

Bacterial colonization of distal airways in healthy subjects and chronic lung disease: a bronchoscopic study

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ABSTRACT: In contrast to the healthy population, distal airway bacterial colonization may occur in patients with chronic lung diseases, who often have altered pulmonary defences. However, the information dealing with this issue is insufficient and is based mainly on nonspecific samples, such as sputum cultures.

Using quantitative cultures of bronchoscopic protected specimen brush (PSB) and bronchoalveolar lavage (BAL) samples, we studied the bacterial colonization of distal airways in 16 healthy subjects, 33 patients with bronchogenic carcinoma, 18 with chronic obstructive pulmonary disease (COPD), 17 with bronchiectasis, and 32 with a long-term tracheostomy due to laryngeal carcinoma. All patients were without exacerbation, and free from antibiotic treatment at least 1 month before the study protocol. Thresholds for quantitative cultures to define colonization were $\geq 10^2$ colony-forming units (cfu)·mL⁻¹ for PSB and $\geq 10^3$ cfu·mL⁻¹ for BAL.

Only one healthy subject was colonized by a potential pathogenic microorganism (PPM) (*Staphylococcus aureus* 4×10^2 cfu·mL⁻¹ in a PSB culture). Colonization was observed in 14 (42%) bronchogenic carcinoma patients (19 non-PPMs, and 10 PPMs); in 15 (83%) COPD patients (22 non-PPMs and 7 PPMs); in 15 (88%) bronchiectasis patients (20 non-PPMs and 13 PPMs); and in 15 (47%) long-term tracheostomy patients (5 non-PPMs and 13 PPMs). The two most frequent non-PPMs isolated in all groups studied were *Streptococcus viridans* and *Neisseria* spp. *Haemophilus* spp., *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* were the most frequent PPMs isolated in bronchogenic carcinoma, COPD, bronchiectasis and long-term tracheostomized patients, respectively. *Pseudomonas aeruginosa* colonization was infrequent in all the groups.

Our results show that distal airway bacterial colonization is a frequent feature in stable patients with chronic lung diseases and also in patients with long-term tracheostomy. However, the pattern of colonization differs among groups studied. The knowledge of different colonization patterns may be important for future antibiotic prophylactic strategies and for the empirical antibiotic regimens when exacerbations occur in these patients.

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Distal airways are usually sterile in healthy nonsmoking individuals as has been demonstrated in various studies [1, 2]. However, when mechanical airway defences are altered, as occurs in chronic bronchitis, chronic obstructive pulmonary disease (COPD), bronchiectasis or bronchial obstruction, or there is a bypass of oropharyngeal defences, e.g. in tracheostomized patients, the distal airways may become colonized by potential pathogenic microorganisms (PPMs) and non-potential pathogenic microorganisms (non-PPMs) [3-5]. The importance of airway bacterial colonization in these populations is not clearly understood, but it has been speculated, particularly in COPD patients, that the persistence of microorganisms in distal airways could worsen the evolution of the chronic underlying disease [6-8]. Furthermore, a

knowledge of the type of colonizing agents may be important to standardize empirical antibiotic strategies when these patients develop infectious exacerbations (tracheo-bronchitis or pneumonia). It seems logical to assume that colonizing microbial flora will be responsible for these infections [9, 10].

Several studies have demonstrated frequent airway colonization in stable COPD and bronchiectatic patients [1, 3, 4, 11, 12]. The information regarding distal airway colonization in tracheostomized patients or those with bronchogenic carcinoma is scarce. Overall, few studies have used reliable bronchoscopic sampling methods, such as protected specimen brush (PSB) or bronchoalveolar lavage (BAL), to study this colonizing flora both in healthy subjects [13] and stable chronic lung disease

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patients [14, 15]. In addition, the relationship between distal airway colonization and oropharyngeal flora has not been assessed. This prompted us to perform a prospective study, using PSB and BAL to investigate distal airway flora in healthy subjects and in patients with bronchogenic carcinoma, stable COPD, bronchiectasis, and long-term tracheostomy. The relationship between this flora and the oropharyngeal bacterial colonization was also investigated.

Material and methods

Patients

We studied 116 patients, who did not require hospital admission. These patients were divided into five categories: 1) healthy nonsmoking volunteers (n=16); 2) patients with bronchogenic carcinoma (n=33) with histological confirmation of malignancy (31 of these patients had concomitant COPD; in addition, they underwent their first bronchoscopic study at the time of inclusion into the present study); 3) patients with COPD (n=18) according to previously defined criteria [16]. All COPD patients were in a stable clinical condition according to classical criteria [17]; 4) patients with bronchiectasis (n=17) (who had clinical features, suggestive chest radiographic findings, and confirmatory computed tomography (CT) scan findings of bronchiectasis) [18]; 5) long-term tracheostomized patients (n=32) due to total laryngectomy because of cancer of the larynx.

Patients included in the study had been free from pulmonary infections during the preceding 30 days. In addition, no patients had received antibiotic treatment during the last 4 weeks. Patients who were admitted to the hospital during the last 3 months, and those with immunosuppression (patients with acquired immune deficiency syndrome (AIDS)) or those receiving chemotherapy or corticosteroids (equivalent prednisone dose ≥ 20 mg·day⁻¹) were excluded. The mean \pm SD forced expiratory volume in one second (FEV₁) was 68 \pm 19, 77 \pm 19, 73 \pm 12 and 92 \pm 7 percentage of predicted for bronchogenic carcinoma patients, COPD patients, bronchiectasis patients, and healthy volunteers, respectively. And, the mean \pm SD FEV₁/forced vital capacity (FVC) was 65 \pm 11, 65 \pm 10, 72 \pm 25 and 80 \pm 8 percentage of predicted, for bronchogenic carcinoma patients, COPD patients, bronchiectasis patients, and healthy volunteers, respectively. COPD patients were classified as having: mild obstruction, FEV₁ 65–80% pred (n=13); or moderate obstruction, FEV₁ 45–65% pred (n=5); and none had severe obstruction, FEV₁ <45% pred. The ex-smoking condition was defined as the cessation of smoking habit at least 5 yrs previously. In patients with bronchiectasis, a confirmatory CT scan was defined as abnormally dilated bronchi near the periphery of the lung fields, failure of bronchi to taper as they left the hilar areas, or cystic dilatation of bronchi, with or without fluid levels. Long-term tracheostomy was defined as the presence of tracheostomy stoma for more than one year.

Study protocol

All patients gave informed consent to participate in the study. Pharyngeal swab samples were taken from

86 patients (10 healthy subjects, 23 bronchogenic carcinoma patients, 13 COPD patients, 11 bronchiectasis patients, and 29 long-term tracheostomized patients).

In COPD patients, the reason for performing a fiberoptic bronchoscopy was mild haemoptysis in 11 cases, suspicion of bronchiectasis in three, and chest radiographic alterations in the remaining four. In the remaining groups, bronchoscopy formed part of either the routine clinical practice in our institution (bronchogenic carcinoma and bronchiectasis), or the study protocol (healthy subjects and long-term tracheostomized patients).

Fibreoptic bronchoscopy (BF30; Olympus, New Hyde Park, NY, USA) was performed transnasally in all groups except in the long-term tracheostomized patients, where the bronchoscope was introduced through the tracheostomy stoma. Four percent nebulized lidocaine (during 15 min) was used as local anaesthetic. No anaesthetics were instilled through the working channel of the bronchoscope. No aspiration was performed through the bronchoscope throughout the procedure. All bronchoscopic procedures were performed in Trendelenburg's position, to avoid oropharyngeal aspiration to the lower airways. A PSB (Microbiology brush; Mill-Rose Laboratory Inc. 7310, Mentor, Ohio, USA) sample was retrieved in all cases (n=116). Samples were obtained in the right lower lobe, except in the group of patients with bronchogenic carcinoma, in whom PSB sampling was performed proximal to the endobronchial lesion, if present, or from the suspected affected bronchus corresponding to the chest radiographic infiltrate.

After PSB sampling, the fiberoptic bronchoscope was wedged into a subsegmentary bronchus from the same area where PSB was performed. BAL samples were obtained in 102 cases (15 healthy subjects, 28 bronchogenic carcinoma, 16 COPD, 13 bronchiectasis, and 30 long-term tracheostomies). Three aliquots of sterile saline (50 mL each) were instilled and aspirated. The first aliquot was discarded. The mean BAL fluid obtained for processing was 27 \pm 12 mL. The PSB procedure was always performed before BAL sampling to avoid bacterial contamination from the bronchoscope channel. For the purpose of the study, colonization of lower airways cultures was considered if PSB and BAL cultures yielded $\geq 10^2$ and $\geq 10^3$ colony-forming units (cfu)·mL⁻¹, respectively. On the other hand, cultures were considered negative if they were sterile or yielded <10² and <10³ cfu·mL⁻¹ for PSB and BAL, respectively. These cut-off points were chosen arbitrarily. Bacteriological results were also evaluated using higher thresholds: $\geq 10^3$ cfu·mL⁻¹ for PSB and $\geq 10^4$ cfu·mL⁻¹ for BAL.

Microbiological processing

Pharyngeal samples were obtained by means of sterile swabs (Eurotubo; Industrias Aulabor, S.A., Barcelona, Spain), homogenized in 1 mL of distilled water, and diluted to concentrations of 10⁻¹, 10⁻² and 10⁻³.

The protected specimen brushes were aseptically cut into a sterile tube containing 1 mL of saline and agitated in a vortex-type mixer for 1 min. Serial dilutions (10⁻¹, 10⁻² and 10⁻³) from each PSB and BAL sample were prepared in sterile normal saline. One hundred

microlitres of each dilution were inoculated into the following agar media: 5% sheep blood; chocolate; Center for Disease Control (CDC); blood agar McConkey; buffered charcoal yeast extract (BCYE- α); and Sabouraud dextrose. All cultures were incubated at 37°C under aerobic and anaerobic conditions and in a CO₂-enriched atmosphere. Cultures were evaluated for growth after 24 and 48 h and discarded, if negative, after 5 days, with the exception of CDC blood agar which was evaluated at 7 days and for Sabouraud medium which was evaluated at 4 weeks. All microorganisms isolated were identified by standard laboratory methods [19].

Bacterial agents were classified into PPMs or non-PPMs. PPMs were those microorganisms recognized as agents causing respiratory infections, whether or not belonging to the gastrointestinal or oropharyngeal flora: Gram-negative rods, such as *Pseudomonas aeruginosa*, Enterobacteriaceae and *Haemophilus* spp.; Gram-positive cocci, such as *Staphylococcus aureus*, *Streptococcus*

pneumoniae; and Gram-negative cocci, such as *Moraxella catarrhalis*. Non-PPMs were those microorganisms belonging to the oropharyngeal or gastrointestinal flora that are not usually involved in respiratory infections in non-immunocompromised patients (*Streptococcus viridans* group, *Neisseria* spp., *Corynebacterium* spp., *Candida* spp., and others) [20].

Results

A total of 116 cases was included in the study. The general characteristics of the study population are summarized in table 1. Healthy subjects were younger than patients from the other four groups (43±16 yrs). Male subjects were more common in the bronchogenic carcinoma group (97%), COPD (83%) and tracheostomy group (91%), when compared to the healthy and bronchiectasis groups.

Table 1. – General characteristics of the study population

	Healthy (n=16)	Bronchogenic carcinoma (n=33)	COPD (n=18)	Bronchiectasis (n=17)	Tracheostomy (n=32)
Age yrs +	43±16	64±10	60±12	57±19	61±9
Gender M/F	7/9	32/1	15/3	8/9	29/3
Smoking habit %					
Never	69	0	0	59	4
Ex-smoker	19	34	50	29	96
Smoker	12	66	50	12	0

COPD: chronic obstructive pulmonary disease; M: male; F: female. +: values are mean, ±SD.

Table 2. – Number of patients with sterile or positive protected specimen brush (PSB) quantitative cultures

PSB cultures	Healthy (n=16)	Bronchogenic carcinoma (n=33)	COPD (n=18)	Bronchiectasis (n=17)	Tracheostomy (n=32)
Sterile	11	16	2	2	19
<10 ² cfu·mL ⁻¹	3	4	1	1	1
10 ² –10 ³ cfu·mL ⁻¹	2	4	5	3	6
10 ³ –10 ⁴ cfu·mL ⁻¹	0	5	6	3	2
10 ⁴ –10 ⁵ cfu·mL ⁻¹	0	4	4	5	3
≥10 ⁵ cfu·mL ⁻¹	0	0	0	3	1

COPD: chronic obstructive pulmonary disease; cfu: colony-forming units.

Table 3. – Microorganisms isolated from PSB quantitative cultures in counts ≥10² cfu·mL⁻¹

	Healthy	Bronchogenic carcinoma	COPD	Bronchiectasis	Tracheostomy
<i>Streptococcus viridans</i> group	1 (33)	8 (32)	11 (41)	7 (29)	2 (15)
<i>Streptococcus</i> group D (non-Enterococcus)	1 (33)	-	-	1 (4)	-
<i>Streptococcus pneumoniae</i>	-	2 (8)	2 (7)	-	1 (8)
<i>Staphylococcus aureus</i>	1 (33)	1 (4)	1 (4)	1 (4)	3 (23)
PC-negative <i>Staphylococcus</i>	-	2 (8)	2 (7)	3 (13)	-
<i>Micrococcus</i> spp.	-	1 (4)	-	-	-
<i>Pseudomonas aeruginosa</i>	-	1 (4)	-	1 (4)	-
<i>Moraxella catarrhalis</i>	-	-	-	-	4 (31)
<i>Haemophilus influenzae</i>	-	3 (12)	2 (7)	10 (42)	2 (15)
<i>Haemophilus parainfluenzae</i>	-	2 (8)	-	-	-
<i>Neisseria</i> spp.	-	5 (20)	6 (22)	1 (4)	-
<i>Corynebacterium</i> spp.	-	-	2 (7)	-	1 (8)
<i>Candida</i> spp.	-	-	1 (4)	-	-
Total	3	25	27	24	13

Values are presented as absolute number of isolations, and percentage of total isolations from each group in parenthesis. PC: plasma coagulase; COPD: chronic obstructive pulmonary disease; PSB: protected specimen brush.

Colonization in healthy subjects

PSB was performed in all (n=16) healthy subjects, whilst BAL was performed in 15 (94%) cases. The quantitative cultures of PSB were negative (sterile cultures or cultures $<10^2$ cfu·mL⁻¹) in 14 (88%) cases. In the remaining two (12%) cases, there were 3 microorganisms in counts $\geq 10^2$ cfu·mL⁻¹ in PSB samples (table 2). These isolates were *S. viridans* group (2×10^2 cfu·mL⁻¹), *Streptococcus* group D (non-Enterococcus) (5×10^2 cfu·mL⁻¹), and *S. aureus* (4×10^2 cfu·mL⁻¹) (table 3).

BAL quantitative cultures were negative in 13 out of 15 (87%) cases (sterile counts or $<10^3$ cfu·mL⁻¹) (table 4). In the remaining two subjects, BAL samples yielded two microorganisms: *S. viridans* (10^3 cfu·mL⁻¹), and *Neisseria* spp. ($\times 10^3$ cfu·mL⁻¹) (table 5).

Using PSB and BAL culture results together, four patients (25%) were colonized, mostly by non-PPM. When cut-off points were raised to $\geq 10^3$ cfu·mL⁻¹ for PSB and to $\geq 10^4$ cfu·mL⁻¹ for BAL, none of the subjects studied demonstrated colonization (tables 2 and 4).

Colonization in patients with bronchogenic carcinoma

PSB samples were negative in 20 out of 33 (61%) patients, and positive in the remaining 13 (39%) (table 2). In the 13 colonized patients (PSB counts $\geq 10^2$ cfu·mL⁻¹), PSB samples yielded 25 microorganisms. Sixteen (64%) of the isolated microorganisms were non-PPMs: *S. viri-*

ans group (8); *Neisseria* spp. (5); plasma coagulase (PC)-negative Staphylococcus (2); and *Micrococcus* spp. (1). The most frequent PPMs were *Haemophilus* spp (n=3) and *S. pneumoniae* (n=2) (table 3).

BAL quantitative cultures were negative in 24 out of 28 (86%) cases (table 4). In four patients, BAL samples yielded positive cultures (n=7 microorganisms): *S. viridans* group (3); *S. aureus* (1); *P. aeruginosa* (1); *Haemophilus influenzae* (1); and *Neisseria* spp. 1 (table 5).

When considering PSB and BAL culture results together, 14 patients (42%) were colonized. When the cut-off points were changed to $\geq 10^3$ cfu·mL⁻¹ for PSB and to $\geq 10^4$ cfu·mL⁻¹ for BAL, nine patients (27%) had distal airway colonization (tables 2 and 4).

Colonization in patients with COPD

PSB samples were negative in 3 out of 18 (17%) patients, and positive in the remaining 15 (83%) (table 2). In the 15 colonized patients, PSB samples yielded 27 microorganisms (table 3). Twenty two (81%) of the isolated microorganisms were non-PPMs: *S. viridans* group (11), *Neisseria* spp. (6); PC-negative Staphylococcus (2); *Corynebacterium* spp. (2); and *Candida* spp. (1). The PPM isolated (n=5) were: *H. influenzae* (2); *S. pneumoniae* (2); and *S. aureus* (1) (table 3).

BAL quantitative cultures were negative in 14 out of 16 (88%) cases (table 4). In two patients, BAL yielded

Table 4. – Number of patients with sterile or positive bronchoalveolar lavage (BAL) quantitative cultures

BAL cultures	Healthy (n=15)	Bronchogenic carcinoma (n=28)	COPD (n=16)	Bronchiectasis (n=13)	Tracheostomy (n=30)
Sterile	6	9	5	1	12
1–10 ² cfu·mL ⁻¹	2	11	7	0	2
10 ² –10 ³ cfu·mL ⁻¹	5	4	2	2	6
10 ³ –10 ⁴ cfu·mL ⁻¹	2	3	2	3	7
10 ⁴ –10 ⁵ cfu·mL ⁻¹	0	1	0	1	2
10 ⁵ –10 ⁶ cfu·mL ⁻¹	0	0	0	5	1
>10 ⁶ cfu·mL ⁻¹	0	0	0	1	0

COPD: chronic obstructive pulmonary disease; cfu: colony-forming units.

Table 5. – Microorganisms isolated from bronchoalveolar lavage (BAL) quantitative cultures

	Healthy	Bronchogenic carcinoma	COPD	Bronchiectasis	Tracheostomy
<i>Streptococcus viridans</i> group	1 (50)	3 (43)	1 (50)	6 (30)	-
<i>Streptococcus pneumoniae</i>	-	-	1 (50)	-	1 (8)
<i>Staphylococcus aureus</i>	-	1 (14)	-	-	4 (33)
PC-negative Staphylococcus	-	-	-	-	1 (8)
<i>Pseudomonas aeruginosa</i>	-	1 (14)	-	1 (5)	-
<i>Moraxella catarrhalis</i>	-	-	-	-	4 (33)
<i>Haemophilus influenzae</i>	-	1 (14)	-	7 (35)	-
<i>Neisseria</i> spp.	1 (50)	1 (14)	-	5 (25)	-
<i>Corynebacterium</i> spp.	-	-	-	1 (5)	2 (17)
Total	2	7	2	20	12

Values are presented as absolute number of isolations, and percentage of total isolations from each group in parenthesis. PC: plasma coagulase; COPD: chronic obstructive pulmonary disease.

positive cultures (n=2 microorganisms): *S. viridans* group (1) and *S. pneumoniae* (1) (table 5).

The total percentage of colonization according to PSB plus BAL results was 83% (n=15). Using the cut-off point of $\geq 10^3$ cfu-mL⁻¹ for PSB and $\geq 10^4$ cfu-mL⁻¹ for BAL 56% of patients (n=10) had distal airway colonization (tables 2 and 4).

Colonization in patients with bronchiectasis

PSB samples were negative in 3 out of 17 (18%) patients, and positive in the remaining 14 (82%) (table 2). In the 14 colonized patients, PSB samples yielded 24 microorganisms (table 3). Twelve (50%) of the isolated microorganisms were non-PPMs: *S. viridans* group (7); *Neisseria* spp. (1); PC-negative Staphylococcus (3); and *Streptococcus* group D (non-Enterococcus) (1). Among the PPMs (n=12 microorganisms), *H. influenzae* (n=10) was the most frequently isolated (table 3).

BAL quantitative cultures were negative in 3 out of 13 (23%) cases (table 4). In 10 patients, BAL samples yielded 20 microbial agents, as shown in table 5.

Taking into account both PSB and BAL culture results, distal airway colonization was observed in 88% of patients (n=15). Using the cut-off point of $\geq 10^3$ cfu-mL for PSB and $\geq 10^4$ cfu-mL⁻¹ for BAL 64% of patients (n=11) had distal airway colonization (tables 2 and 4).

Colonization in long-term tracheostomized patients

PSB samples were negative in 20 out of 32 (62%) patients, and positive in the remaining 12 (38%) (table 2). In the 12 colonized patients, PSB samples yielded 13 microorganisms (table 3). Three (23%) of the isolated microorganisms were non-PPMs: *S. viridans* group (2); and *Corynebacterium* spp. (1). The predominant PPM (n=10) were: *M. catarrhalis* (4) and *Staphylococcus aureus* (3) (table 3).

BAL quantitative cultures were negative in 20 out of 30 (67%) cases (table 4). In 10 patients, BAL samples yielded 12 microbial agents as shown in table 5.

The total percentage of colonization (PSB plus BAL cultures) was 47% (n=15). When using higher cut-off points for PSB ($\geq 10^3$ cfu-mL⁻¹) and BAL ($\geq 10^4$ cfu-mL⁻¹) the total percentage of distal airway colonization was 28% (n=9) (tables 2 and 4).

Microbial isolates from PSB (<10² cfu-mL⁻¹) and BAL samples (<10³ cfu-mL⁻¹)

In healthy subjects, three cases yielded *S. viridans* from their PSB cultures in counts <10² cfu-mL⁻¹. The following microorganisms were isolated from BAL cultures of healthy subjects in counts <10³ cfu-mL⁻¹ in seven cases. *S. viridans* (4); *Streptococcus* group D (1); PC-negative Staphylococcus (1); and *S. viridans* + PC-negative Staphylococcus + *Neisseria* spp. (1).

In the group of patients with bronchogenic carcinoma, the following microorganisms were isolated from PSB cultures in counts <10² cfu-mL⁻¹ in three patients: *S. pneumoniae* (1); PC-positive Staphylococcus (1); *Neisseria* spp. (1). The following microorganisms were isolated from BAL cultures in counts <10³ cfu-mL⁻¹ in 15 cases: *S. viridans* (6); PC-negative Staphylococcus (1); PC-positive Staphylococcus (1); *S. pneumoniae* (1); *H. influenzae* (1); *S. viridans* + PC-negative Staphylococcus (1); *S. viridans* + PC-positive Staphylococcus (1); *Neisseria* spp. (1); *S. viridans* + *Neisseria* spp. (1); and *Enterobacter sakazakii* (1).

In COPD patients, only one case yielded *S. viridans* from PSB cultures <10² cfu-mL⁻¹. As regards BAL cultures <10³ cfu-mL⁻¹, nine patients yielded the following microorganisms: *S. viridans* + *Neisseria* spp. (3); *S. viridans* (2); *Neisseria* spp. (2); *S. pneumoniae* (1); and PC-negative Staphylococcus (1).

As regards patients with bronchiectasis, only one case yielded *S. viridans* from PSB cultures <10² cfu-mL⁻¹. From BAL cultures (<10³ cfu-mL⁻¹), two patients yielded *S. pneumoniae* + PC-negative Staphylococcus, and the other yielded *P. aeruginosa* + *Neisseria* spp.

Finally, patients with long-term tracheostomies yielded *Neisseria* spp. from PSB cultures in counts <10² cfu-mL⁻¹ in only one case. BAL cultures <10³ cfu-mL⁻¹ from eight patients yielded: PC-positive Staphylococcus (2); *Neisseria* spp. (2); *S. pneumoniae* (1); *Streptococcus* group G (1); *S. viridans* + PC-positive Staphylococcus + *Corynebacterium* spp. (1); *M. catarrhalis* (1).

Qualitative agreement between pharyngeal swab, PSB, and BAL isolate

Pharyngeal swabs were taken from 86 patients yielding a total of 217 microorganisms. In all these patients PSB and BAL samples were also obtained. The results of the qualitative concordance between pharyngeal swabs

Table 6. – Qualitative concordance between microbial species isolated from pharyngeal swabs, protected specimen brush (PSB) samples and bronchoalveolar lavage (BAL) samples

Group	PhS n*	PSB			BAL		
		Coincident microorganisms with PhS		Non-PPMs	Coincident microorganisms with PhS		
		n*	PPMs		n*	PPMs	Non-PPMs
Healthy subjects (n=10)	33	5	0	4 (80)	11	0	10 (91)
Bronchogenic carcinoma (n=23)	62	27	0	12 (44)	20	0	13 (65)
COPD (n=13)	30	19	0	11 (58)	11	0	7 (64)
Bronchiectasis (n=110)	29	15	0	5 (33)	24	3 (12)	10 (42)
Tracheostomy (n=29)	63	16	2 (12)	5 (31)	28	2 (7)	8 (28)

*: number of isolates. The values in parenthesis are percentages. PPMs: potential pathogenic microorganisms; non-PPMs: non-potential pathogenic microorganisms; PhS: pharyngeal swabs; COPD: chronic obstructive pulmonary disease.

and PSB and BAL cultures are presented in table 6. The percentage of concordance was higher in healthy subjects (80% for PSB and 91% for BAL) compared to the remaining groups (table 6). Non-PPMs accounted for most qualitative concordances among the three types of cultures (pharyngeal swab, PSB and BAL).

Discussion

The main finding of the present study was that distal airways are frequently colonized in clinically stable populations with bronchogenic carcinoma (42%), COPD (83%), bronchiectasis (88%) and in long-term tracheostomized patients (47%). Healthy subjects were insignificantly colonized. The most frequent microorganisms found in each group were non-PPMs, such as *S. viridans* group and *Neisseria* spp., except for tracheostomized patients, in whom PPMs (*M. catarrhalis* and *S. aureus*) were the most frequent colonizers. These results reveal the possible pathogens related to bacterial exacerbations in the populations studied.

Healthy nonsmokers are free from bacterial colonization of the lower airways. Nevertheless, there is very little information dealing with this issue [13]. In the present study, we found that only one healthy subject had a true respiratory pathogen isolated in significant numbers (4×10^2 cfu·mL⁻¹ *S. aureus*) from his lower airways. KIRKPATRICK and BASS [13], using PSB and BAL to study the distal airways of eight healthy people, found that only one BAL specimen yielded one PPM. Similar results were also reported in a recently published article [14]. The present study and the latter two underline the efficacy of lung defences in healthy subjects in maintaining the near-sterility of the lower airways.

For several years, it has been believed that COPD patients had their distal airways colonized by bacteria [3, 4, 12]. In addition, an increase in the airway bacterial burden was a constant feature during exacerbations [15]. However, this general conviction comes from studies that have used diagnostic methods with high risk of oropharyngeal microbial contamination, such as sputum cultures [3]. A recent article from MONSO *et al.* [15], using fiberoptic PSB samples, demonstrated that 25% of 40 stable COPD patients had colonization of the distal airways (cut-off $\geq 10^3$ cfu·mL⁻¹), although they did not distinguish potential pathogenic from non-potential pathogenic microorganisms. Similar results were reported by RUISE *et al.* [14] studying 18 COPD patients. The present results in the COPD group, although using a lower threshold, demonstrated a higher rate of colonization (83%). However, 76% of the colonizing agents were non-PPMs belonging to the oropharyngeal flora (tables 3 and 5). We do not know the significance of the presence of these non-PPMs in the lower airways. The measurement of inflammatory cytokine production by the airways could clarify the meaning of both types of microorganisms (PPMs and non-PPMs) colonizing the distal airways of stable COPD patients. More studies are still warranted to confirm the frequently and importance of non-PPM colonizing microorganisms in COPD patients. Additionally, it could help to explain the "vicious circle" hypothesis regarding the role of bacterial infection in COPD [21].

The PPMs (*S. pneumoniae*, *H. influenzae* and *S. aureus*)

found in the present study represented 24% of the total number of colonizing agents corresponding to seven COPD patients. We did not observe Gram-negative bacilli, such as *P. aeruginosa*, which is in agreement with other studies in the literature [1–4, 11, 14]. However, we do not know whether more severely ill COPD patients, needing antibiotics frequently and/or receiving long-term corticosteroids, could have enteric Gram-negative bacilli. Enteric Gram-negative bacilli were also found infrequently during COPD exacerbations requiring or not requiring hospitalization [15, 22]. Nonetheless, in COPD exacerbated patients requiring mechanical ventilation, *P. aeruginosa*, *Proteus mirabilis* and *Escherichia coli* accounted for 18% of the total microorganisms recovered [23], suggesting a relationship between the severity of the exacerbation and the presence of these microorganisms. In addition, this latter finding may have important implications for antibiotic therapy. A possible limitation of the present study as regards COPD patients could be the bias due to studying this population because of the distinct clinical indications. However, the majority of patients were investigated because of mild haemoptysis. Moreover, all patients were free from exacerbation.

Results of the study of colonization pattern in patients with bronchogenic carcinoma did not differ from those found in COPD patients, indicating that both populations were very similar as demonstrated by the results of forced spirometry. However, one case of *P. aeruginosa* was found in the bronchogenic carcinoma group. Another study of patients with bronchogenic carcinoma found similar PSB isolates as in the present study, and yet the authors studied the contralateral lung [24]. Overall, the presence of bronchogenic carcinoma does not seem to modify the colonizing bacterial flora in COPD patients. However, others have shown that Gram-negative bacilli and anaerobes may be found in distal airways below the bronchial obstruction [25]. We believe that a knowledge of bacterial colonization in patients with bronchogenic carcinoma may be important for the development of prophylactic strategies, especially when they are undergoing thoracic surgery.

The most frequent PPM found in distal airways from patients with bronchiectasis was *H. influenzae*. This finding coincides with most series dealing with bronchoscopic and nonbronchoscopic sampling in bronchiectatic populations [6, 26–28]; and indicates that the spectrum of empirical antibiotic treatment in bronchiectasis has to cover this microorganism. *P. aeruginosa* was extremely infrequent in the present series (one case; <6%). This figure is significantly low compared to the 10% incidence [6, 28], or to the 24–31% incidence [26, 29] reported by other authors. Explanations for discrepancies among studies regarding *P. aeruginosa* in bronchiectasis are based on the different diagnostic methods used, and also on difference in severity of airway obstruction. For instance, EVANS *et al.* [6] confirmed that bronchiectatic patients colonized by *P. aeruginosa* had lower FEV₁ (28% pred) when compared to those who did not yield this microorganism (59% pred). Therefore, empirical coverage against *P. aeruginosa* is mandatory in exacerbated patients with bronchiectasis and severe obstruction. In fact, a large study on severe community-acquired pneumonia in which only five patients had

P. aeruginosa, revealed that all five had bronchiectasis [30].

We also investigated tracheostomized nonhospitalized patients. Surprisingly, *M. catarrhalis* accounted for the majority of PPMs, followed by *S. aureus*. There is a disparity between the bacterial flora found in this group and that found in the remaining groups, indicating that long-term tracheostomized patients constitute a peculiar group in terms of distal airway colonization. Unfortunately, we do not know how many patients from this group had COPD. This is a significant finding for the empirical antibiotic strategy when these patients develop tracheobronchitis or pneumonia. Moreover, there is very little information regarding airway colonization in nonhospitalized long-term tracheostomized patients. A recent paper from HARLID *et al.* [31] demonstrated a 30% colonization of the distal airways in this type of patient. The predominant microorganisms isolated were *S. aureus*, enteric Gram-negative bacilli, and *P. aeruginosa*. As regards the latter microorganism, there is clear evidence that tracheostomized patients (particularly post-Intensive Care Unit (ICU)) have a predisposition to become colonized by *P. aeruginosa* [32–34]. The colonization probably starts in the stoma and migrates down the trachea, where microorganisms have a peculiar affinity for epithelial adherence [35, 36]. We found a discrepancy between pharyngeal and distal colonization in these patients, thus, we have to assume that the stoma is the introductory airway colonization gate, as has been suggested by other authors [31]. Unfortunately, we only have stoma swab cultures in few patients from this group.

Overall, the percentage of positive cultures was much lower in BAL compared to PSB samples except in patients with bronchiectasis. These results clearly suggest that bacterial colonization is located mainly at the bronchial level. However, in bronchiectasis it seems to be a persistent bacterial burden in alveolar spaces. These patients could have more substantial alterations in their lung defences compared to the remaining populations studied.

A potential criticism of the present study may be the choice of low thresholds for quantitative cultures of PSB ($\geq 10^2$ cfu·mL⁻¹) and BAL ($\geq 10^3$ cfu·mL⁻¹). In fact, other studies [14, 15] have used higher thresholds ($\geq 10^3$ cfu·mL⁻¹ for PSB). Since the latter cut-off point for PSB was originally [37] defined to distinguish colonization from infection, and the present study was designed to study colonization of distal airways, we thought that using lower thresholds would avoid missing important microbiological information regarding colonization. Conversely, we could have chosen lower thresholds to differentiate colonization from infection, consequently increasing the rate of colonization, particularly with BAL. However, we have ignored the meaning of microorganisms isolated in very low counts (PSB $< 10^2$ cfu·mL⁻¹ and BAL $< 10^3$ cfu·mL⁻¹).

It is commonly believed that prior abnormal oropharyngeal colonization is the main mechanism for subsequent distal airway colonization [38]. In healthy subjects, 80% of the microorganisms found in distal airways were also isolated from the oropharynx (table 6). However, the percentage of qualitative agreement between both sites decreased to around 50% in other patient groups, and was even lower in patients with long-term

tracheostomy, in agreement with a previous study [39]. Discrepancies between pharyngeal and distal airway cultures were even higher for *H. influenzae* in patients with bronchiectasis. The lack of concordance between pharyngeal swabs and PSB or BAL cultures could be explained as follows: 1) pharyngeal swabs may not be the best samples since they can miss bacteriological information; some authors recommend pharyngeal gargles [40]; 2) pharyngeal colonization may, perhaps, be a transient feature, whilst distal airway colonization is a constant one; 3) other oropharyngeal-related reservoirs for distal airway colonization that were not evaluated in the present study could play a role, *e.g.*, sinuses [41] and dental plaque [42]; and, finally, 4) some microorganisms, such as *P. aeruginosa* and *H. influenzae* have special affinity due to their ability of adhere to tracheal cells without previous oropharyngeal colonization, particularly in specific populations, such as patients with COPD or bronchiectasis [31, 34].

In summary, our results confirm that colonization of distal airways is a frequent finding in stable patients with chronic lung disease or long-term tracheostomy. Our findings reinforce the idea that populations with alterations in their lung defences are chronically colonized. The clinical importance of this chronic colonization warrants future research.

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