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Pseudomonas aeruginosa in cystic fibrosis: cross-infection and the need for segregation

D. Tubbs, W. Lenney, P. Alcock, C.A. Campbell, J. Gray and C. Pantin

North Staffordshire Royal Infirmary, Stoke-on-Trent, Staffordshire, U.K.

Evidence-based reasons for segregation of patients colonized with *Pseudomonas aerugionsa* in the outpatient setting are unclear. To clarify local decisions, *Pseudomonas* genotyping of the local environment, patients and patient contacts was undertaken in 1993. The hospital environment was re-swabbed in 1997. *Pseudomonas* genotyping of old and new patients attending the North Staffordshire cystic fibrosis clinic has subsequently been undertaken and more recently been repeated on an annual basis to assess whether the same *Pseudomonas* genotypes can be found in both the environment and in patients, and whether the same *Pseudomonas* genotype can be transferred from one patient to another.

No *Pseudomonas* genotype found in the local environment in 1993 or in 1997 has been found in any of our patients. Nine children attending the same special school for many years and sharing the same physiotherapy facilities showed no evidence of cross-infectivity. Except for siblings living in the same household our cross-infectivity rate is very low and where cross-infection has potentially occurred the level of contact between these patients has been minimal. This study does not support the suggestion that patients with cystic fibrosis attending the North Staffordshire clinic and colonized with *Pseudomonas aeruginosa* should be segregated from non-colonized patients.

Key words: cystic fibrosis; *Pseudomonas aeruginosa*; cross-infection; genotype; segregation.

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Introduction

Pseudomonas aeruginosa (PA) is the most important pathogen in patients with cystic fibrosis (CF). It is a Gram-negative motile rod which is undemanding in its requirements and can live almost anywhere. In a hospital setting it is most commonly found in a damp or moist environment and is rarely found in healthy individuals. Colonization of patients with PA is unusual except in CF where the abnormal mucus of CF patients makes an ideal environment. Once in the lungs, it adheres to the respiratory epithelium and its exoproducts affect the immune response. When established in the respiratory tract PA adapts and changes to a mucoid form, becoming more difficult to eradicate and resulting in colonization which is usually defined as three positive sputum samples within a period of 6 months. This is the definition we have used in North Staffordshire in relation to our patients. Jacques *et al.* (1) showed that in 6–13-year-old CF patients colonized with PA, lung function was significantly worse than in those not colonized. Ballmann *et al.* (2) differentiated between patients colonized with non-mucoid and mucoid forms, and found the mean annual deterioration in FEV_1 worsened with a change to the mucoid form.

The medical literature is unclear about the relationship between PA isolated in the environment and subsequent transmission to patients with CF (3–6), cross-infectivity between CF patients (7–10) and the possible benefits of patient segregation (10,11). Against this background there is a difference of opinion as to whether patients colonized with PA should be segregated from those not colonized by organizing separate outpatient clinics in an attempt to reduce cross-infection and subsequent colonization in the vulnerable patients.

Phenotypic and genotypic methods for PA identification have been described. Genotypic methods measure the precise genetic make-up of the organism and are more accurate (12). We have studied PA in our patients using a genotypic method to assess whether cross-infectivity has occurred to a significant degree over time. We felt that before recommending segregation, which would involve considerable disruption to our outpatient service, we needed clear scientific evidence that such disruption would be beneficial to our patients and would warrant the

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Correspondence should be addressed to: W. Lenney, Academic Department of Child Health, City General Hospital Site, North Staffordshire Royal Infirmary, ST4 6QG, U.K.

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organizational upheaval which would necessarily take place. Initial testing began in 1993. We report our findings over a 6-year period between 1993 and 1998.

Methods

PATIENTS AND METHODS

A cross-sectional study was initiated in 1993 to identify which of our CF patients were growing PA and whether there were any similarities in genotype. There were 46 patients in this original study accounting for 100% of the patients attending the North Staffordshire Cystic Fibrosis Clinic at that time. Their ages ranged from 1 to 54 years. Between 1993 and 1998 26 new patients joined the North Staffordshire CF Clinic from within and outside the county borders.

In 1993 swabs were taken in the environment and from patients in an attempt to identify any possible transmission of PA from the environment to patients. Swabs were also taken from close family members and the degree of contact was noted between a patient, family members and other CF patients attending the outpatient clinic. Members of the CF team also had nose and throat swabs cultured for PA. It was known in 1993 that nine patients had attended the same special school for children with physical disabilities, two being in the same class and two travelling to school together. The degree of contact between patients was arbitrarily assessed using the following simple scale:

(i) No contact;
(ii) Attended the same CF clinic;
(iii) Occasional social visits to each others houses;
(iv) Inpatient care in the same ward at the same time

  (a) once or twice,
  (b) frequently;

(v) Close relationships outside hospital;
(vi) CF patients within the same family.

Our cystic fibrosis clinic is of such a size that we feel we know our patients well and through the members of the cystic fibrosis team we can accurately place our patients into one of the above six arbitrary categories. The environmental sites swabbed in 1993 were from the paediatric and adult clinics, the paediatric and adult wards and the local special school. The main sites swabbed were sinks, baths, toilets, lung function equipment, treatment room surfaces etc. (There is a separate adult and paediatric CF clinic sited in the same outpatient building. The adult and paediatric wards are housed in different buildings some 200 m apart.) The patients had nose and throat swabs taken and wherever possible sputum samples were taken for culture. Whenever new patients were diagnosed locally or new patients were transferred from other districts to the North Staffordshire CF clinic for their care (between 1993 and 1998) it was noted whether they were already colonized with PA and genotyping of the organism was undertaken at the earliest opportunity.

Some of the patients in the original 1993 cross-sectional study have had genotype re-testing to see if they have acquired any new strains. It has now become our policy to annually re-type each patient's PA strain to identify the rate of new strain acquisition. The hospital environment was re-swabbed in 1997.

LABORATORY METHODS

Bacteriology

(a) Culture. All samples were cultured for PA using standard techniques. Sputum was diluted 1 : 1 with 'sputasol' (Oxoid SR89), homogenized and cultured directly on to 5% horse blood, MacConkey agar (Oxoid CM7) and *Burkholderia cepacia* agar (Mast DM253) plates. Plates were examined after being left overnight and after 48 h incubation at 35–37°C for the presence of PA. Nasal swabs, throat swabs and environmental swabs were cultured on Columbia blood agar base (Oxoid CM331), 5% horse blood and MacConkey agar and examined in a similar fashion. Colonies were identified by morphology, oxidase reaction and pigmentation. A limited number of samples was also identified by the response pattern to the AP1 20NE (Biomerieux, France) system. All PA isolates were stored at −20°C on porous ceramic beads in a cryovial containing glycerol.

(b) Genotyping. We used restriction endonuclease analysis to obtain the PA genotype employing rare cutting enzymes to produce large fragments which were then separated by pulse field electrophoresis. Discrimination was defined by the number of band differences between different isolates. All strains were prepared on the same gel with an 80% or greater match to indicate the same PA genotype. The pulse-field gel electrophoresis method used was essentially that of Grothues (13), modified by Kaufmann and Pitt (14). The bacteria were grown overnight on nutrient agar plates and then embedded into agarose blocks. They were then incubated with proteinase K-EDTA and subsequently digested with infrequently cleaving enzyme X bal. The fragments were then separated and analysed by contour clamped homogenous electric field gel electrophoresis CHEF Mapper (Biorad).

Results

GENOTYPE RESULTS

Table 1 shows three pairs of patients (1993 cross-sectional study) with the same three PA genotypes together with the degree of patient contact. Table 2 shows the subsequent PA genotypings between 1993 and 1998 found in more than one patient together with the degree of patient contact. Patients 47, 48 and 49, 38 and 39, are families of siblings living in the same household. Patients 42 and 22 are patients with prolonged and close contact.

Nine children attended the same special school, two were in the same class and two had travelled to school together using the same transport system for 2 years. All children shared the same school physiotherapy facilities on a daily basis. Six became colonized with PA whilst at the school, colonization taking place between 1 and 7 years after school entry. None of these patients shared the same PA genotype!

Table 1. The three pairs of *Pseudomonas* genotypes found in paired patients with their degree of contact as found in the 1993 cross-sectional study

Genotype	Patient individual number	Degree of contact
S1	42	iv (b)
S1	22	
S2	44	i
S2	36	
S3	28	ii
S3	48 (Dec. 93)	

Degree of contact: (i) no contact; (ii) attended the same CF clinic; (iii) occasional social visits to each others houses; (iv) inpatient care in the same ward at the same time (a) once or twice or (b) frequently; (v) close relationships outside hospital; (vi) CF patients within the same family.

NEW PATIENTS

Twenty-six new patients began attending the North Staffordshire CF Clinic after the original 46 patients in the 1993 cross-sectional study. Ten were adults, eight having been colonized with PA before arrival having previously attended other CF clinics. Of the 16 paediatric patients, two came from outside clinics with an established diagnosis of CF. One patient arrived allegedly non-colonized but his fourth throat swab 3 months after arrival grew PA genotype S8. The same type had been found in one patient in the 1993 cross-sectional study 9 months earlier. The only contact between the two patients was one outpatient clinic attendance 1 month prior to the new patient's PA isolation. The other new paediatric patient grew PA soon after arrival; the genotype was unrelated to any other genotype in our other patients. Of the remaining 14 newly diagnosed paediatric patients nine have grown PA. One is genotype S2 which has been found in four other patients, the degree of contact between all of them being

Table 2. The subsequent clusters of 10 PA genotypes found in association with patients and their degree of patient contact

Genotype	Individual patient number	Degree of contact
S1	42	iv (b)
S1	22	
S2	44	i with all others having genotype S2
S2	36	i with 56, 64, ii with 15 (1 clinic visit in common)
S2	15	i with 44, 2 with 36, 56, 64
S2	56 (Dec 94)	i with 44, 2 with 36, 15, 64
S2	64 (June 96)	i with 44, 2 with 36, 15, 56, 64
S3	28	ii (only possible contact: - one OP visit)
S3	48 (March 95)	i
S3	60 (Dec 96)	
S4	mother of 46	i
S4	58 (not confirmed on second dendogram)	
S5	47 (May 94)	vi
S5	48 (May 94)	
S6	47 (May 94)	vi
S6	48 (May 94)	
S7	35 (Nov 93)	iv with 35, 48 and 49
S7	48 (Dec 93)	vi with 48 and 49
S7	49 (May 94)	
S8	25 (Nov 93)	ii
S8	53 (Aug 94)	
S9	38 (Jan 95)	vi
S9	39 (Jan 95)	
S10	63	i
S10	35	

very low (i–ii). Six have grown genotypes unrelated to other patients. Genotypes from the other two patients are not available.

PATIENTS AND CONTACT CULTURES

In the 1993 cross-sectional study in which 25 of 46 patients were colonized with PA, 16 patients produced 27 PA isolates from sputum cultures and 17 isolates from mouth/throat swabs. Swabs were taken from 222 patient contacts of whom only one grew PA. This was from the mother of a patient who at that time was not colonized with PA.

CONTACT QUESTIONNAIRE

The results of the questionnaire used in 1993, to assess the degree of contact between patients, indicated the majority had little contact with each other outside hospital and apart from two holidays none had contact with patients from other areas.

ENVIRONMENTAL CULTURES

In the 1993 cross-section study 182 environmental swabs were taken yielding 35 positive PA cultures. The follow-up environmental study in 1997 produced six positive cultures from 46 swabs. None of the arbitrary PA genotypes in either 1993 or 1997 were the same as any of the PA genotypes isolated from any of the CF patients.

Discussion

THE ENVIRONMENT AND PATIENTS WITH CF

In a multi-centre study in 1990 (3) one strain of PA was found in 28% of isolates from patients and in 21% of isolates from the environment (including a river, a swimming pool and a drain >300 km away). A second strain was found in four patients and in standing water. A cross-sectional study examined PA isolates from patients, from sinks and from the hands of hospital personnel (4). Twenty per cent of patients and 89% of sink swabs grew PA. On arrival at work all personnel had negative cultures but 42·5% became positive during duty. Four genotypes were isolated from the environment, none of which were found in the patients. Transmission from sinks to hands was shown, with PA surviving on the hands for at least 60 min.

PA briefly survives in aerosols or on dry surfaces (5) but in dried sputum can survive for 1 week and in sinks for over 6 months. In the CF lung one strain persisted for more than 8 years during which time it changed to the mucoid form.

Doring *et al.* (6) showed coughing of patients contaminated surfaces and sinks, which then contaminated hands. Hand shaking was shown to transfer PA to clean hands for at least 30 min. Eight PA genotypes found in the environment resulted in six being isolated from patients on the wards. Environmental genotypes were also found on health staff workers.

In our 1993 and 1997 environmental studies many PA genotypes were isolated but none of them was also cultured from patients with CF. We therefore have no evidence that the hospital environment (or the environment in the special school attended by nine of our patients) has contributed to the colonization of our patients with PA. Similarly it is also true we have no evidence to suggest that patients have contaminated the environment!

CROSS-INFECTION—THE NEED TO SEGREGATE PATIENTS

Twenty-five patients from Hanover were genotyped (7). Nine of 13 families with more than two affected children had closely related PA strains. In three families, resistant strains in one child spread to another within 6 months. One strain was found in four patients from unconnected families who attended the same CF outpatient clinic. Eight of 10 patients spending 6 weeks in a rehabilitation centre became colonized with different genotypic strains, three of which were mucoid. PA infection rate rose from 37% in 1983 to 60% in 1986.

Cross-colonization has been shown to occur in children during recreational stays, the rate increasing the longer the stay (8). Four months later the same strain was found in 57% of patients, the remainder having different strains.

An epidemic of multi-resistant PA was reported in Liverpool in 1995 (9). Sixty-five of 92 patients grew the

TABLE 3. The pattern of colonization seen in patients with cystic fibrosis aged 0–18 years from 1993 to 1998

	1993	1996	1997	1998
Number of patients	39	45	46	48
Number PA colonized	14	23	27	28
Colonized 0–4 years	0 of 5	1 of 7	2 of 8	3 of 12
Colonized 5–9 years	5 of 15	4 of 10	4 of 8	5 of 8
Colonized 10–14 years	6 of 15	10 of 17	11 of 17	10 of 15
Colonized 15–18 years	3 of 4	8 of 11	10 of 13	10 of 11
% colonized	36	51	59	58

multi-resistant PA but retrospective analysis indicated the same organism was first detected in 1987. Subsequently, four to 12 patients were infected per year. Eighty-five per cent of these were the same strain which was not found in the hospital environment. It seems probable that this multi-resistant PA was transmitted by patient to patient contact. Hoiby and Pedersen (10) used a mathematical model to show that as their clinic size increased so did the likelihood of cross-infection. The probability of acquisition of PA was found to fall after patients were segregated. Mean age of non-colonized patients rose after segregation. Swabbing the environment revealed six out of 1000 genotypes were also found in patients.

Tümmler *et al*. (7) showed that between 1983 and 1986 PA colonization rose from 37% to 60%. Twelve of 14 newly colonized patients had PA genotypes identical to those of previously colonized patients. Segregation and general hygiene measures were taken in 1986 and 2 years later all unrelated patients grew PA of different strains.

Our study shows an increase in the PA colonization rate in our paediatric patients, from 36% in 1993 to 58% in 1998 (Table 3). We have also seen an increasing colonization rate in younger patients and indeed more rapidly following their first PA isolation. This is despite actively and aggressively pursuing a policy of immediate treatment with nebulized colomycin and oral ciprofloxacin for 3-month periods in recent years. Of the newly diagnosed paediatric patients, however, most who have grown PA have revealed strains unrelated to our other patients, only one revealing a strain (S2) previously found. Possible contact with that patient could only have occurred during one previous outpatient visit. In contrast, nine patients who regularly attended the same special school for many years and shared, on a daily basis, the same physiotherapy facilities are colonized with different PA strains. This indicates that colonized patients tend to keep their own strains and do not easily or frequently pass on their own strain to others. Certain 'epidemic' strains may well behave differently.

As with other studies, we have shown that siblings living in the same household often share the same PA genotype. Patients attending the North Staffordshire CF Clinic who share the same PA genotype, and are not siblings living in the same household, have been found to have little contact with each other. There is no evidence that the clinic environment is acting as a vehicle for transfer from patient to patient.

We do not know the total number of PA strains occurring in our CF patients and we are therefore unable to calculate the likelihood of two patients having the same PA strain by chance. However, from the evidence in this study there seems little reason to recommend the urgent segregation of CF patients in the outpatient setting into those colonized with PA and those not colonized. The logistics of this are significant, the practical upheaval, the rearrangement of the clinics and the planning of the timetable for all members of the CF team outweigh such changes. Compared with the largest CF centres in the U.K. our clinic size is relatively small but comparable in size to many others in the country. The size of the clinic may well be important in determining the possible need for segregation. Hygiene in the clinic is also important. We attempt to keep our weekly clinic limited to eight patients maximum per clinic, to spread out the patients arrival times so only a small number are present at any one time and to have separate cubicles for the doctors, nurse, physiotherapist, dietician and psychologist.

It is interesting to note that although we try to segregate CF inpatients by placing them in individual cubicles when they are admitted for inpatient treatment, they are frequently found together in the same cubicle watching TV or playing on the same PlayStation™.

We accept that all clinics do not have the facility for genotyping PA strains and this needs to be considered when recommending whether to segregate patients. We also admit that in North Staffordshire we have never isolated an epidemic strain of PA or indeed an epidemic strain of *Burkholderia cepacia*. Had this occurred the conclusions may well be different. Our present segregation policy (for both inpatient and outpatient care) includes the isolation of patients with *Burkholderia cepacia* (epidemic strains or not) or multi-resistant *Staphylococcus aureus* (MRSA). Given our low PA cross-infectivity results and considering the above practicalities, this study does not support segregating patients colonized with PA in our outpatient setting.

Conclusions

(1) We have shown no relationship between PA strains isolated from the environment and PA strains isolated from our CF patients.
(2) We have not shown any convincing evidence to recommend the immediate isolation of patients colonized with PA from those non-colonized in an attempt to reduce the risk of cross-infection in the outpatient setting.

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