Sputum *versus* bronchoscopy for diagnosis of *Pseudomonas aeruginosa* biofilms in cystic fibrosis

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ABSTRACT: The present authors hypothesised that bronchoscopy with protected specimen brush may sample biofilm-forming bacteria adherent to the airway wall, whereas traditional sputum collection may not.

Pseudomonas aeruginosa obtained from sputum, bronchoalveolar lavage and protected brush, taken from the right upper lung bronchus of 12 adult patients with cystic fibrosis, were compared. Retrieved bacteria were genotyped, and grown in planktonic cultures and as biofilms, and susceptibilities to individual antibiotics and to antibiotic combinations were determined.

Bacterial cultures obtained using bronchoscopy did not yield any new strains of bacteria that were not also found in sputum. A total of 10 patients (83%) had a single strain of *P. aeruginosa* found using sputum, bronchoalveolar lavage and protected brush techniques, and two patients (17%) had two strains recovered in sputum, but only one strain was recovered using bronchoscopic techniques. Susceptibility to single antibiotics and to antibiotic combinations were not different between planktonically or biofilm-grown bacteria derived from sputum, as compared to those obtained by bronchoalveolar lavage and protected brush.

In conclusion, sputum collection provides as much information as bronchoscopy for characterising the genotype and antibiotic susceptibility of chronic *Pseudomonas aeruginosa* infection in patients with stable cystic fibrosis.
Eur Respir J 2004; 24: 631–637.

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Pseudomonas aeruginosa is the most common pathogen that chronically infects patients with cystic fibrosis (CF) [1]. Recent evidence suggests that *P. aeruginosa* may actually grow as dense communities of exopolysaccharide-encapsulated biofilms adherent to CF patients′ airways [2–4].

Patients with CF have sputum cultures taken regularly during clinically stable periods, and susceptibility results from these cultures are used to guide initial antibiotic therapy during exacerbations. One problem with relying on sputum samples to guide therapy is that biofilm bacteria may not be adequately sampled *via* sputum, since sputum expectoration only samples *P. aeruginosa* that have detached and dispersed from the airway biofilm [5, 6]. *In vitro* studies have shown that biofilm-forming *P. aeruginosa* are significantly less susceptible to antibiotics compared to nonadherent forms [3, 7]. However, studies have not previously been done to determine whether bacteria retrieved from sputum are more susceptible to antibiotics compared to bacteria retrieved directly from the airway biofilm.

Techniques for acquiring respiratory specimens that are more invasive, and potentially more accurate than sputum, have been investigated to determine their ability to detect bacterial flora present in patients′ lungs. For example, semiquantitative broncheoalveolar lavage (BAL) has previously been evaluated for the detection of infections in the lower respiratory tract [8–11]. Bronchoscopy with protected specimen brush (PSB) has also been utilised to collect uncontaminated airway specimens for culture [12–14]. In contrast to BAL, where saline washings of bronchial secretions are aspirated for sampling, in PSB bronchoscopy the airway lumen is directly brushed to remove bacteria and the PSB is then retracted into its sterile sheath to minimise contamination with other airway secretions. Thus, it is possible that PSB may directly recover *P. aeruginosa* bacteria that have aggregated as biofilms and adhered to the surface of the airway.

The purpose of the present study was to determine whether the PSB can sample directly from the airway biofilm and, thus, more accurately represent the state of *P. aeruginosa* in CF patients′ lungs. The authors wanted to determine whether bacteria adherent to the airways, retrieved *via* protected brush specimens, are genotypically or phenotypically different from the bacteria that are traditionally retrieved from sputum cultures. If this were the case, then this might suggest that bronchoscopy specimens are preferable over sputum for the accurate diagnosis of CF pulmonary infections.

Materials and methods

Subjects

Patients were eligible for this study if they were aged >16 yrs, had a confirmed diagnosis of CF with chronic *P. aeruginosa*

infection, were able to spontaneously produce sputum, had a forced expiratory volume in one second (FEV1) $\geqslant 40\%$ of predicted and were clinically stable and free of any oral or *i.v.* antibiotics for >4 weeks prior to study entry. The study was approved by the Ottawa Hospital Research Ethics Board and all subjects provided informed, written consent.

Specimen collection

Patients were required to spontaneously produce sputum immediately prior to bronchoscopy being performed. The same investigator performed the bronchoscopy on all 12 patients. Following expectoration of sputum, the upper airway was anaesthetised with 2% lidocaine spray. Patients were then sedated with intravenous fentanyl and diazepam, using an average dose of 50 µg and 5 mg, respectively. Topical anaesthesia of the vocal cords and the trachea was achieved using 2% lidocaine, applied through the broncho-scope from above the cords. The bronchoscope (IT-160 and BF-200; Olympus Corporation, Hinode, Japan) was then introduced through the vocal cords and the tip was wedged in the anterior segment of the right upper lobe bronchus. Use of suction was avoided before wedging to keep the suction channel free of contamination. BAL was performed by instilling and aspirating five 20-mL aliquots of sterile 0.9% NaCl (CHASTRE *et al.* [12]). The BAL fluid was pooled and immediately placed on ice. Subsequent to the BAL procedure, the PSB (8115; Primed Instrument Incorp., Mississauga, ON, Canada) was introduced through the working channel of the bronchoscope and advanced into the anterior segment of the right upper lobe bronchus. After dislodging the distal catheter plug, the brush was applied to the wall of the subsegmental airway and agitated against the airway wall to procure lower airway secretions (CHASTRE *et al.* [13]). The brush was then cut and deposited into 1 mL of sterile saline and placed on ice.

Specimen culture

Sputum specimens were washed with an equal volume of sterile saline and the wash liquid discarded. An appropriate amount of sputum was then added to an equal amount of trypticase soy broth (TSB; Becton-Dickinson Company, Cockysville, MD, USA) and ground in a tissue grinder (Radnoti Glass, Monrovia, CA, USA). The specimens were further diluted 1:10, 1:1,000 and 1:100,000 with TSB, and 10 µL of each dilution were inoculated onto trypticase soy agar with 5% sheep blood agar (BA) and McConkey agar plates (PML Microbiologicals, Mississauga, ON, Canada).

BAL fluid was diluted 1:100 and 1:100,000 with TSB and 10 µL of the undiluted specimen, and each of the two dilutions were inoculated onto media as described for sputum. PSB specimens were vortexed vigorously in 1 mL of sterile saline to assure complete release of all material from the brushes. Dilutions and inoculation of microbiological media was done as described for BAL fluids.

The BA and McConkey plates were incubated for 48–72 h at 35°C in ambient air. Colonies resembling *Pseudomonas* spp. were selected from either the BA or McConkey plates for identification and susceptibility testing. A total of five different colonies were randomly selected from each specimen type regardless of whether or not they represented the same or different morphological types. In this way, the cultures were deliberately over-sampled in order to maximise the recovery of as many different strain types as possible. All isolates selected for work-up were identified as *P. aeruginosa* by conventional microbiological methods, as described elsewhere [15, 16]. All isolates were kept frozen at -70°C in TSB containing 15% glycerol (Sigma-Aldrich, St. Louis, MO, USA) until required for further testing.

Pulsed-field gel electrophoresis-typing of isolates

Molecular genotyping of *P. aeruginosa* isolates was carried out by pulsed-field gel electrophoresis (PFGE) as described by AARON *et al.* [17]. Restriction fragment profiles were visually compared and interpreted based on guidelines recommended by TENOVER *et al.* [18]. Isolates with identical restriction fragment profiles were considered to represent a single strain. Isolates with restriction profiles that differed by 1–3 fragments (bands) were considered to be closely related strains and were considered to have evolved from a single clone. Isolates with restriction profiles differing by four bands or more were considered to be different strains.

Minimal inhibitory concentration determinations (planktonic growth)

Minimal inhibitory concentrations (MICs) were determined for six anti-pseudomonal antibiotics: meropenem (AstraZeneca Pharmaceuticals, Wilmington, DE, USA), tobramycin (Sabex, Boucherville, QC, Canada), amikacin (Bristol laboratories, Montreal, QC, Canada), piperacillin (Wyeth-Ayerst, Montreal, QC, Canada), ciprofloxacin (Miles Inc., West Haven, CT, USA), and ceftazidime (GlaxoSmithKline, Mississauga, ON, Canada) for each isolate of *P. aeruginosa*. MICs were performed by broth microdilution and interpreted using National Committee for Clinical Laboratory Standards guidelines [19].

Multiple combination bactericidal testing (planktonic growth)

Multiple combination bactericidal testing (MCBT) was performed to assess the susceptibility of each isolate to multiple combinations of antibiotics, as previously described [20]. Ten antibiotics were tested against each *P. aeruginosa* in 94 different combinations. The combinations consisted of group-ings of two or three of the following: azithromycin (Pfizer, New York, NY, USA), meropenem, ticarcillin-clavulanic acid (GlaxoSmithKline, Mississauga, ON, Canada), piperacillin-tazobactam (Wyeth-Ayerst), trimethoprim-sulfamethoxazole (GlaxoSmithKline), amikacin, ceftazidime, ciprofloxacin, chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA) and tobramycin.

Biofilm testing

All of the *P. aeruginosa* isolates recovered from sputum, BAL and PSB specimens of the 12 patients were grown as biofilms using a modified Calgary Biofilm device [21]. Single and combination antibiotic susceptibility testing of *P. aeruginosa*, grown as biofilms, has been previously validated and shown to be reproducible [7, 21].

Briefly, for biofilm growth, the isolates were grown for 24 h adherent to polystyrene pegs that sat in standard 96-well plates (Nalge Nunc International, Roskilde, Denmark) at 35°C in ambient air. A rocking table was used to produce shear forces across each peg, resulting in the formation of equivalent biofilms at each peg site. The current authors have previously shown by electron microscopy that this method results in biofilm growth on the pegs [7]. The biofilm culture was then transferred to a standard 96-well plate in which dilutions of antibiotics had been prepared and then incubated

overnight at 35°C in ambient air. The MIC for the biofilm was determined to be the concentration of antibiotic at which biofilm growth (absence of turbidity in the well) was inhibited. The biofilm was then removed from the pegs by sonication and the viability of the bacterial biofilm (minimum biofilm eradication concentration) was assessed by plate counts [7].

Statistical analysis

A generalised estimating equations (GEE) model was used to compare the sputum isolates to the BAL and PSB isolates to determine whether there were any significant differences in their MIC values. The GEE model is an extension of the generalised linear model and allows for correlation between individuals and does not require a normal distribution of data. Results were analysed modelling antibiotics individually, and also treating antibiotic type as a covariate to compare susceptibility of sputum, BAL and PSB isolates across all six antibiotics. MCBT results were analysed with paired t-tests to determine whether there were significant differences between sputum and BAL, and sputum and PSB results. A p-value <0.05 was considered statistically significant.

Results

Study population

The study population consisted of nine male and three female patients with CF whose average age was 29 yrs. All patients had been chronically infected with mucoid

P. aeruginosa isolates for an average duration of 8 ± 5 yrs (range: 3–19 yrs). Relevant patient characteristics are given in table 1. All 12 study patients tolerated the bronchoscopy well without complications.

Table 1. – Baseline characteristics of cystic fibrosis (CF) patients

Characteristic	
Age yrs	29.3±8.0
Male sex	9 (75)
FEV1	
L	2.41±0.80
% pred	64.6±21.2
FVC	
L	3.86±0.76
% pred	85.6±19.8
Duration of *P. aeruginosa* infection yrs	8.2±5.1
Pancreatic insufficient	11 (92)
Diabetes	0 (0)
Liver disease	1 (8)
Medications for CF	
Bronchodilators	8 (67)
Inhaled corticosteroids	5 (42)
Inhaled tobramycin	6 (50)
Vitamins	12 (100)
Pancreatic enzymes	11 (12)

Data are presented as mean±SD or n (%). n=12. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; *P. aeruginosa*: *Pseudomonas aeruginosa*.
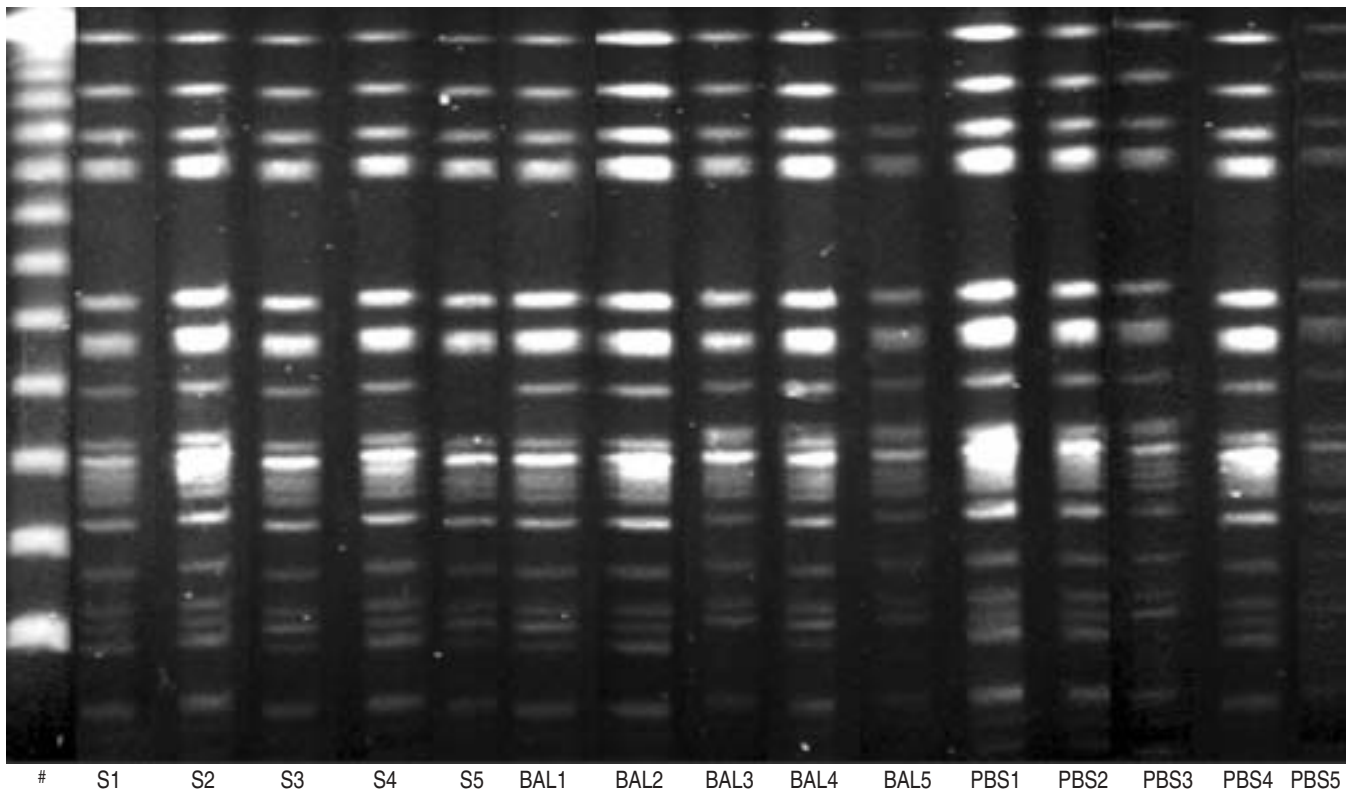

Fig. 1. – Pulsed-field gel electrophoresis (PFGE) of *Pseudomonas aeruginosa* isolates from patient 4. The genomic DNA for the 15 *P. aeruginosa* isolates from each patient (five for each of the three sampling techniques) were analysed by PFGE following restriction digestion with SpeI (restriction endonuclease). All the isolates from this patient were either derived from a single strain or a closely related strain of *P. aeruginosa*, since none of the isolates differed by more than three restriction bands. S: sputum; BAL: bronchoalveolar lavage; PSB: protected specimen brush. #: 48.5-kb DNA ladder.

Quantitative cultures

Quantitative culture analysis of the 15 planktonically grown isolates (five sputum, five BAL and five PSB) retrieved from each of the 12 CF patients revealed that they all had ≥1,000,000 cfu·mL^{-1} of *P. aeruginosa* present in their samples. This amount of bacteria is well above the 10,000 cfu·mL^{-1} cut-off currently mandated for accurately diagnosing lung infections with PSB and BAL [11, 12].

Bacterial genotyping

Bacterial cultures obtained using bronchoscopy did not yield any new strains of bacteria that were not also found in sputum. PFGE revealed that 10 patients (83%) had a single strain of *P. aeruginosa* found in all sputum, BAL and PBS cultures (fig. 1). Two patients (17%) had two strains of *P. aeruginosa* recovered from sputum, but only one strain was recovered from BAL and PSB (fig. 2).

Table 2 summarises the genotypes of the patients′ isolates. A total of five patients (patients 1, 3, 5, 7 and 11) were discovered to have a common 'A′ strain of *P. aeruginosa* growing from sputum.

Single-antibiotic susceptibilities

Table 3 summarises the single antibiotic MIC data as log$_2$ values for the planktonically grown isolates. Antibiotic

Table 2. – Summary of *Pseudomonas aeruginosa* strains

Patient	Genotype of *P. aeruginosa* retrieved		
	Sputum	BAL	PSB
1	A	A	A
2	F/G[#]	F	F
3	A	A	A
4	H	H	H
5	A	A	A
6	J	J	J
7	A	A	A
8	D	D	D
9	B	B	B
10	C	C	C
11	A/D[#]	A	A
12	E	E	E

Genotypes are arbitrarily designated an alphabetic letter based on their restriction fragment profiles. A single letter indicates that all five isolates for that sampling technique yielded a single strain of bacteria. Patients 2 and 11 had two strains of *P. aeruginosa* bacteria present in sputum. Five patients (patients 1, 3, 5, 7, 11) shared a common "A" strain of *P. aeruginosa*. BAL: bronchoalveolar lavage; PSB: protected specimen brush. [#]: patients with two strains of *P. aeruginosa* in sputum.

susceptibilities were independent of the source of the *P. aeruginosa* isolate (p=0.76 and p=0.65 for the comparison of sputum and BAL MICs, and sputum and PSB MICs, respectively). There were no significant differences in antibiotic susceptibility between the three sampling techniques for
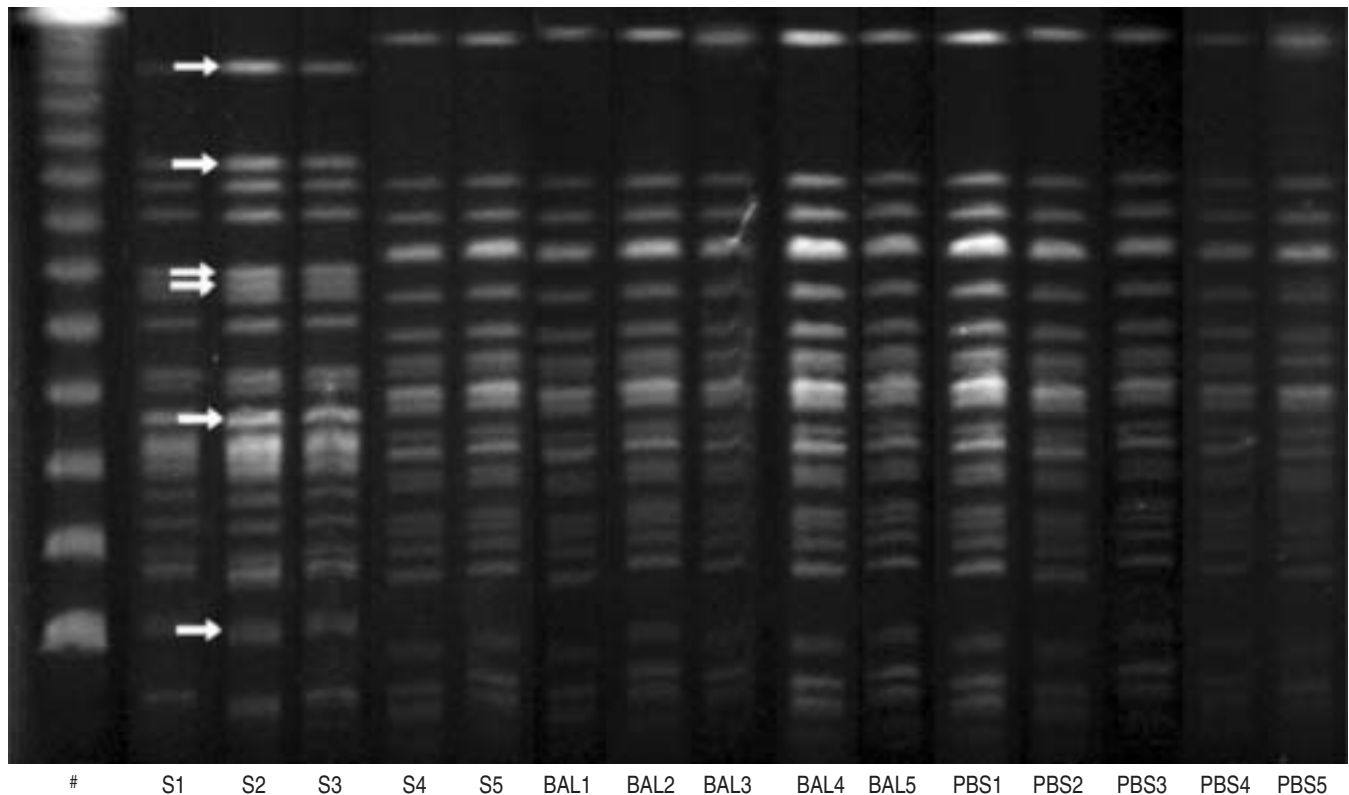

Fig. 2. – Pulsed-field gel electrophoresis (PFGE) of *Pseudomonas aeruginosa* isolates from patient 2. The genomic DNA for the 15 *P. aeruginosa* isolates from each patient (five for each of the three sampling techniques) were analysed by PFGE following restriction digestion with SpeI (restriction endonuclease). Isolate profiles in lanes 5–16 are derived from the same strain of *P. aeruginosa*. However, isolates 1, 2 and 3 from sputum are a different strain of *P. aeruginosa* than the other isolates (see white arrows for illustrative examples of different bands). S: sputum; BAL: bronchoalveolar lavage; PSB: protected specimen brush. [#]: 48.5-kb DNA ladder.

Table 3. – Single antibiotic susceptibility results presented as minimal inhibitory concentration (MIC) $\log_2$ values for the planktonically grown isolates obtained from cystic fibrosis patients

Antibiotic	MICs for isolates			p-values			
				Sputum *versus* BAL		Sputum *versus* PSB	
	Sputum	BAL	PSB	IA	AA	IA	AA
Tobramycin	1.82±1.50	1.42±1.50	1.47±1.49	0.07	0.76	0.09	0.65
Meropenem	-1.50±1.74	-1.67±1.78	-1.15±2.38	0.41		0.31	
Amikacin	4.78±1.19	4.77±1.48	5.00±1.46	0.94		0.49	
Ceftazidime	3.28±1.68	3.90±2.25	3.50±2.53	0.04		0.62	
Piperacillin	5.15±2.31	5.30±2.26	5.28±2.47	0.55		0.75	
Ciprofloxacin	-0.67±2.10	-0.53±1.89	-0.53±2.10	0.53		0.45	

MIC values (mean±SD) are shown as $\log_2$ values of the dilution used. n=12. Comparative p-values are derived from the generalised estimating equations model. BAL: bronchoalveolar lavage; PSB: protected specimen brush; IA: individual antibiotics; AA: all antibiotics.

all six antibiotics tested. The one exception was ceftazidime, where isolates obtained *via* BAL were less susceptible to ceftazidime than those obtained from sputum (p=0.04).

Table 4 summarises the single antibiotic MIC data as $\log_2$

Table 4. – Single antibiotic susceptibility results presented as minimal inhibitory concentration (MIC) $\log_2$ values for the biofilm grown isolates obtained from cystic fibrosis patients

Antibiotic	MICs for isolates			p-values	
	Sputum	BAL	PSB	Sputum *versus* BAL	Sputum *versus* PSB
Tobramycin	6.0±0.0	6.0±0.0	6.0±0.0	0.99	0.99
Meropenem	5.0±0.0	5.0±0.0	5.0±0.0	0.99	0.99
Amikacin	7.0±0.0	7.0±0.0	7.0±0.0	0.99	0.99
Ceftazidime	8.0±0.0	8.0±0.0	8.0±0.0	0.99	0.99
Piperacillin	8.0±0.0	8.0±0.0	8.0±0.0	0.99	0.99
Ciprofloxacin	3.93±0.23	3.87±0.21	3.80±0.4	0.37	0.10

MIC values (mean±SD) were obtained from paired t-test analysis and are shown as $\log_2$ values of the dilution used. n=12. Comparative p-values are derived from the generalised estimating equations model. BAL: bronchoalveolar lavage; PSB: protected specimen brush.

values for the isolates grown as biofilms. For all these isolates, there were no significant differences in susceptibility between BAL- and PSB-acquired samples in comparison to the isolates from sputum. Minimum biofilm eradication antibiotic concentrations were even higher than the biofilm MICs and they similarly showed no differences between sputum, BAL or PSB-derived isolates (data not shown).

Combination antibiotic susceptibilities

Table 5 summarises the MCBT data. A total of 94 double and triple antibiotic combinations were assessed for bactericidal activity against each isolate. There were no significant differences observed between the number of susceptible antibiotic combinations for the planktonically grown sputum isolates (mean: 75.9±7.5) compared to BAL isolates (mean: 76.2±8.6, p=0.80) or PSB isolates (mean: 76.1±11.0, p=0.89).

Similar results were seen for biofilm-grown isolates. Biofilm-grown isolates were less susceptible to antibiotic combinations than planktonic-grown isolates. However, there were no significant differences observed when comparing the number of susceptible antibiotic combinations for the biofilm-grown sputum isolates (mean: 31.0±9.0) compared to BAL

Table 5. – Multiple combination bactericidal testing results

Patient	Mean number of bactericidal combinations for the five isolates from each patient					
	Planktonic			Biofilm		
	Sputum	BAL	PSB	Sputum	BAL	PSB
1	81	81	83	21	13	24
2	84	88	87	24	30	25
3	76	69	73	23	6	22
4	61	60	72	35	23	40
5	63	70	54	18	36	21
6	83	85	85	48	26	38
7	71	71	71	30	37	40
8	80	81	91	38	45	41
9	75	69	64	24	24	13
10	78	85	85	37	38	31
11	76	74	66	38	34	30
12	82	82	83	35	17	11
Mean±SD	75.9±7.5[#]	76.2±8.6	76.1±11.0[¶]	31.0±9.0[+]	27.5±11.4	28.1±10.4[§]

Each cell represents the mean number of susceptible drug combinations (out of a total of 94) for the five isolates from each patient (five from sputum, five from bronchoalveolar lavage (BAL) and five from protected specimen brush (PSB)). Paired t-tests were used to compare differences between the mean number of susceptible drug combinations between sputum and BAL, and sputum and PSB. [#]: p=0.80, *versus* planktonic BAL; [¶]: p=0.89, *versus* planktonic sputum; [+]: p=0.34, *versus* biofilm BAL; [§]: p=0.29, *versus* biofilm sputum.

isolates (mean: 27.5±11.4, p=0.34) or PSB isolates (mean: 28.1±10.4, p=0.29).

Discussion

Prior to this study, no previous investigations have been conducted to explore whether bronchoscopy can obtain cultures of bacteria directly from *in vivo* biofilms and whether these bacteria are genetically different or more resistant to antibiotics than those obtained from sputum cultures. In this group of 12 stable CF patients, neither the PSB nor the BAL isolates provided any additional relevant information regarding strain type and antibiotic sensitivity of the *P. aeruginosa* bacteria in comparison to those samples isolated from sputum.

Bronchoscopy specimens did not yield any *P. aeruginosa* strains that were not found in sputum. A possible explanation for this is that bronchoscopy sampling was limited to the right upper lobe only in the present study to avoid contamination of the sample port from suctioning multiple sites. Expectorated sputum samples a greater proportion of the airways bilaterally and may, potentially, provide a more accurate picture of the flora present. Alternatively, it is possible that the additional *P. aeruginosa* strains recovered in the sputum from two patients, but not recovered from their right upper lobes using bronchoscopy, may have originated from these patients′ upper airways or sinuses.

A total of 10 out of 12 patients had a single distinct strain of *P. aeruginosa* in all their isolates, and sputum cultures from only two patients produced two distinct strains. The presented results are consistent with those generated from previous investigations of *P. aeruginosa* in chronically infected CF patients [17, 22, 23]. These studies have all reported that, over time, most adult CF patients tend to harbour a single clone or subclone of *P. aeruginosa* during periods of clinical stability, and that the same bacterial clone retrieved during periods of clinical stability is also retrieved during pulmonary exacerbations [17].

The current study used standard serial dilution microbiological techniques to isolate, identify and quantify organisms. Serial dilution techniques may not identify organisms that are present only in very low numbers. However, isolates were deliberately over-sampled from the culture plates in order to maximise the recovery of as many different strain types as possible.

A total of five out of the 12 patients shared the same strain of *P. aeruginosa* in all specimen types. This indicates that there may be a strain of *P. aeruginosa* circulating through the clinic at our centre causing infection in a proportion of our CF patients. This observation is consistent with the findings of several other studies [24–28]. These studies have all provided convincing evidence that cross-infection in CF patients may exist and imply that limited shared contact in a clinic setting may be sufficient to transfer strain types between CF patients. ARMSTRONG *et al.* [28] and JONES *et al.* [29] both suggested that the most likely mode of spread was direct patient-to-patient contact.

When the single antibiotic susceptibilities for *P. aeruginosa* obtained from sputum were compared to those obtained by bronchoscopy, no significant differences in MIC values were observed. Similar results were found when comparing susceptibilities of isolates to antibiotic combinations. Based on these observations, isolates obtained through bronchoscopy techniques would not provide any additional advantages over sputum isolates for assessing antibiotic susceptibility of *P. aeruginosa* in CF patients.

BAUGHMAN *et al.* [11] published a study in which sputum culture results were compared to BAL semiquantitative culture results from 28 bronchoscopies done in 11 adult patients with CF. They showed that in 11 instances BAL was able to either detect a new pathogen, or detect an additional pathogen that sputum cultures did not. This led to an alteration in therapy in 48% of the patients investigated. The major difference in the study by BAUGHMAN *et al.* [11], in comparison to the current report, was that there were inadequate sputum cultures for comparison in 11 out of their 28 BAL procedures. Since many of their patients could not produce sputum, bronchoscopy revealed pathogens not otherwise identified.

The present study was restricted to clinically stable patients. The authors chose to conduct this study in clinically stable patients due to safety concerns and also to mimic clinical practice. Patients with CF have sputum cultures taken regularly during clinically stable periods and susceptibility results from these cultures are used to guide initial antibiotic therapy during exacerbations. A previous study [17] has confirmed that the vast majority of *P. aeruginosa* isolates retrieved during exacerbations are identical to those retrieved during clinical stability. Thus, the patients presented here are representative of those who would benefit the most from an alternative, and potentially more accurate, airway sampling technique during clinically stable periods to direct their therapy when they become ill.

Antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolated from sputum remains a requirement for directing therapy in chronically infected cystic fibrosis patients. However, antibiotic administration hardly ever eliminates carriage of this bacterium, but rather reduces symptoms of pulmonary exacerbations. Given the current findings, the authors feel that the failure of antibiotic therapy to sterilise the adult cystic fibrosis airway is not due to any inherent limitation in the ability of sputum to adequately sample the airway flora. In conclusion, two bronchoscopic techniques (bronchoalveolar lavage and protected specimen brush) failed to show any benefit over sputum in sampling the type of *Pseudomonas aeruginosa* infecting the lungs of stable cystic fibrosis patients.

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