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Prevalence of potential bacterial respiratory pathogens in the oral cavity of hospitalised individuals

David Zuanazzi[a], *Renata Souto*[a], *Marcelo Barbosa Accioly Mattos*[a], *Maura Rodrigues Zuanazzi*[a], *Bernardo Rangel Tura*[b], *Carmelo Sansone*[a], *Ana Paula Vieira Colombo*[a,*]

[a] *Federal University of Rio de Janeiro (UFRJ), Brazil*
[b] *National Institute of Cardiology (INC), Brazil*

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ABSTRACT

Objective: To assess the prevalence of oral colonisation by bacterial respiratory pathogens in hospitalised patients.

Methods: Thirty patients undergoing myocardium revascularisation surgery were evaluated. At baseline (pre-operative phase), full-mouth clinical periodontal assessment was performed. Saliva and biofilm samples were obtained from subjects at baseline and at the post-operative phase, after orotracheal extubation. DNA was extracted from samples and species of *Acinetobacter*, *Pseudomonas*, *Staphylococcus aureus* and *Dialister pneumosintes* were detected by PCR or culture (for staphylococci isolates).

Results: Most of the subjects were males, with history of hypertension and smoking. Thirteen were edentulous (ED) and 17 were dentate (DE), with moderate chronic periodontitis. The most prevalent bacteria in saliva were *Staphylococcus* spp. (85.7%), *Pseudomonas* spp. (83.8%), and *Acinetobacter* spp. (53.3%). There was a trend for *D. pneumosintes* to be more frequently detected in DE (43.7%) than ED (11.5%) patients. In plaque samples, DE with >14 teeth showed a higher prevalence of *Pseudomonas* spp. (100%) than individuals with ≤14 teeth (69.1%; $p = 0.048$). Conversely, *P. aeruginosa* was more prevalent in subjects with fewer teeth (35.5%) than with >14 teeth (5.7%; $p = 0.037$). All staphylococci isolates were coagulase-negative, and about 11% were positive for the *mec*A gene. These *mec*A-positive isolates showed a tendency to increase in all samples, whereas *P. aeruginosa* reduced after surgery. A strong correlation between the presence of *Acinetobacter* spp. and *Pseudomonas* spp. was observed (rho = 0.886, $p < 0.05$).

Conclusions: The oral cavity of hospitalised patients harbours high frequencies of bacterial respiratory pathogens, supporting its potential role as a reservoir for these species.

1. Introduction

Nosocomial infections are major causes of morbidity and mortality among hospitalised patients, especially in the intensive care unit (ICU).[1] Bacterial pneumonia may represent about 13–48% of all infections in hospitals and health care institutions.[2,3] This disease is a result of infection of the lower respiratory tract, usually by aspiration of microorganisms colonising the oropharyngeal area.[4] In particular, elderly patients in nursing homes and hospitals have a

* *Corresponding author at*: R. Gal. Dionísio, 60 apt. 604 – Humaitá, CEP: 22271-050 - Rio de Janeiro, RJ, Brazil. Tel.: +55 21 25376815; fax: +55 21 25376815.
E-mail addresses: anapaulacolombo@yahoo.com, apcolombo@micro.ufrj.br (A.P.V. Colombo).
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higher risk of developing respiratory infections due to silent aspiration.[5]

The microbial diversity of the human oral cavity has been recognised for decades, and over 700 species have been recently identified in this habitat.[6] In addition to the resident oral species, studies have shown that the oral cavity may harbour ''non-oral'' bacteria, including various medically important pathogens.[7–9] For instance, previous investigations have reported that respiratory pathogens such as *Acinetobacter* spp., *Dialister pneumosintes*, *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Haemophilus* spp., and *Staphylococcus aureus* can colonise dental plaque of hospitalised patients.[1–4,9–11] Of interest, colonisation frequency seems to increase in individuals presenting poor oral health, significant amounts of dental plaque and/or periodontal diseases.[8,9,11,12] Dental plaque may influence the initiation and progression of pneumonia due to translocation of the bacteria from the biofilm to the respiratory tract.[2] Saliva may also play a significant role in the development of pneumonia by acting as a vehicle to bacteria residing in the oral cavity, which can be aspirated into the lungs.[13] Oral anaerobic bacteria have also been isolated from patients with ventilator-associated pneumonia, reinforcing the role of the oral microbiota in these infections.[14]

Considering that the mouth may be a reservoir for respiratory pathogens, the purpose of the present investigation was to determine the prevalence of potential bacterial respiratory pathogens, including *Acinetobacter* ssp., *D. pneumosintes*, *Pseudomonas* spp., and *S. aureus* in dental biofilm and saliva of hospitalised patients. In addition, associations between the frequency of these organisms and epidemiological parameters, general health and periodontal *status* were investigated.

2. Materials and methods

2.1. Study population

The study population consisted of hospitalised patients recruited from the Coronary Heart Disease Unit of the National Institute of Cardiology (INC) in Rio de Janeiro, Brazil, between January and August of 2007. All patients were admitted to the hospital to undergo elective myocardial revascularisation surgery. Of 97 patients screened, 30 patients fulfilled the study criteria and consented to participate. Exclusion criteria included any inflammatory or infectious disease other than coronary artery or periodontal diseases, as well as any periodontal treatment or use of local or systemic antimicrobial agents within 6 months prior to the entry into the study. Informed consent was obtained from all enrolled individuals. The study protocol was approved by the Review Committee for Human Subjects of the INC.

2.2. Oral examination

Demographic and medical history data were obtained from patient's records. Dental examination included periodontal clinical measurements, performed at six sites per tooth in all teeth except third molars,[15] including probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP),[16] and dental plaque (DP).[17] For dental plaque, we evaluated six sites per tooth instead of four surfaces. After initial clinical evaluation, subjects were categorised into dentate (DE) or fully edentulous (ED) according to the presence or total absence of teeth, respectively. All ED subjects wore upper and lower full prosthesis. DE subjects were divided into two groups based on the number of remaining teeth; i.e. subjects presenting $\leq$14 teeth and $>$14 teeth.[18] All clinical examination and microbiological sampling were conducted by a single trained examiner.

2.3. Sampling

Patients were sampled at two time points: at baseline (pre-operative phase), approximately 2 weeks after hospital admission, while they waited for surgery; and at the post-operative phase, at the Intensive Care Unit (ICU), within 12 h after extubation. The intubation occurred in the beginning of the surgery and lasted no more than 20 h. The interval between the first and second sampling was about 1 week. Antibiotic prophylaxis was given to all patients according to the hospital standard protocol (1 g cefazolin intravenously 20–30 min before, and up to 24 h after surgery).[19] For sampling, patients were not allowed to clean their teeth or to eat 30 min before sampling. For saliva sampling they rinsed out their mouths with 10 mL of 0.9% sterile saline for 60 s, and the mouthwashes were collected in sterile plastic tubes.[20] Afterwards, supra- and sub-gingival biofilm samples were obtained from 4 to 6 mesial sites in different teeth of each dentate subject as follows: first upper molars or the most posterior teeth; and lower right central incisors or the most anterior teeth. Before sampling, the area was dried and isolated with sterile gauze, and supra and subgingival plaque were collected using individual sterile periodontal curettes (*Gracey* no 9–10—Hu-Friedy, Chicago, IL.). Each sample was placed in tubes containing 1.5 mL of mycoplasma broth (Sigma–Aldrich, USA) with 10% DMSO (dimethyl sulfoxide 100 mg/mL), and stored at $-20\,^{\circ}C$. All saliva and plaque samples were divided in two aliquots: one for extraction of bacterial DNA and the other for cultivation and isolation of *Staphylococcus* spp.

2.4. Isolation of Staphylococcus spp.

Each sample was inoculated in tryptic soy broth (TSB - Difco™, Sparks, USA) containing 6.5% NaCl, and incubated overnight at 37 °C. After growth, 100 μL of the suspension were plated on mannitol salt agar selective media (BBL™, Sparks, USA) and incubated for up to 48 h at 37 °C. Colonies from each plate were identified initially on the basis of their appearance, Gram stain, catalase and oxidase reactions, and colour changes on the selective media. Representative colonies were placed in TSB overnight at 37 °C for enrichment. One millilitre was obtained for bacterial DNA extraction as described below.

2.5. Bacterial DNA extraction and detection of Acinetobacter spp., D. pneumosintes, Pseudomonas spp., and Staphylococcus spp. by PCR

Bacterial DNA was extracted from saliva and dental biofilm samples using the Mo Bio UltraClean microbial DNA kit (Mo Bio

Table 1 – PCR primer sequences selected for detection of respiratory pathogens.

Species	Sequence	Amplicon (bp)	Reference
Acinetobacter spp.	27f: 5′-AGA GTT TGA TCC TGG CTC AG-3′ 1492r: 5′-TAC GGC TAC CTT GTT ACG ACT T-3′	1450	21
	Ac436f: 5′-TTT AAG CGA GGA GGA GG-3′ Ac676r: 5′-ATT CTA CCA TCC TCT CCC-3′	280	22
D. pneumosintes	160f: 5′-TTC TAA GCA TCG CAT GGT GC-3′ 1265r: 5′-GAT TTC GCT TCT CTT TGT TG-3′	1105	23
Pseudomonas spp.	PS1: 5′-ATG AAC AAC GTT CTG AAA TTC TCT GCT-3′ PS2: 5′-CTT GCG GCT GGC TTT TTC CAG-3′	250	24
	PAL1: 5′-ATG GAA ATG CTG AAA TTC GGC-3′ PAL2: 5′-CTT CTT CAG CTC GAC GCG ACG-3′	510	
Staphylococcus spp.	Staphylococci 5′-CCT ATA AGA CTG GGA TAA CTT CGG G-3′ 16S rRNA 5′-CTT TGA GTT TCA ACC TTG CGG TCG-3′	791	25
	ClfA 5′-GCA AAA TCC AGC ACA ACA GGA AAC GA-3′ 5′-CTT GAT CTC CAG CCA TAA TTG GTG G-3′	638	
	mecA 5′-AAC TGG AGG AAG GTG GGG GAT-3′ 5′-AGG AGG TGA TCC AAC CGC A-3′	371	
	Eubacteria 5′-TCC AGG AAT GCA GAA AGA CCA AAG C-3′ 16S rRNA 5′-GAC ACG ATA GCC ATC TTC ATG TTG G-3′	499	

Laboratories, Inc., Carlsbad, CA), and stored at −20 °C until PCR amplification was performed.

Acinetobacter spp. detection was carried out by a nested PCR.[21,22] The first amplification used the bacterial 16S rRNA universal primers 27f and 1492r (Table 1). Approximately 100 ng of sample DNA was added into a 50 μL PCR mixture containing 0.5 pmol of each primer, 400 μM of each dNTP, 3 mM $MgCl_2$, PCR Platinum® Taq buffer (20 mM Tris–HCl [pH 8.4], 50 mM KCl), and 1.5 U Platinum® Taq DNA polymerase (Invitrogen, Grand Island, NY). The amplification programme included an initial step of 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min, and a final step of 72 °C for 5 min. The second amplification was performed with the primers Ac436f and Ac676r (Table 1) in a reaction volume of 50 μL containing 2 pmol of primer Ac436f, 1 pmol of primer Ac676r, 200 μM of each dNTP, 2 mM $MgCl_2$, PCR Platinum® *Taq* buffer, and 1.5 U Platinum® *Taq* DNA polymerase (Invitrogen). The amplification programme was carried out according to Vanbroekhoven et al.[22]

Detection of *D. pneumosintes* was carried out in a 25 μL reaction mixture containing 100 ng of DNA template, 2 pmol of each primer (Table 1), 200 μM of each dNTP, 1.6 mM $MgCl_2$, PCR Platinum® *Taq* buffer, and 1 U Platinum® *Taq* DNA polymerase (Invitrogen). The amplification programme was performed according to Doan et al.[23]

A multiplex PCR using two pairs of primers, PS1, PS2, PAL1 and PAL 2 (Table 1) was carried out for detection of *Pseudomonas* spp., including the species *P. aeruginosa*.[24] The PCR was performed in a 100 μL reaction mixture containing 100 ng of DNA, 0.5 pmol of each primer PS1 and PS2, 0.7 pmol of each primer PAL1 and PAL2, 200 μM of each dNTP, 1.7 mM $MgCl_2$, PCR Platinum® *Taq* buffer, and 2 U Platinum® *Taq* DNA polymerase (Invitrogen). The amplification reaction included an initial step of 95 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 40 s, 57 °C for 1 min, 72 °C for 1 min, and a final step of 72 °C for 2 min.

For detection of *Staphylococcus* spp., a multiplex PCR using four pairs of primes (Table 1) was carried out. These primers were designed to distinguish between *S. aureus* and coagulase-negative staphylococci (CNS), as well as to detect the presence of the methicillin resistance gene, *mecA*.[25] The PCR reaction was performed in a 100 μL mixture containing 100 ng of DNA extracted from isolates, 1 pmol of each primer, 200 μM of each dNTP, 3 mM $MgCl_2$, PCR Platinum® *Taq* buffer and 2.5 U Platinum® *Taq* DNA polymerase (Invitrogen). The amplification included an initial step of 94 °C for 3 min followed by 36 cycles of denaturation at 94 °C for 1.5 min, 55 °C for 1 min, 72 °C for 1 min, and a final step of 72 °C for 10 min.

All PCR amplifications were performed in a termocycler (Primus 25/96, MWG-Biotech-INCs, High Point, NC, USA). The amplicons were analysed on a 1.5% agarose gel electrophoresis stained with 0.5 mg/mL ethidium bromide, and visualised on a UV transilluminator. A 1 kb DNA ladder digest (Life-Technologies, Gaithersburg, MD) was used as a standard molecular weight. Positive controls included DNA extracted from the reference strains *Acinetobacter baumannii* (ATCC 19606), *P. aeruginosa* (ATCC 27853), *D. pneumosintes* (ATCC 33048), *Staphylococcus epidermidis* (ATCC 14579) and *S. aureus* (ATCC 25923).

2.6. Statistical analysis

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS Inc. v.15 Chicago, IL, USA). In the DE patients, full-mouth clinical measurements were computed for each subject and then average across subjects within the groups. Differences on clinical parameters between groups were sought using Mann–Whitney and χ^2-tests. The frequency of detection of all bacteria was computed for each subject, and significant differences between groups were sought using the Mann–Whitney, χ^2 and Fisher's exact tests. Differences in the prevalence of bacteria before and after surgery within groups were tested by McNemar or Wilcoxon tests. Associations between clinical and microbiological parameters were examined by Spearman correlation analysis. Any difference of $p < 0.05$ was considered statistically significant.

Table 2 – Demographic features and full-mouth periodontal clinical parameters of the study population.

Parameters	Dentate		Edentulous
	≤14 teeth N = 10	>14 teeth N = 7	N = 13
Age (mean ± SEM) in years	63.5 ± 2.8	58.3 ± 3.7	66.2 ± 2.6
Males (%)	70	85.7	76.9
Smoking (%)			
Smoker	30	28.6	38.5
Non-smoker	30	28.6	38.5
Former smoker	40	42.8	23.1
Hypertension (%)	80	71.4	92.3
Diabetes (%)			
Type I	0	14.3	0
Type II	40	0	23.1
Non-diabetic	60	85.7	76.9
Mean (±SEM)			
Number of teeth[a]	6.7 ± 1.3	22.5 ± 1.5	
PD (mm)	3.0 ± 0.1	2.7 ± 0.1	
CAL (mm)[a]	5.1 ± 0.4	3.7 ± 0.3	
Mean (±SEM) % of sites			
PD 1–3 mm	88.1 ± 2.9	92 ± 2.4	
PD 4–6 mm	10.3 ± 2.7	6.9 ± 2.3	
PD >6 mm	1.5 ± 1.1	1.03 ± 0.6	
CAL 1–3 mm[a]	47.6 ± 8.5	75 ± 6.5	
CAL 4–6 mm	29 ± 5.8	20.3 ± 4.6	
CAL >6 mm[a]	23.3 ± 6.1	4.6 ± 2.1	
BOP[a]	58.8 ± 10.4	25.5 ± 4.7	
DP[b] score 1	48.2 ± 3.6	58.2 ± 6.4	
Score 2[a]	29.7 ± 5.2	10.8 ± 3.2	
Score 3	10.5 ± 4.3	2.4 ± 1.2	

PD: pocket depth; CAL: clinical attachment level; BOP: bleeding on probing; DP: dental plaque.
[a] Refers to significant differences between groups ($p < 0.05$; Mann–Whitney test).
[b] Refers to Silness and Löe plaque index.

3. Results

3.1. Clinical and demographic parameters

Table 2 shows the demographic features and full-mouth periodontal clinical parameters of the 30 subjects separated into two groups according to presence (DE) or absence of teeth (ED). This population was comprised mainly of males (77%), over 45 years of age (mean age 63.5 ± 1.7 years), with history of hypertension. Approximately 26% of the individuals were diabetic. No significant differences between groups were found for these parameters (χ^2 test). The DE group was further divided into two groups, according to number of remaining teeth. All patients presented chronic periodontal disease. Subjects with ≤14 teeth presented significantly more loss of clinical attachment, BOP and visible DP than subjects with >14 teeth ($p < 0.05$; Mann–Whitney test).

3.2. Microbiological data

3.2.1. Saliva samples

Table 3 shows the frequency of bacteria in saliva samples from DE and ED patients. Overall, the most prevalent bacteria in saliva samples at baseline (pre-operative stage) were *Pseudomonas* spp. (83.3%), CNS (81.5%) and *Acinetobacter* spp. (63.3%). All *Staphylococcus* spp. cultivated were CNS, and *S. aureus* was not detected in any sample. No significant differences in the frequency of bacteria between DE and ED were seen at pre-surgery; however, *D. pneumosintes* showed a tendency to be more prevalent in DE than ED patients ($p = 0.051$; Fisher's Exact Test). After surgery, the number of patients sampled decreased due to inappropriate time of extubation, or refusal of the patient to participate in the study. The prevalence of CNS, particularly carrying the *mec*A gene seemed to increase, whereas *P. aeruginosa* had a marked decrease in all patients after surgery. *Acinetobacter* spp. and *D. pneumosintes* were reduced, and *Pseudomonas* spp. increased in frequency in ED subjects. However, no significant changes in the prevalence of any tested bacteria were found between baseline and post-surgery in both groups (Wilcoxon test).

3.2.2. Plaque samples

The mean frequency of all tested bacteria in biofilm samples of dentate individuals with high (>14) and low number of teeth (≤14) is depicted in Fig. 1. At pre- and post-surgery, the most prevalent bacteria in all patients were *Pseudomonas* spp.

Table 3 – Frequency (%) of detection of respiratory pathogens in saliva samples of all subjects before (pre) and after surgery (post).

Parameters	Saliva					
	Edentulous		Dentate		Total	
	Pre N = 13	Post N = 7	Pre N = 17	Post N = 13	Pre N = 30	Post N = 20
D. pneumosintes	23.1	0	41.2	46.2	33.3	30
Acinetobacter spp.	80	40	50	44.4	63.6	42.9
Pseudomonas spp.	84.6	100	82.4	76.9	83.3	84.2
P. aeruginosa	18.2	0	21.4	10	20	6.3
CNS	83.3	100	80	84.6	81.5	89.9
CNS carrying *mecA*	11.1	25	18.2	20	15	21.4

CNS: all *Staphylococcus* spp. isolated were coagulase-negative staphylococci.

(80.3 ± 37%), *Acinetobacter* spp. (67.2 ± 33%), and CNS (66.2 ± 34%). *Pseudomonas* spp. was the only group of microorganisms that occurred significantly more often in subjects with >14 teeth (100%) than in subjects with ≤14 teeth (69.1 ± 12%; $p = 0.048$). Conversely, *P. aeruginosa* was significantly more prevalent in subjects with fewer teeth (35.5 ± 9.3%) compared to the ones with >14 teeth (5.7 ± 5.7%; $p = 0.037$; Mann–Whitney test). At post-surgery, similar distributions of the bacteria were observed between dentate subgroups, except for *D. pneumosintes* which was slightly more prevalent in subjects with ≤14 teeth (Fig. 1). Regarding changes after surgery, *Acinetobacter* spp., *D. pneumosintes* and CNS showed a tendency to increase in prevalence in dentate patients with fewer teeth. Likewise, the frequency of CNS isolates carrying the *mecA* gene seemed to increase in this group. Although the frequency of *Pseudomonas* spp. did not change very much after surgery, *P. aeruginosa* had a marked decrease from 35.5% to 5.7% in subjects with ≤14 teeth. Nevertheless, none of these changes reached statistical significance ($p > 0.05$; Wilcoxon signed rank test).

A strong association between the presence of *Pseudomonas* spp. and *Acinetobacter* spp. was found, after controlling for gender, smoking, age, and number of teeth (rho = 0.886, $p < 0.02$). Detection of *Staphylococcus* spp. correlated strongly with the presence of *P. aeruginosa* (rho = 0.962, $p < 0.002$), and modestly with BOP (rho = 0.317, $p < 0.013$). On the other hand, increase in supragingival plaque accumulation correlated with lower prevalence of *P. aeruginosa* (rho = −0.859, $p < 0.03$) and *mecA Staphylococcus* spp. (rho = −0.443, $p < 0.01$), after controlling for the variables cited above. When comparing saliva and dental plaque samples for the prevalence of the tested bacteria, *Staphylococcus* spp. were isolated significantly

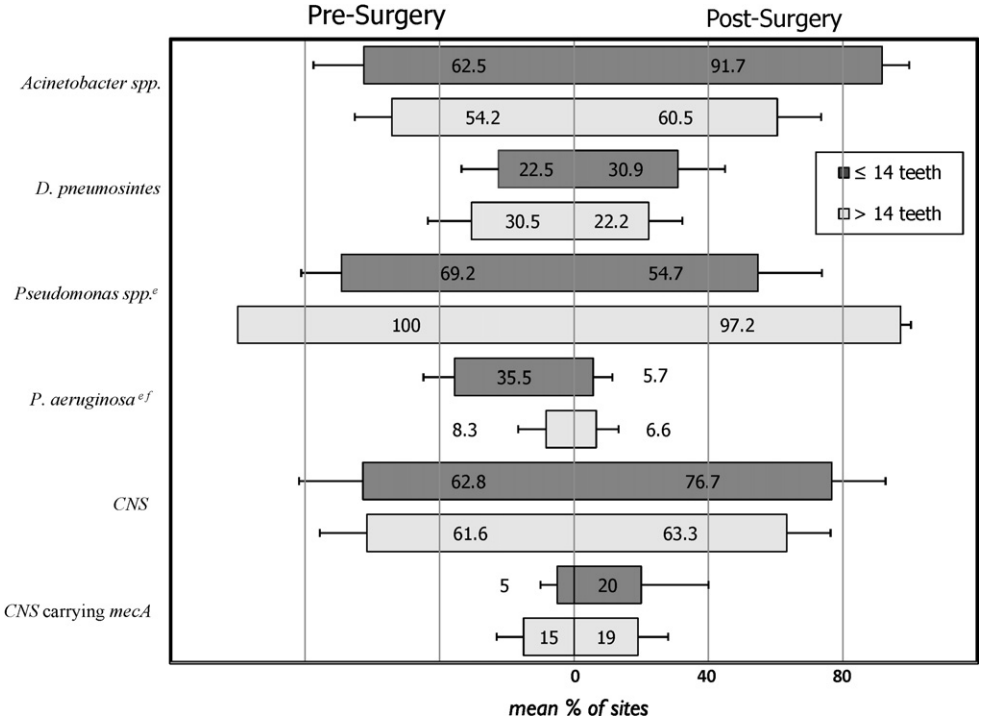

Fig. 1 – Mean frequency (±SEM) of respiratory pathogens in plaque samples of dentate patients with ≤14 or >14 teeth, before and after surgery. CNS: coagulase-negative staphylococci; [e]Significant differences between dentate subgroups at pre-operative time point ($p < 0.05$; Mann–Whitney test). [f]prevalence of *P. aeruginosa* within *Pseudomonas* spp. positive samples.

more often from saliva (81.5%) than plaque (56.5%; $p = 0.03$, Fisher's exact test). No significant associations were observed between supra and subgingival plaque for any of the species examined.

4. Discussion

Studies have indicated that the oral cavity is a potential reservoir for respiratory infection, particularly in elderly people with poor oral health.[2,11,26–33] In the current investigation, a markedly high prevalence of bacterial respiratory pathogens, such as *Pseudomonas* spp. and *Acinetobacter* ssp. was found in saliva and dental plaque of hospitalised patients, corroborating the data reported by other investigators.[1,10,26–33] These findings may reflect the profile of this particular population. Most of the individuals were old males, with history of hypertension and smoking. Approximately 40% were fully edentulous, while all dentate individuals presented moderate chronic periodontitis and poor oral hygiene (high % of sites with visible plaque and BOP). Little information about the periodontal conditions of the patients is provided in other studies analysing respiratory pathogens in the oral cavity. However, the association between respiratory pathogens and periodontal diseases has been reported by several investigators.[7,9,34–37] The elevated frequency of these pathogens in patients with periodontitis suggests that the presence of a complex subgingival microbiota together with the chronic inflammatory process may provide a favourable environment for the establishment of these microorganisms.

In saliva samples, no major differences were found between DE and ED patients, however *D. pneumosintes* showed a tendency to be more prevalent in DE subjects. Moreover, this species was slightly more frequent in DE subjects with >14 teeth. In fact, Ferraro et al.[8] detected *D. pneumosintes* mainly in subgingival biofilm rather than saliva samples from periodontitis and healthy patients. More recently, it has been found in the oral cavity and bronchoalveolar lavage (BAL) samples from subjects with ventilator-associated pneumonia (VAP).[31]

Although the role of *Staphylococcus* spp. in the ecology of the oral flora is controversial, their occurrence is unquestionable. A number of investigations have reported frequencies ranging from 83% to 100% of *Staphylococcus* spp. in the oral cavity of both healthy adults and elderly individuals; however, the prevalence of these species in hospitalised subjects is variable.[26,28–30,38,39] We found very high frequencies of *Staphylococcus* spp. in saliva (81.5%) and plaque (62%) samples in our population. Surprisingly, *S. aureus* was not detected in any of our patients, and all isolates were CNS. Although CNS are not associated with nosocomial respiratory infections, their role as major pathogens in other nosocomial infections has significantly increase in the last years.[40,41] Possible reasons for the absence of isolates of *S. aureus* could be the number of subjects or samples assessed, as well as the methodology employed. Regardless of the species, the major concern for treating staphylococci nosocomial infections is related to the continued emergence of multi-drug resistant strains. These species can carry the *mec*A gene, which is the primary determinant of oxacillin-resistance in both *S. aureus* and CNS species.[42] In the present study, CNS carrying the *mec*A were isolated from 15% of saliva and 11.4% of plaque samples. Similarly, Abe et al.[26] isolated *mec*A-positive *S. aureus* in gargled samples of 15% of elderly subjects requiring daily nursing care.

Despite the high frequency of *Pseudomonas* spp., *P. aeruginosa* was detected in 20% of saliva and 24.6% of plaque samples. Using cultivation methods, some studies showed lower detection rates for *P. aeruginosa* (5–12%) in institutionalised or hospitalised elderly patients.[26,27,34,35] On the other hand, Didilescu et al.[10] reported a high frequency of this species in plaque samples (38.2%) of hospitalised patients with chronic lung disease by the checkerboard DNA–DNA hybridisation technique. Other species of *Pseudomonas* such as *P. putida* and *P. fluorescens* may play a role in episodes of nosocomial infections.[43] Recently, *P. fluorescens* was detected in tongue and BAL samples of subjects with VAP,[31] confirming that the oral cavity can harbour different *Pseudomonas* spp.

As observed in several other reports,[1,10,30] *Acinetobacter* spp. were also detected quite frequently in dental plaque (59%) and saliva (63.6%) of our patients. These species are considered major nosocomial pathogens,[44] accounting for about 80% of reported infections. This species has been detected in the subgingival biofilm of individuals with chronic periodontitis,[7,9] and has also been associated with treatment failure in patients with refractory periodontitis,[45] probably due to its high rates of resistance to many commonly prescribed broad-spectrum antibiotics.[46] Due to the close phenotypic and genotypic similarity of among *Acinetobacter* spp., identification of these species by traditional biochemical methods, as well as DNA-based methods has been difficult and often ambiguous. The ever-changing classification and confusing nomenclature further complicates accurate identification of this group of organisms. In this study, the primers employed in the PCR did not discriminate among species of *Acinetobacter*, so that we cannot state that *A. baumannii* was the major species detected in our samples. Further analyses of these samples (>200 samples) would require cloning and sequencing for species identification. A very strong positive association between oral colonisation by *Pseudomonas* spp. and *Acinetobacter* spp. was found in the present investigation. Previous studies have indicated that species of *Acinetobacter* and *Pseudomonas* may cooperatively interact with each other, exhibiting direct metabolic communications and genetic exchanges when they are inserted in highly organised mixed communities.[47,48]

Post-operative sampling was performed to evaluate possible changes in oral colonisation by these respiratory pathogens from the pre-operative time point. There was a reduction in the number of subjects and samples evaluated at this second moment, mainly because of the difficult in sampling the patients in the ICU, lack of cooperation or refusal to participate. Thus, these results should be interpreted cautiously. No significant changes were observed for any bacteria from first to second sampling. Overall, there was a trend for the frequency of these microorganisms to increase more in dental plaque than saliva samples, possibly because of rapid plaque accumulation. Detection rates of *mec*A-positive CNS increased, whereas *P. aeruginosa* reduced after surgery. Increase in colonisation by respiratory pathogens during hospitalisation has been reported in other studies.[1,10] Nevertheless, these studies usually evaluated patients hospitalised

for long periods of time in ICUs. In our study, the second sampling was carried out in a very short span of time, within 12 h after extubation. Furthermore, only the second sampling took place at the ICU, while the first one was performed at the pre-operative hospital room. Changes in prevalence of different species during hospitalisation may also be influenced by prophylactic use of broad-spectrum antibiotics. Nevertheless, no remarkable changes in the frequency of respiratory species in dental plaque or saliva were observed after antibiotic administration in our study population. Therefore, established pathogens in dental biofilm may be more difficult to eradicate due to their higher resistance to antimicrobials.[49]

In summary, hospitalised patients harboured high frequencies of potential bacterial pathogens associated with respiratory infections in saliva and dental plaque. These data suggest that the oral cavity may be a reservoir for these species, increasing the risk for nosocomial pneumonia in susceptible individuals.

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Ethical approval: The study protocol was approved by the Review Committee for Human Subjects of the National Institute of Cardiology (INC) of Rio de Janeiro, under the number CEP 0100/05.05.2006.

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