

Bacterial reservoirs in cystic fibrosis

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Abstract

To establish whether colonisation of the upper respiratory tract or bacterial contamination of inhaler devices or solutions predisposes to colonisation of the lower respiratory tract in patients with cystic fibrosis, bacterial isolates from groups of children who were positive (n=13) or negative (n=18) for *Pseudomonas aeruginosa* were studied. Cultures of swabs from inhaler devices, toothbrushes, and upper airways were compared with cough swabs or sputum cultures. No pathogens were obtained from inhaler equipment administering unit dose medications. Upper airway carriage of *Staphylococcus aureus* and *Haemophilus influenzae* was identified in both groups but correlated poorly with sputum isolates. *P. aeruginosa* was found only in the upper respiratory tract of children with established colonisation of the lower airways. No *P. aeruginosa* isolates were obtained from the upper airways of the group with negative sputum, including one patient who became colonised by *P. aeruginosa* during the study.

Our results did not support the suggestion that colonisation of the upper respiratory tract by *P. aeruginosa* predisposes to colonisation of the lower airways. Failure to isolate pathogenic organisms consistently from the upper airways in patients with positive sputum argues against a local epithelial factor predisposing to bacterial colonisation.

The upper respiratory tract has a normal flora that varies with time and includes some of the pathogens associated with cystic fibrosis, although there are species differences—for example, capsulate or non-capsulate *Haemophilus influenzae*.¹ *Pseudomonas* spp may also be part of the normal upper airways' flora in both normal subjects and patients with cystic fibrosis.² It has been suggested that, in cystic fibrosis, colonisation of the upper respiratory tract may precede spread to the lower airways.³ Once established in the lungs the organism is extremely difficult to eradicate, therefore treating the infection at an early stage, possibly when it is confined to the upper airways, is an attractive proposition.

Many children with cystic fibrosis also use inhaled treatment. Shortly after the introduction of nebulisers, an increase of necrotising Gram negative pneumonias was noted in normal patients.⁴ Gram negative organisms, in particular *Pseudomonas* spp were also found in nebuliser reservoirs and solutions.^{5,6} Therefore we con-

sidered it appropriate to investigate the upper respiratory tract flora in children with cystic fibrosis to determine whether colonisation of the upper airways with *Pseudomonas aeruginosa* could be identified before sputum conversion and to examine inhaler equipment for contamination by pathogenic organisms.

Patients and methods

Chronic colonisation by *P. aeruginosa* was identified in 29 (40%) of the 73 children attending the cystic fibrosis clinic at Sheffield Children's Hospital. Thirteen consecutive clinic attenders in this group were studied (patients with positive sputum). Of the remainder, 18, from whom *P. aeruginosa* had never been grown (patients with negative sputum) also agreed to participate in the study. At a monthly clinic visit dry swabs were collected from the anterior and posterior nasal space and the back of tongue. After physiotherapy a sputum sample or cough swab, or both, was taken as appropriate. Parents were requested by letter to bring nebulisers, Spinhalers (Fisons), Rotahalers (Allen and Hanburys), jet inhalers, and toothbrushes with them to the clinic. Swabs were taken from these devices for bacteriological culture. Media used were blood agar incubated in air plus 5% carbon dioxide, heated blood agar with a bacitracin disk (8 µg) incubated anaerobically, CDMI agar,⁷ and cystine-lactose electrolyte deficient agar cultured aerobically. After incubation for 24 hours, plates were examined and colonies of potential pathogens were tested by standard procedures for reaction in Gram stain and production of coagulase, deoxyribonuclease, and oxidase for the recognition of *Staphylococcus aureus* and *P. aeruginosa*. *H. influenzae* was identified by a requirement for X and V factors in a disk test. Biochemical identification of 'coliform bacilli' included tests for indole, the Voges-Proskauer reaction, citrate utilisation, urease, and the o-nitrophenol-galactopyranoside test; these were confirmed, when necessary, by the API 20E system (Api-Bio Merieux).

Results

Results were recorded as shown in table 1. The recovery of the major bacterial pathogens for both groups is summarised in table 2. As expected the children in the group negative for *P. aeruginosa* were younger (mean age 8.3 years, range 3.3-14.6) with minimal or non-productive cough compared with the patients who were positive (mean age 11.9 years, range 6.5-17.5). Sputum cultures were often difficult to obtain

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Table 1 Example of bacterial harvest from a single site (toothbrush), showing the number of patients from results of both direct culture and broth incubation for groups of patients positive and negative for *P aeruginosa*

	Direct culture		Broth incubation	
	Positive patients	Negative patients	Positive patients	Negative patients
<i>Staphylococcus epidermidis</i>	3	2	2	3
<i>Streptococcus viridans</i>	3	1		4
Non-haemolytic Anaerobes: spore bearer			3	
Diphtheroids	1		1	1
<i>Neisseria</i> spp				1
<i>Escherichia coli</i>	1	3	1	3
Coliform bacilli	2		3	4
<i>Klebsiella</i> spp	2		1	
<i>Pseudomonas fluorescens</i>	3	1		
<i>Aeromonas hydrophila</i>	1			
<i>H parainfluenzae</i>	1	1		
Non-lactose fermenting bacilli			1	2
No growth	4		1	

Other organisms sought but not isolated included: *S aureus*, α and β haemolytic streptococci, *Streptococcus pneumoniae*, *Streptococcus faecalis*, *H influenzae*, *Haemophilus parahaemolyticus*, bacteroides, and fungi.

Table 2 Number of patients with major bacterial isolates in groups positive and negative for *P aeruginosa*

	Toothbrush*	Rotahaler†	Nasal spray‡	PEP mask§	Nebuliser	Anterior nasal¶	Posterior nasal**	Tongue††	Cough swab‡‡	Sputum
Patients negative for <i>P aeruginosa</i> (n=18)										
<i>S aureus</i>						3	3		2	1
<i>H parainfluenzae</i>	1	Nil from 6	Nil from 2	Nil from 2	Nil from 2	2	3	3	1	3
<i>H influenzae</i>										
<i>P aeruginosa</i>										
<i>Pseudomonas cepacia</i>										
<i>Pseudomonas</i> spp										
Patients positive for <i>P aeruginosa</i> (n=13)										
<i>S aureus</i>		Nil from 6	Nil from 2	Nil from 2	Nil from 8	1	1	2	4	5
<i>H parainfluenzae</i>	1									
<i>H influenzae</i>										
<i>P aeruginosa</i>										
<i>Pseudomonas cepacia</i>										
<i>Pseudomonas</i> spp										

Samples taken from: *between bristles with moist swabs; †inside mouthpiece with moist swab; ‡outside and inside nosepiece with moist swabs; §internal green and yellow surfaces of valve with moist swab, internal wall of resistor arm with moist swab; ||lower chamber with moist swab; ¶dry swab before physiotherapy; **3 cm posterior and lateral with dry swab; ††back of tongue with dry swab before physiotherapy; ‡‡deep throat with dry swab after physiotherapy; |||patient after physiotherapy.

in the former group; only cough swabs were possible in 10.

There was a high correlation between cultures from cough swabs and isolates from the dorsum of tongue in both groups and also with sputum cultures in the group positive for *P aeruginosa*. *S aureus* was isolated from anterior and posterior nasal swabs from three of the negative group but not from the sputum. One was a regular user of a nasal spray from which *S aureus* was also cultured. A further three patients had positive cultures from either sputum or cough swabs but not nasal carriage. One child was sputum positive for *H influenzae* with nasal carriage, two others had positive sputum cultures but no nasal isolates. *H influenzae* was isolated from the posterior nasal space of two further children but they gave negative sputum cultures. *Haemophilus parainfluenzae* was grown from sputum or cough swabs from five patients, only one of whom had nasal carriage. A single patient carried the organism in the posterior nares and dorsum of tongue but had clear sputum cultures. *P aeruginosa* was not isolated from the upper airway of any child in the group with negative sputum, including the one patient who became sputum positive during the study.

In the group positive for *P aeruginosa* there was good correlation between the results of cough swabs and sputum isolates. Except for two children who could not produce a sputum

sample, *P aeruginosa* was isolated from sputum cultures of all who had positive cough swabs. *P aeruginosa* was also found in nasal swabs from one patient.

Discussion

Pulmonary infection with *P aeruginosa* is a major cause of morbidity and subsequent mortality in patients with cystic fibrosis.⁸ Once the organism is established in the lungs attempts to eradicate it are rarely successful, although transient bacterial clearance and improvement in respiratory function can be achieved with either inhaled⁹ or intravenous antibiotics.¹⁰ However, most patients will revert to being sputum positive within three months of treatment.⁹

If colonisation of the upper airways precedes pulmonary invasion, it may be argued that appropriate antibiotic treatment at this stage may prevent or delay pulmonary disease. Parallels with *P aeruginosa* colonisation of endotracheal tubes in intensive care settings have been drawn, with the success of selective bacterial decontamination in these patients being proposed as a precedent for similar treatment in cystic fibrosis.¹¹ Support for this theory is drawn from studies by Lindemann *et al*¹² and Komiyama *et al*³ who were able to obtain positive cultures from the oral cavity in cystic

fibrosis sufferers. Komiyama *et al* also obtained growths from dental plaque but *P aeruginosa* was not isolated from this site by Lindemann *et al*. As the mean age in the former study was 19 years and in the latter was 13.5 years, it is likely that many if not all of the cystic fibrosis subjects studied had chronic *P aeruginosa* lung colonisation. The importance of positive cultures from the oral cavity in such patients is therefore uncertain. Neither study purports to have examined children before they became sputum positive. Iacocca *et al* have already shown that isolation of an organism from deep throat or nasopharyngeal swabs often reflects the presence of the organism below the larynx¹³; our findings show that the organism occurs only in the upper airways of patients with established lung colonisation. We were unable to demonstrate *P aeruginosa* in the upper respiratory tract of any child in the absence of pulmonary involvement, even in the single child who became sputum positive during the study. The results of a single trial of selective decontamination of the nasopharynx have also proved unsuccessful (AM Dalzell, DP Heaf, HKF van Saene. Abstract presented at Cystic Fibrosis Research Workers Conference, Manchester 1988).¹⁴

The possibility that bacterial contamination of inhalation equipment may occur and act as an infectious reservoir was also explored. Aerosol solutions are frequently used in cystic fibrosis to administer bronchodilators and mucolytics before physiotherapy. Nebulised broad spectrum antibiotics are widely employed to reduce both the frequency of relapse in established *P aeruginosa* infection and to prevent the progression from colonisation to infection.¹⁴ Microbial contamination of nebuliser treatment equipment has, however, been observed with both home and hospital usage.^{5 6 15 16} Studies suggest that contamination is most likely with multiple dose solutions rather than unit dose medications. However, both bronchodilator and antibiotic solutions can be affected; the presence of bacteriostatic agents in the drug solutions appears protective. Studies of equipment from patients with asthma or chronic obstructive airways disease have usually yielded environmental organisms, however *Pseudomonas* spp were found in 8–54%, the latter higher figure occurring in a burns unit.¹⁷ The lack of positive cultures from inhalation equipment in this study reflects the use of single dose medications. Multiple dose aerosols were given only as nasal sprays. Three of the study population used these devices, one of which showed contamination with *S aureus*; this child also had nasal carriage. Although little can be drawn from such a small sample, the limited ability to clean adequately such multidose systems should be considered. We could find no evidence to

implicate aqueous sprays from toothbrushes as a source of infection.

Overall we have shown a poor correlation between upper airways carriage of bacteria and sputum positivity. There was a good correlation between sputum positivity and isolation of pathogens from cough swabs, however, confirming the appropriateness of this technique in young children unable to produce sputum voluntarily. We have found no evidence to suggest that colonisation of the upper airways, particularly with *P aeruginosa*, precedes lower respiratory tract invasion. Therefore, prophylaxis to prevent this colonisation would seem inappropriate until a longitudinal study establishes a link between the two events. With <5% of patients with cystic fibrosis becoming positive for *P aeruginosa* each year, a multi-centre study would appear necessary. As the floor of the nose shows the same abnormality of ion transport as the lungs in cystic fibrosis,¹⁸ the lack of colonisation suggests that local epithelial factors do not predispose to chronic infection.

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