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ORIGINAL ARTICLE

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Respiratory pathogens in dental plaque of hospitalized patients with chronic lung diseases

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Abstract Bacterial cultivation studies have shown that dental plaque is a reservoir for respiratory pathogens in intensive care unit patients and in elderly who are debilitated, hospitalized or in a nursing home, placing them at risk of bacterial pneumonia. No information is available, however, concerning dental plaque as a reservoir of putative respiratory pathogens in hospitalized patients with chronic lung diseases. Supragingival plaque colonization of 34 hospitalized chronic lung-diseased Romanian citizens, excluding those with tuberculosis and less than 20 teeth, was therefore assessed by checkerboard DNA–DNA hybridization using a selected panel of whole genomic DNA probes produced from eight respiratory pathogens and eight oral pathogens. Thirty-one lung-healthy dental outpatients served as reference population. Respiratory pathogens were detected in plaque from 29 of the 34 (85.3%) hospitalized patients and 12 of the 31 (38.7%) reference population subjects. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter cloacae* occurred significantly ($p<0.05$) more frequent among the hospitalized patients. Hospitalized chronic lung-diseased patients harbored in their supragingival plaque samples bacteria known to cause nosocomial pneumonia significantly ($p<0.001$) more frequent than lung-healthy dental outpatients. Our results indicate that dental plaque in patients with chronic lung diseases often serves as a reservoir of bacteria known to cause nosocomial pneumonia in susceptible individuals.

Keywords Dental plaque · Dental biofilm · Respiratory pathogens · Nosocomial pneumonia · Chronic lung diseases

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Introduction

As described in a systematic review of 24 articles dealing with risk factors for nosocomial (hospital acquired) bacterial pneumonia, recent studies showed that the oral cavity serves as a reservoir of infection for nosocomial respiratory pathogens [24]. Oral colonization by respiratory pathogens is common in institutionalized patients, especially intensive care unit (ICU) patients, and in the elderly who are debilitated, hospitalized or in a nursing home [12, 18, 20]. Data of the systematic review also support the idea of an association between nosocomial pnemonia and poor oral health for subjects at high risk [24]. The risk factors include presence of underlying disease such as chronic obstructive pulmonary disease (COPD), congestive heart failure, diabetes mellitus, age >70 years, mechanical ventilation, a history of smoking, previous antibiotic treatment, immunosuppresion, depressed consciousness, cross-infection, enteral tube feeding, gastroesophageal reflux, a long preoperative stay, and/or prolonged surgical procedures [5, 26]. Two reports demonstrated a specific association between periodontal disease and COPD [10, 23].

Microorganisms can colonize the lower airways by various routes [11]. Most bacterial nosocomial pneumonias

occur by aspiration of bacteria colonizing the oropharynx or upper gastrointestinal tract of the patient [29]. Also, oral bacteria can be released from dental plaque into the oral fluids which are then aspirated into the lower respiratory tract to cause pneumonia [22].

The vast majority of pulmonary infections are due to aerobic bacteria that are found in the oral flora but are not related to oral diseases [22]. The major aerobic and facultative bacteria causing aspiration pneumonia and/or empyema are various *Streptococcus* species (sp.), *Staphylococcus aureus* and various gram-negative bacilli including *Klebsiella pneumoniae*, *Enterobacter* sp., *Serratia marcescens*, and *Pseudomonas aeruginosa*. Occasionally, other gram-negative bacilli, such as *Escherichia coli* and *Proteus* sp., may be implicated. The obligate anaerobic bacteria most often involved in aspiration pneumonia and lung abscesses are oral species (pigmented and non-pigmented *Prevotella* sp., *Fusobacterium* sp., *Peptostreptococcus* sp. and *Actinobacillus actinomycetemcomitans*) [3, 8].

The colonization of the oral cavity by putative respiratory pathogens has been studied. *P. aeruginosa* was isolated from the tongue, but not from dental plaque, of 14 out of 20 patients with cystic fibrosis, whereas none of the healthy controls harbored this species [13]. Several known pulmonary pathogens, such as *K. pneumoniae* and *S. marcescens*, were isolated from dental plaque of ICU patients but not from patients in a dental clinic [19].

The aims of the present study were: (1) to assess the oral hygiene of a population of hospitalized chronic lung-diseased (HCLD) patients, (2) to examine supragingival plaque from these patients for selected respiratory and oral pathogens, and (3) to compare the data of the HCLD patients with similar data obtained from a population of lung-healthy dental outpatients.

Materials and methods

Subjects

Thirty-four HCLD patients, aged 21–79 years (mean 58.3± 12.4 years) and of which 58.8% were males, were recruited to the study among the patients at the Institute of Pneumology, Bucharest, Romania. Of these, 23 were smokers, 14 received systemic steroids and eight used a steroid inhaler. Their participation was based on informed consent and the Directorate of the Institute of Pneumology approved the study. Reasons for their hospitalization were as follows: COPD (n=14), carcinoma (n=5), bronchiectasis (n=4), chronic pneumonia (n=4), pulmonary suppuration (n=3), pleurisy (n=2), atelectasis (n=1), and pulmonary fibrosis (n=1). Nineteen of the patients had received broad-spectrum antibiotics during the preceding 6 months. An outpatient group from a private dental clinic, comprising 31 subjects ranging in age from 20 to 70 years (mean 50.5± 14.1 years) and a gender distribution of 51.6% males, volunteered as reference population.

For all subjects, demographic data were gathered from the medical or dental records and verified by subject interviews. The following demographic characters were recorded: date of birth, gender, and hospital admission date (HCLD patients only). Medical history data included the presence and history of pulmonary disease and present use of specific medication as well as of antibiotics during the preceding 6 months. HCLD patients with tuberculosis and less than 20 teeth were excluded from the study. So were also dental outpatients with underlying pulmonary diseases, less than 20 teeth and/or recent dental treatment.

Oral examination

All study subjects were examined orally and the amount of supragingival plaque was assessed visually on six teeth (16, 11, 24, 36, 31, and 44) and given scores from 0 to 3: 0 = no visible plaque, 1 = thin plaque adhering to the marginal portion of the tooth only, 2 = moderate accumulation of soft deposits on the gingival portion of the tooth surface, 3 = abundance of soft material on the gingival portion of the tooth surface. For those subjects missing any of the six index teeth, the tooth remaining closest to the missing index tooth was scored. A single score was given for each selected (index or replacement) tooth, representing the surface of that tooth harboring the greatest amount of plaque. The buccal and lingual surfaces at the gingival margin of the teeth were scored according to the plaque index first described by Silness and Löe in 1964 [25]. HCLD patients were examined orally at bedside aided by a hand-held light. Oral examination of the outpatients was carried out in a dental chair with light from the dental unit lamp.

Plaque sampling

All test subjects were sampled once; in HCLD patients who were hospitalized for more than 10 days, a second plaque sample was also obtained. As much as possible, supragingival plaque was collected from one surface (buccal or lingual) of the selected teeth using sterile curettes. Before sampling, the area was first dried and isolated with sterile cotton rolls. The collected plaque was immediately transferred into an Eppendorf tube containing 100 μl of sterile TE (10 mM Tris–HCl, 1.0 mM EDTA, pH 7.6) buffer. The six plaque samples from each subject were then pooled, giving one pooled sample from each study subject. The tubes with the pooled samples were labeled with subject identification numbers and the plaque was then suspended in the TE buffer by vigorous vortexing before adding 100 μl of a 0.5 M NaOH solution. All samples were thereafter transported to the Department of Oral Sciences–Oral Microbiology, University of Bergen, Norway, and maintained at 4°C until analysis for the selected probe bacteria.

All oral examinations and microbiological sampling were conducted by a single examiner (A.C.D.).

Probe bacteria and preparation of DNA probes

Sixteen reference strains were selected as probe bacteria (Table 1). Selection was based on literature information about their implication in the etiology of nosocomial pneumonia [2, 17, 22].

Whole genomic DNA from both gram-negative and gram-positive bacteria was extracted using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). The quantity of extracted DNA was determined spectrophotometrically as the absorbance at 260 nm and its purity by electrophoresis in 1% agarose gel. Digoxigenin labeling of the probes was done using the random primer technique [6]. In brief, first, the nylon membranes were treated with Blocking Reagent to prevent the non-specific binding of antibody contained in Anti-Digoxigenin-AP to the membrane; second, the membranes were incubated with a dilution of Anti-Digoxigenin-AP; and third, the membranes carrying the hybridized probes and bound antibody conjugate were reacted with a chemiluminescent substrate (CSPD) and exposed to the X-ray film to record the chemiluminescent signals (Boehringer, Mannheim, Germany). For detection of chemiluminescent signals, the membranes were exposed to Lmi-Film (Boehringer).

The validation of our probes was made by hybridizing each of the 16 DNA probes with its homologous strain and the other 15 heterologous strains. Weak cross-reactions were observed between the two members of *S. mitis* group: *S. mitis* and *S. sanguinis*.

Table 1 The 16 bacteria used for production of the whole genomic DNA probes

Lung pathogens	Oral pathogens
Staphylococcus aureus ATCC[a] 6538	*Streptococcus mitis* CCUG 31611
Acinetobacter baumanii CCUG[b] 19096	*Streptocuccus sanguinis* ATCC 1055
Klebsiella pneumoniae ATCC 13883	*Streptococcus constellatus* NTCC 2226
Enterobacter cloacae CCUG 6323	*Peptostreptococcus micros* CCUG 17638
Escherichia coli ATCC 25922	*Actinomyces israelii* CCUG 18307
Haemophilus influenzae NCTC[c] 8468	*Eubacterium nodatum* CCUG 15996
Serratia marcescens CCUG 1647	*Prevotella intermedia* VPI[d] 4197
Pseudomonas aeruginosa CCUG 2080	*Fusobacterium nucleatum* ATCC 10953

[a]American Type Culture Collection (Rockville, MD, USA)
[b]Culture Collection, University of Gothenburg (Gothenburg, Sweden)
[c]National Collection of Type Cultures (London, UK)
[d]Virginia Polytechnic Institute (Virginia, USA)

Checkerboard DNA–DNA hybridization

Assessment of the presence and levels of the probe bacteria in the supragingival plaque samples was done by using the checkerboard DNA–DNA hybridization method described by Socransky et al. [27]. Briefly, the samples were boiled for 10 min and neutralized using 800 μl of 5 M ammonium acetate. The released DNA was then placed into the extended slots of a Minislot-30 apparatus (Immunetics, Cambridge, MA, USA), concentrated onto a 15×15 cm positively charged nylon membrane (Boehringer) and fixed to the membrane by cross-linking under ultraviolet light followed by baking at 120°C for 20 min. Two lanes on each membrane had standards consisting of a mixture at 10^5 and 10^6 cells of each probe species. The membrane with fixed DNA was placed in a Miniblotter 45 (Immunetics), with the DNA lanes at 90° to the channels of the device. The DNA probes and hybridization solution were mixed and injected into the individual lanes of the Miniblotter. To detect hybrids, chemiluminescent detection was processed as described above. Evaluation of the chemiluminescent signals was performed visually by comparing the obtained signals of the test samples with those generated by the pooled standardized reference samples (the mixtures containing 10^5 and 10^6 cells, respectively).

Readings were recorded and assigned as checkerboard scores as follows: 0 = no indicated signal; 1 = a signal weaker than that of the low standard (i.e., less than 10^5 cells); 2 = a signal equal to that of the low standard (=10^5 cells); 3 = a signal stronger than that of the low standard but weaker than that of the high standard (more than 10^5 but less than 10^6 bacteria); 4 = a signal equal to that of the high standard (=10^6 bacteria); and 5 = a signal stronger than that of the high standard (more than 10^6 bacteria).

Data analysis

Data were expressed as means, standard deviations and ranges as appropriate. The means were computed for each subject and then averaged across subjects. Intergroup comparisons were done by using *t* test, contingency tables, and chi-square test. Fisher's exact test was used when the expected frequency of any cell in the table was less than 5. Contingency tables and the McNemar test were used for intragroup comparisons. Spearman's rank correlation coefficient was used for testing a possible association between dental plaque scores and the number of respiratory pathogen strains detected in the plaque samples. A p value less than 0.05 was considered statistically significant.

The checkerboard DNA–DNA hybridization scores scaled from 0 to 5 (described above) were used for the calculation of proportions and levels of the 16 bacterial species examined. Score 1 was used as cutoff level [4] to contrast colonization (checkerboard scores 1–5) vs non-colonization (checkerboard score 0) and to minimize cross-reactions (see above).

Table 2 Numbers of individuals testing positive for and prevalences (%) of respiratory pathogens in the 34 hospitalized chronic lung-diseased (HCLD) patients and the 31 lung-healthy dental outpatients

Respiratory pathogens	Number (%) of HCLD patients who tested positive	Number (%) of dental outpatients who tested positive
P. aeruginosa[a]	13 (38.2%)	4 (12.9%)
S. aureus[b]	19 (55.9%)	7 (22.6%)
A. baumannii[c]	15 (44.1%)	3 (9.7%)
E. cloacae[d]	23 (67.7%)	5 (16.1%)
K. pneumoniae	6 (17.7%)	1 (3.2%)
H. influenzae	3 (8.8%)	1 (3.2%)
S. marcescens	19 (55.9%)	11 (35.5%)
E. coli	1 (2.9%)	1 (3.2%)

[a] p=0.02, Fisher's exact test
[b] p=0.006, chi-square test
[c] p=0.002, Fisher's exact test
[d] p<0.001, chi-square test

SPSS 9.0 for Windows statistical software (SPSS Inc. 1998–1999) was used for data management and statistical tests.

Results

Oral hygiene, registered as plaque scores, was poor among the HCLD patients. Their mean plaque score was significantly higher than that of the dental outpatients (2.1±0.7 vs 1.4±0.7, p<0.001, t test). Among the HCLD patients, smokers (n=23) demonstrated a mean plaque score of 2.3 and non-smokers (n=11) 1.6. The differences were significant (p=0.012).

Respiratory pathogens were detected in a significantly higher number of plaque samples from HCLD patients (29

Table 4 Prevalence (%) of bacteria in supragingival plaque samples obtained from 21 hospitalized chronic lung-diseased patients examined twice within 10 days

Bacteria	First sampling (%)	Second sampling (%)
A. baumannii[a]	42.9	47.6
P. aeruginosa[a]	19.1	28.6
E. cloacae[a]	61.9	81.0
K. pneumoniae[a]	9.5	33.3
S. marcescens[a]	47.6	76.2
E. coli[a]	4.8	14.3
H. influenzae[a]	9.5	19.0
S. aureus[a]	42.9	57.1
E. nodatum[b]	14.3	9.5
S. constellatus[b]	28.6	19.1
P. intermedia[b]	38.1	28.6
S. mitis[b]	57.2	80.9
P. micros[b]	19.1	4.8
A. israelii[b]	61.9	33.3
S. sanguinis[b]	61.9	61.9
F. nucleatum[b]	38.1	28.6

[a] Respiratory pathogen
[b] Oral pathogen

Table 3 Prevalence (%) of the 16 tested bacteria by checkerboard scores in the 34 hospitalized chronic lung-diseased patients (LDP) and the 31 lung-healthy dental outpatients (HP)

Bacteria	Checkerboard scores[a]											
	0		1		2		3		4		5	
	LDP (%)	HP (%)	LDP (%)	HP (%)	LDP (%)	HP (%)	LDP (%)	HP (%)	LDP (%)	HP (%)	LDP (%)	HP (%)
A. baumannii[b]	55.9	90.3	44.1	9.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. aeruginosa[b]	61.8	87.1	32.4	9.7	0.0	0.0	2.9	0.0	2.9	3.2	0.0	0.0
E. cloacae[b]	32.3	83.9	67.7	9.7	0.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0
K. pneumoniae[b]	82.3	96.8	17.7	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. marcescens[b]	44.1	64.6	55.9	29.0	0.0	3.2	0.0	3.2	0.0	0.0	0.0	0.0
E. coli[b]	97.0	96.8	3.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. influenzae[b]	91.2	96.8	8.8	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. aureus[b]	44.1	77.4	47.1	22.6	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. nodatum[c]	79.4	74.2	20.6	22.6	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0
S. constellatus[c]	73.5	71.0	26.5	29.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. intermedia[c]	58.8	38.7	23.5	29.0	3.0	9.7	11.8	22.6	2.9	0.0	0.0	0.0
S. mitis[c]	35.3	45.2	41.2	45.2	8.8	6.4	5.9	3.2	2.9	0.0	5.9	0.0
P. micros[c]	82.4	90.3	17.6	9.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. israelii[c]	32.4	64.5	58.8	25.8	2.9	9.7	5.9	0.0	0.0	0.0	0.0	0.0
S. sanguinis[c]	35.3	45.2	44.1	38.7	8.8	12.9	8.8	3.2	0.0	0.0	3.0	0.0
F. nucleatum[c]	64.7	54.8	29.4	29.1	5.9	12.9	0.0	3.2	0.0	0.0	0.0	0.0

[a] See Materials and methods
[b] Respiratory pathogen
[c] Oral pathogen

Table 5 Prevalences (%) of the 16 tested bacteria by checkerboard scores in the 21 hospitalized chronic lung-diseased patients examined twice (I=first sample, II=second sample) within 10 days after hospitalization

Bacteria	Checkerboard scores[a]											
	0		1		2		3		4		5	
	I (%)	II (%)	I (%)	II (%)	I (%)	II (%)	I (%)	II (%)	I (%)	II (%)	I (%)	I (%)
A. baumannii[a]	57.1	52.4	42.9	38.1	0.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0
P. aeruginosa[a]	80.9	71.4	19.1	4.8	0.0	4.8	0.0	19.0	0.0	0.0	0.0	0.0
E. cloacae[a]	38.1	19.0	61.9	52.4	0.0	14.3	0.0	14.3	0.0	0.0	0.0	0.0
K. pneumoniae[a]	90.5	66.7	9.5	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. marcescens[a]	52.4	23.8	47.6	47.6	0.0	28.6	0.0	0.0	0.0	0.0	0.0	0.0
E. coli[a]	95.2	85.7	4.8	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. influenzae[a]	90.5	81.0	9.5	19.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. aureus[a]	57.1	42.9	42.9	57.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. nodatum[b]	85.7	90.5	9.5	9.5	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. constellatus[b]	71.4	80.9	28.6	19.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. intermedia[b]	61.9	71.4	19.0	19.0	0.0	4.8	14.3	4.8	4.8	0.0	0.0	0.0
S. mitis[b]	42.8	19.1	33.3	47.6	9.5	9.5	4.8	19.0	4.8	0.0	4.8	4.8
P. micros[b]	80.9	95.2	19.1	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. israelii[b]	38.1	66.7	57.1	23.8	4.8	9.5	0.0	0.0	0.0	0.0	0.0	0.0
S. sanguinis[b]	38.1	38.1	47.6	52.4	4.7	9.5	4.8	0.0	0.0	0.0	4.8	0.0
F. nucleatum[b]	61.9	71.4	28.6	23.8	9.5	4.8	0.0	0.0	0.0	0.0	0.0	0.0

[a]See Materials and methods
[b]Respiratory pathogen
[c]Oral pathogen

out of 34, 85.3%) than from dental outpatients (12 out of 31, 38.7%, $p<0.001$, chi-square test). No significant correlation was found, however, between the dental plaque scores and the numbers of respiratory pathogens detected. The prevalence of respiratory pathogens in the plaque samples and the statistical significant differences between them in the two study groups are presented in Table 2. The prevalence rates of the tested species expressed as checkerboard scores are given in Table 3.

Twenty-one HCLD patients were resampled within 10 days. There was a trend of increasing colonization by each respiratory pathogen in the second sample as compared to the first one (Table 4). However, such an increase was statistically significant only for *S. marcescens* ($p=0.03$, McNemar test). The oral pathogens *E. nodatum*, *S. constellatus*, *P. intermedia*, *P. micros*, *A. israelii*, and *F. nucleatum* were detected in a lower number of HCLD patients in the second sampling than in the first one. However, no statistically significant differences were recorded (McNemar test). Table 5 shows the shifts in prevalences of the tested species during the time period between the two plaque samplings. No plaque sample of the first collection from the HCLD patients demonstrated checkerboard scores for the respiratory pathogens higher than 1, whereas seven HCLD patients were found at the second sampling to be colonized by respiratory pathogens at scores higher than 1.

Discussion

Associations between oral health and respiratory infection [14, 15], and between periodontal disease and risk factors for bacterial nosocomial pneumonia and COPD [24] have recently been extensively reviewed. To our knowledge, our study is the first report on oral hygiene and dental plaque in HCLD patients. Our primary aim was to assess supragingival plaque and its colonization by respiratory pathogens and some putative oral pathogens in HCLD patients as compared to healthy individuals seen in a dental clinic. Oral pathogens were included in the study because some of them have been isolated from infected lungs [2, 17]; also, they served as reference bacteria both in the checkerboard assay and for the evaluation of the plaque samples. Many of the respiratory pathogens were detected in a larger number of HCLD patients than dental outpatients, and *S. aureus*, *P. aeruginosa*, *A. baumannii*, and *Enterobacter cloacae* occurred significantly more frequently among the HCLD patients than in the outpatients. Only for *E. coli* was the same number of colonized subjects noted in both groups.

HCLD patients had higher prevalences of respiratory pathogens in supragingival plaque (85.3%) than those found by Scannapieco et al. [19] and Fourrier et al. [9] in ICU patients (65% and about 40%, respectively). One explanation for this difference is that all ICU patients, compared to only nine of our HCLD patients, were sampled on hospital admission. Our results indicate that colonization of dental plaque increased during hospitalization. This is in agreement with the findings of Fourrier et al. [9] that the dental plaque of 46% of their ICU patients was colonized by aerobic pathogens when the ICU stay exceeded 10 days. Moreover, the checkerboard levels of respiratory pathogens detected at the second sampling also increased: seven patients were found to be colonized at levels higher than 1 compared to none at the first sampling. One explanation could be that there was a lack of attention to oral hygiene of the HCLD patients. In a prospective study, using PCR and cultivation techniques, Abe et al. [1] examined the prevalence of potential respiratory pathogens in gargled samples from elderly persons. Results showed that professional oral health care in elderly requiring daily nursing care reduced the cell numbers of potential respi-

ratory pathogens. Yoneyama et al. [30] concluded that oral care lowered the risk of pneumonia in institutionalized elderly. Fourrier et al. [9] reported that the pathogen causing pneumonia first colonized dental plaque.

With respect to the 16 bacteria tested, there was a shift in the composition of dental plaque between the two samplings, with a trend of increasing colonization by respiratory pathogens and decreasing colonization by oral pathogens in the second sampling. Altogether, our data suggest that a decrease in the normal flora of supragingival plaque occurred coincidentally with greater colonization by gram-negative bacilli. Scannapieco et al. [19] found that oral colonization by respiratory pathogens was positively associated with the use of antibiotics. It can therefore be speculated that the use of systemic antibiotics in 19 of the 34 HCLD patients might have influenced their supragingival microbiota. Our study did not address this question specifically, but it is known that dental plaque prevents antibiotic efficacy [28]. Few studies have investigated microbial changes in subgingival plaque microbiota in patients receiving systemic antibiotics. Periodontal pathogens were not significantly altered by oral administration of doxycycline using conventional therapeutic dosage [7].

The oral hygiene of the HCLD patients (mean plaque score 2.1) was poorer than that reported for ICU patients (mean plaque score 1.9) [19]. No oral hygiene data from HCLD patients have been published until now. The mean plaque score of the HCLD patients was significantly higher than that of the dental outpatients. Similar results were reported by Scannapieco et al. [19], who found a mean plaque score for the ICU patients to be significantly higher compared to the control population of preventive dentistry clinic patients. In another study, Scannapieco et al. [21] found that 41 individuals with a confirmed chronic respiratory disease had significantly higher oral hygiene index scores than had 193 subjects without respiratory disease. A plausible explanation for this is decreased oral hygiene standard while staying in the hospital.

Several techniques can be used for the assessment of the composition of the plaque microbiota. Among them the culture procedures have traditionally been regarded as the reference ("gold standard") in the analysis of the plaque microbiota [16]. In our study, transportation of plaque specimens from Romania to Norway could have compromised some of the bacterial species and, consequently, the culture technique was not used. Therefore, we chose to use the checkerboard DNA–DNA hybridization method in order to analyze the Romanian supragingival plaque samples. To our knowledge, this is the first published attempt to detect respiratory pathogens in supragingival plaque by using the checkerboard DNA–DNA hybridization method.

One limitation of this study was the age difference between the HCLD patients and the outpatients (mean age 58.3 vs 50.5 years). A more appropriate comparison would also have been done with a group of age- and disease-matched outpatients. Another suggestion for comparison of HCLD patients would be the use of a group of patients in the same hospital, hospitalized for different conditions other than chronic pulmonary diseases.

In conclusion, our results suggest that dental plaque of both lung-diseased and lung-healthy subjects harbor respiratory pathogens, with a significantly higher rate for lung-diseased patients. We therefore consider that special attention must be paid to the oral hygiene care in individuals at risk of acquiring lower respiratory infections. This is in line with several controlled trials demonstrating that interventions that reduce the oral microbial burden or oral respiratory pathogen colonization prevent nosocomial pneumonia [24].

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