	−0.115	−0.147	0.365	0.063	−0.105	−0.030
		p value	0.717	**0.050***	0.683	0.334	0.182	0.975	0.649	0.561	**0.029***	0.714	0.541	0.863
	A.a	R	−0.092	0.177	−0.082	−0.148	−0.132	0.524	0.526	−0.093	−0.079	0.529	0.016	−0.060
		p value	0.718	0.482	0.747	0.559	0.601	**0.026***	**0.025***	0.715	0.646	**0.001***	0.926	0.727
	T.f	R	0.059	0.654	−0.113	−0.129	−0.307	−0.037	0.801	0.198	−0.192	0.051	0.014	−0.084
		p value	0.815	0.003*	0.654	0.609	0.215	0.884	**<.0001***	0.430	0.261	0.768	0.934	0.627
	P.g	R	0.079	−0.331	−0.165	0.468	−0.169	−0.134	0.509	0.586	−0.101	−0.085	0.005	−0.047
		p value	0.757	0.180	0.514	0.050*	0.504	0.595	**0.031***	0.025*	0.559	0.623	0.978	0.785
			Dentate				Edentulous				Total population (dentate + edentulous)			
Both tube sides	Total bacterial load	R	0.078	0.440	0.080	0.232	0.404	−0.125	−0.173	−0.177	0.449	−0.026	−0.156	−0.064
		p value	0.759	0.067	0.752	0.353	0.096	0.621	0.492	0.481	**0.006***	0.881	0.364	0.709
	A.a	R	−0.109	0.105	−0.039	−0.074	−0.134	0.525	0.531	−0.095	−0.090	0.514	0.029	−0.051
		p value	0.667	0.677	0.879	0.770	0.596	**0.025***	**0.023***	0.708	0.602	**0.001***	0.869	0.768
	T.f	R	0.317	0.420	−0.191	−0.199	−0.299	−0.147	0.626	0.452	−0.158	−0.070	−0.020	−0.103
		p value	0.199	0.083	0.449	0.428	0.228	0.561	**0.006***	0.060	0.357	0.687	0.907	0.550
	P.g	R	0.123	−0.119	−0.255	−0.184	−0.182	−0.146	0.519	0.511	−0.119	−0.102	0.003	−0.053
		p value	0.628	0.638	0.308	0.464	0.470	0.563	**0.027***	**0.029***	0.489	0.554	0.986	0.761

A.a Aggregatibacter actinomycetemcomitans, *T.f* Tannerella forsythia, *P.g* Porphyromonas gingivalis

*Values in bold have a statistically significant correlation, $P < 0.05$

Discussion

The findings of the present study suggest that besides other bacterial species periodontal pathogens can play a role in the specific bacterial consortium of ET. Further, although dental biofilm is the primary habitat of bacteria related to periodontal diseases, the present study revealed at most a discrete influence by teeth on ET bacterial levels. In fact this last observation denies one of the study hypotheses. However, mouth as a whole is a key source for bacterial contamination of ET.

In addition to ET colonization, the oral cavity is an important reservoir of infection for ventilator-associated pneumonia [11]. Therefore, oral hygiene in ICU patients becomes relevant although hospitalisation per se tends to reduce its pattern [23]. Unfortunately, poor oral hygiene has been associated with systemic findings [24, 25], which can be critical in ICU patients.

In our dentate population, we had an opportunity to confirm this statement once a high level of plaque (0.96) index was observed. As expected, this high plaque level was followed by a high gingival index mean value (0.91). However, this high plaque index did not seem to influence ET colonization by periodontal pathogens since data from ET did not differ between dentate and edentulous.

Although there are recommendations to focus oral care efforts on reducing dental plaque accumulation, the most common tool used is the foam swab [26]. Unfortunately, in our study we noticed that ICU nurses rarely used a toothbrush alone or associated with chlorhexidine. In a recent review [27] it was published that effective oral hygiene care is associated with a 40 % reduction in the odds of developing ventilator-associated pneumonia in critically ill adults.

According to Scannapieco et al. [12], dental plaque accumulates rapidly in the mouths of critically ill patients, and as the amount of plaque increases, colonization by target pathogens is likely. Periodontal pathogens are Gram negative bacteria that according to Munro and Grap [28] can predominate in the oral microbiota of critically ill adults within 48 h of hospital admission. Despite *A. actinomycetemcomitans* being the most isolated ($p < 0.05$) pathogen in the gingival sulcus/pocket of dentate patients, all three periodontal pathogens investigated were detected (Table 2). In addition, in the analysis of target periodontal pathogens in the oral cavity of both dentate and edentulous groups (cheek + tongue samples), we observed the presence of *A. actinomycetemcomitans, P. gingivalis,* and *T. forsythia* (see Table 3), indicating that the target putative periodontal pathogens were present in both groups, even in those subjects without teeth. As the presence of teeth is not a requirement for colonization of periodontal pathogens, oral health care of non-dental sites should be similar for dentate and edentulous ICU intubated patients.

In the present study we highlight that colonization of the oral cavity by periodontal pathogens occurs in edentulous patients. Our group [29, 30] and others [31] have previously documented that periodontal pathogens can be detected from the oral mucous membranes of edentulous individuals.

Our results showed that periodontal pathogens can migrate from oral hard and soft tissues to colonize ET. Probably, due to a direct contact between live and artificial surfaces this colonization starts in the oral side of ET to later progress until the endotracheal side of ET. It was observed in ET from both dentate and edentulous groups without any significant difference. These data confirm that biofilm developed in initially sterilized ET can harbor oral microorganisms and more specifically periodontal pathogens. As our study population stayed in the ICU from 5 to 7 days with minimal oral care, their poor oral hygiene facilitated plaque accumulation and ET contamination. Unfortunately, as reviewed by Par et al. [32], poor oral care may increase the incidence of ventilator-associated pneumonia in critically ill patients. In addition, our analytic results demonstrated that the total bacterial load from oral samples was positively correlated with the total bacterial load from tube samples for both dentate and edentulous patients. An analysis of the systemic condition of our population (Table 1) showed that out of 36 patients examined, 11 (31 %) developed ventilator-associated pneumonia among which nine patients (82 %) evolved to death. In addition, out of 26 patients that did not survive, 15 (58 %) were dentate with periodontal disease. However, our sample size is not large enough to establish any relationship between periodontal conditions and ventilator-associated pneumonia. Furthermore, in the study population, presence of teeth and early nosocomial pneumonia did not increase the risk of death. On the other hand, patients from a prospective cohort in Spain who developed nosocomial pneumonia had a 2.6-fold higher risk of dying compared with those who did not [33].

A. actinomycetemcomitans was first isolated from nonoral infections, but different mechanisms, such as aspiration of *A. actinomycetemcomitans* as well as *P. gingivalis*, have been linked to respiratory infections [34]. In the present study, female gender and the association between the Caucasian race and dentate status influenced the levels of *A. actinomycetemcomitans* in the sampled tubes. In no hospitalized individuals, these clones have a colonization largely restricted to individuals of African descent [35] and are not spread within the Caucasian inhabitants from German cities. Nonwhite ethnicity was also associated with this bacterium [36]. Combined data from dentate and edentulous patients and isolate data from edentulous patients showed that higher oral levels of *A. actinomycetemcomitans* were correlated with higher levels of this same species in tube samples. Wang et al. [5] studied invasive infections of *A. actinomycetemcomitans* and reported oral

lesions as the probable sources of the microorganism to distant sites. *A. actinomycetemcomitans* has also been associated with pneumonia [6]. Therefore, the observed tube colonization by periodontal pathogens could be the first step for further bacterial dissemination. Future studies should be conducted to comprehensively evaluate this possible route. The presence of pathogens also indicates that it is fundamentally important for physicians and nurses to plan active strategies to reduce these microorganisms in ICU intubated patients.

Conclusions

Periodontal pathogens have the ability to colonize ET as well as the oral cavity of ICU patients independently of whether teeth are present or not. Periodontal pathogen profiles of endotracheal tubes tend to be similar between dentate and edentulous ICU patients.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no competing interests.

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