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Epidemiology of *Pseudomonas aeruginosa* in a cystic fibrosis rehabilitation centre

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ABSTRACT: ***Pseudomonas aeruginosa*** **is the leading pathogen in cystic fibrosis (CF) lungs. Since there is great concern about clonal spread in CF centres, this study examined the *P. aeruginosa* genotypes of colonised residents of a CF rehabilitation centre.**

The isolates from the sputum of 76 *P. aeruginosa*-colonised patients were genotyped by fluorescent amplified fragment length polymorphism on arrival and departure.

A total of 71 different *P. aeruginosa* genotypes were identified from 749 isolates. Forty-nine patients had one genotype, 20 had two genotypes and seven had three. Forty-four patients had one or more genotypes in common with other patients (*i.e.* cluster types). Thirty-two patients were colonised by a single genotype not shared by any other patient. Thirty-eight of the 44 patients with a cluster type already carried their cluster type strain(s) on arrival. Patient-to-patient transmission could not be excluded for eight patients. For five of these, this infection was transient. None of the environmental *P. aeruginosa* isolates had a genotype similar to the patients' genotypes.

In summary, most patients were colonised by only one or two *P. aeruginosa* genotypes and the risk of persistent patient-to-patient transmission was low during the study period (4%). Most patients with a cluster-type strain carried this strain on arrival, indicating that transmission could have happened in the past. No environmental contamination could be established.

KEYWORDS: **Cystic fibrosis, epidemiology, *Pseudomonas aeruginosa***

Pseudomonas aeruginosa has been the leading pathogen in cystic fibrosis (CF) lung pathology over the last three decades [1–3]. After initial infection, colonisation (as defined by the criteria of DÖRING *et al*. [4]) leads to the destruction of lung tissue and reduction of lung function, which may result in early death. The US CF Foundation database reported that, in 1996, the median survival of CF patients who were colonised with *P. aeruginosa* was 28 yrs, while the median survival for noncolonised patients was 39 yrs [5]. Loss of lung function has been clearly demonstrated by KEREM *et al*. [6], who showed that patients who were colonised with *P. aeruginosa* at the age of 7 yrs had a mean forced expiratory volume in one second (FEV_1) that was 10% lower than that of noncolonised patients. Although the emergence of a mucoid colonial morphotype is a more unfavourable prognostic factor than the presence of nonmucoid *P. aeruginosa* [7], the latter forms an important microbial reservoir from which mucoidy, bacterial biofilms and chronic colonisation are established [8].

Prevention of chronic *P. aeruginosa* colonisation by appropriate antibiotic therapy is now common practice once a ''new'' infection by the organism has been identified [9, 10]. Currently, spread of highly transmissible strains in some CF centres has caused great concern, particularly when such strains are multi-resistant and responsible for primary infection [11–16]. Other studies, however, have failed to find evidence of clonal spread [17–20].

In Belgium, many patients are referred to the CF rehabilitation centre ''Zeepreventorium'' in De Haan, for either a short or prolonged stay, in order to learn specific physiotherapeutic techniques, such as autogenic drainage. This situation has led to justifiable concern among patients and physicians about the risk of cross-infection. Therefore, during 2001 and 2002, *P. aeruginosa* isolates from 76 patients, together with environmental isolates, were genotyped to investigate the risk of patient-to-patient transmission.

METHODS

Patients

All 76 *P. aeruginosa*-colonised patients who attended the rehabilitation centre from January 8 to April 30, 2001, and from September 1, 2001, to October 31, 2002, with a total duration of stay of 8,218 days (median 63 days), were enrolled in

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this study. Patients were aged 5–38 yrs (mean 20.5 yrs) and 33 were male (table 1). Seven patients stayed in the centre during the first period, 42 during the second period and 27 during both periods. Patients were housed in separate rooms, but dining and living facilities were shared. Patients infected with *Burkholderia cepacia* complex are not admitted to the rehabilitation centre. Patients were mostly referred from one of the seven Belgian CF centres, but also some were from German and French centres.

From 1992, infection control measures were instigated to reduce cross-infection from the environment, *i.e.* decontamination of the sinks in each patient room, patient segregation during physiotherapy according to *P. aeruginosa* colonisation and exclusion of patients harbouring *B. cepacia* complex from the centre. Nevertheless, *P. aeruginosa*-colonised patients continued to share the same physiotherapy room, each patient using his/her own nebuliser and physiotherapeutic devices, which were decontaminated separately after each session.

Sampling

Sputum samples were taken from all patients at least on arrival and departure, and were collected at the end of a physiotherapeutic session to ensure that the samples originated from the deeper airways. Environmental samples (10 mL) were taken during the study period from sink drains of the bedrooms and the recreation rooms. Some patients were sampled >20 times during the study period.

Microbiology

Sputum samples were inoculated onto McConkey agar (BBL Becton Dickinson, Cockeysville, MD, USA). After 2 days of incubation at 37℃, different-looking lactose-negative colonies were picked, subcultured on 5% sheep blood agar (BBL Becton Dickinson) and tested for oxidase. Only oxidase-positive colonies were further investigated, using tRNA-PCR [21].

Genotyping

For each patient, all *P. aeruginosa* isolates exhibiting different colonial morphology on McConkey agar were genotyped by arbitrarily primed PCR, using alkaline cell lysis for DNA extraction [22], and randomly amplified polymorphic DNA analysis (RAPD) Ready-to-Go beads (Amersham Biosciences AB, Uppsala, Sweden) and primer ERIC2 (AAGTAAGTGACTGGGGTGAGCG) at an annealing temperature of 35℃, as described previously [23]. This enabled the number of isolates that were subsequently genotyped by the more laborious fluorescent amplified fragment length polymorphism (fAFLP) method to be reduced, since only single representatives of each RAPD type were genotyped using this procedure.

Total bacterial DNA was isolated from fresh cultures on Tryptic Soy Agar using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). fAFLP was carried out as described below. A combined restriction-ligation procedure was used, in which 10 ng of total genomic DNA was incubated with 2 pmol of EcoRI adapter, 20 pmol of MseI adapter, 1 U of EcoRI (Amersham Biosciences), 1 U of MseI (New England Biolabs, Beverly, MA, USA), 50 mM NaCl, 50 ng bovine serum albumin per μL (Roche, Basel, Switzerland) and 4 U of T4 DNA ligase (Amersham Biosciences), in a total volume of 10 μL of 1× reaction buffer for 3 h at 37℃, after which the mixture was diluted 20 times with Tris-buffer (Tris 10 mM, EDTA 0.1 mM, pH 8.0). For the selective amplification of the restriction fragments, 5 μL of the diluted restriction-ligation mixture was used for amplification in a volume of 10 μL under the following conditions: 0.4 μM 6-tetrachlorofluorescein-labelled EcoRI+0 primer, 1.2 μM MseI+C primer (E1; Eurogentec, Seraing, Belgium), 0.2 mM deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 1× reaction buffer and 1 U of GoldStar™ DNA polymerase (Eurogentec). After 2 min of incubation at 72℃ and at 94℃, the cycling conditions were 36 cycles of 30 s at 94℃, 30 s at 65–56℃ and 60 s at 72℃. During the first 13 cycles, the annealing temperature was lowered by 0.7℃ per cycle. After an additional 10-min incubation at 72℃, the samples were cooled. An overview of PCR primers and adapter sequences is shown in table 2.

A combination of 12 μL deionised formamide, and 0.3 μL GS-400 high-density size standard and 0.2 μL GS-500 size standard (which both contain ROX-labelled fragments in the range of 50–500 bp) were added to each 1 μL of PCR product. This mixture was then electrophoresed on an ABI PRISM 310 (Applied Biosystems, Foster City, CA, USA).

A similarity matrix was calculated using the BaseHopper programme [21], and from this a similarity tree was constructed by neighbour joining, using the programme PHYLIP [24]. Fingerprints that were clustered to >90% similarity were visually checked to enable final decisions with regard to similarity. Visual checking of fingerprints that were assessed by the software as having <90% similarity showed that such fingerprints always differed from each other by at least three major peaks. Visual interpretation of fingerprints assessed by the software as having ~90% similarity led, in some cases, to the conclusion that the fingerprint was identical. Therefore, a final decision with regard to clonality of isolates possessing fingerprints with ⩾90% similarity, according to the software, was based on human interpretation.

Statistical analysis

Data are presented as mean±SD or median (interquartile range or SEM). Statistical analysis of the epidemiological data was carried out using the unpaired t-test when groups were normally distributed and the Mann-Whitney rank-sum test when the normality test failed. A 95% confidence interval for the difference in median time in the centre between the group with a possible patient-to-patient transmission and the group without transmission was obtained using a bootstrapping procedure [25].

RESULTS

A total of 749 *P. aeruginosa* isolates from 76 patients for which the colony morphology on McConkey agar was different were genotyped by arbitrarily primed PCR (RAPD). For each patient, at least one representative of each different RAPD type was further genotyped by fAFLP, enabling digital comparison of the genomic fingerprints. Figures 1 and 2 represent some of the fAFLP-fingerprints obtained, with figure 2 representing details of three different fAFLP fingerprints in a superimposed manner.

Only 71 different *P. aeruginosa* genotypes were found among the 749 isolates, indicating that, in individual patients, isolates

TABLE 1 Clinical characteristics of the patients

Patient No.	Age yrs	Sex	Colonised since	Stay duration days (periods)	FVC %	FEV_1 %	Cluster type
1	15	M	1992	14	127	137	Z and Y
2	17	F	1984	18	100	63	Z
3	15	F	1996	144	107	100	Y
4	15	M	1991	48 (2)	95	89	Y
5	19	F	1998	89 (2)	134	112	Y
6	13	F	1991	236 (2)	86	76	Y
7	26	M	1992	98 (2)	41	25	Y
8	21	F	1996	25	77	50	Y
9	5	F	NA	38	NT	NT	Y
10	25	M	<2001	178 (4)	69	32	Y and R
11	7	F	2000	115	45	38	Y
12	10	F	<1999	68 (3)	73	51	X
13	16	F	<2000	84 (3)	69	51	X
14	18	F	<1994	253 (3)	83	65	W
15	8	F	NA	86 (3)	105	83	W
16	21	M	<1999	430 (3)	52	23	U
17	26	F	<1999	213 (4)	48	18	U
18	17	F	<1997	39 (3)	70	37	U
19	26	M	<1997	78 (2)	107	82	U and P
20	31	M	1998	31	NA	NA	U
21	19	M	1992	84	46	31	T
22	8	M	<2001	160 (6)	NT	NT	T
23	9	M	1996	391 (7)	NA	NA	T
24	15	F	1991	25	88	48	T
25	30	M	<1991	41 (2)	53	22	R
26	30	M	<1998	276	48	16	Q
27	18	F	1993	94 (4)	72	48	Q and M
28	16	F	1994	39 (2)	102	100	Q
29	13	M	1996	56 (2)	92	95	P and O
30	27	M	<2000	65 (2)	62	36	P
31	27	M	<2000	122 (3)	35	14	P
32	21	F	<2000	41 (2)	55	41	P and M
33	14	F	NA	102 (2)	116	99	P and O
34	22	F	1985	35	34	22	O
35	26	F	1983	79 (2)	74	47	O and M
36	23	F	<1999	11	103	47	O
37	18	M	<1999	104 (4)	96	85	O
38	18	F	<2000	546 (3)	83	60	O
39	16	M	<1999	211 (2)	71	54	J
40	13	F	<1999	285 (5)	62	46	J
41	33	M	<1990	44	44	22	M
42	28	F	1977	38	63	29	M
43	20	F	<2001	45 (2)	73	76	K
44	15	F	<2001	42 (2)	100	84	K
45	22	F	1991	49	49	25	
46	18	F	<2000	89 (4)	76	49	
47	34	F	<1999	59 (2)	65	34	
48	20	F	<2000	37	40	25	
49	16	F	<1993	321 (3)	52	36	
50	22	M	<1996	37 (2)	62	35	
51	37	F	<1989	27	89	56	
52	22	F	1995	44	104	85	
53	32	M	1993	456	47	28	
54	20	M	<1999	158 (2)	51	35	
55	33	M	1984	28	83	57	
56	12	F	1989	35	67	48	
57	27	F	1993	62 (2)	67	48	
58	15	M	1993	25	82	70	
59	29	F	NA	59	56	22	
60	21	M	1998	36	32	20	
61	26	M	1993	84 (2)	30	21	
62	22	M	NA	31	43	21	
63	28	M	<1990	81 (2)	47	23	
64	26	M	<2000	111 (2)	46	21	
65	23	F	1990	21	96	75	
66	22	M	1992	56 (2)	93	48	
67	10	M	<2000	85 (4)	126	115	
68	12	F	NA	28	65	48	
69	34	F	1985	49 (2)	85	62	
70	26	M	<1988	35	27	17	
71	26	F	1984	59 (2)	42	24	
72	14	M	<2000	79 (2)	100	27	
73	5	M	NA	204 (2)	64	65	
74	18	F	NA	23	103	96	
75	23	F	1991	64 (2)	53	25	
76	19	F	1982	17	46	49	

FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; M: male; F: female; NA: data not available; NT: not tested because of young age or mental retardation.

with different colonial morphology mostly belonged to the same genotype. Fifty-seven of these genotypes were only found in a single patient (distinct genotypes), while 14 were found in more than one patient (cluster genotypes). More than half of the patients (49) carried only one genotype, 20 carried two genotypes and seven carried three genotypes.

Thirty-two patients (42%) were colonised by one or more strains with distinct genotypes, 32 patients (42%) had one or more genotypes belonging to at least one cluster, and 12 patients (16%) carried both distinct and cluster strains.

Of the 44 patients with cluster strains, 36 carried strains that belonged to only one cluster and eight had strains belonging to two clusters. There was a statistical difference between the age

TABLE 2 Adapter and primer sequences used for fluorescent amplified fragment length polymorphism-based genotyping

Adapters and primers	Sequence
EcoRI adapter1	5′ CTCGTAGACTGCGTACC
EcoRI adapter2	5′ AATTGGTACGCAGTCTAC
MseI adapter1	5′ GACGATGAGTCCTGAG
MseI adapter1	5′ TACTCAGGACTCATC
EcoRI+0 primer	5′ (tet)GACTGCGTACCAATTC
MseI+C primer	5′ GATGAGTCCTGAGTAAC

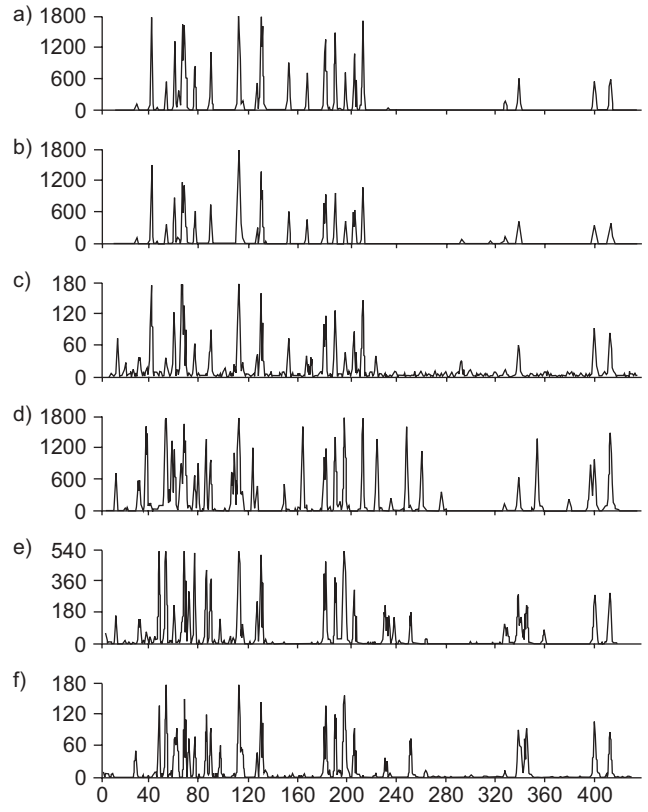

FIGURE 1. Fluorescent amplified fragment length polymorphism fingerprints of *Pseudomonas aeruginosa* isolates from different patients, illustrating different genotypes: a–c) three different patients belonging to cluster Y; d) one patient belonging to cluster U; e, f) two siblings belonging to cluster J.

of the patients with cluster strains (19±6.95 yrs (1.05)) *versus* the age of patients with distinct strains (22.3±7.49 yrs (1.32), p=0.04). The patients with a cluster strain had similar lung function test results when compared with those with distinct strains (FEV1: 49.0% (31.5–82.5) *versus* 35.5% (24.5–56.5), p=0.092; FVC: 76.5±25.7 (4.1) *versus* 65.3±24.8% (4.4), p=0.065).

The 32 patients who had distinct genotypes had a median number of genotypes of 1.3, while the patients with strains belonging to one or more clusters carried a median number of 1.6 genotypes per patient (NS). The 32 patients with distinct genotypes had a median stay duration in the institute of 177 days, *i.e.* during the study period and in the past, while the 44 patients with shared genotypes had a median stay in the institute of 197 days (NS). During the study period, the two groups had a similar duration of stay (group of patients with distinct genotype 52.5 days (35–84) *versus* 81.5 days (40–152) in the group with cluster genotypes, p=0.092).

Among the 44 patients with cluster genotypes, there was one group of 10 patients (including one sibling pair) with the same genotype, one group of seven patients, one of six, two of five, one of four, one of three and six groups of two patients (including two sibling pairs). All three pairs of siblings shared at least one genotype with their sibling. Of the 44 cluster patients, 38 had a shared genotype already on arrival.

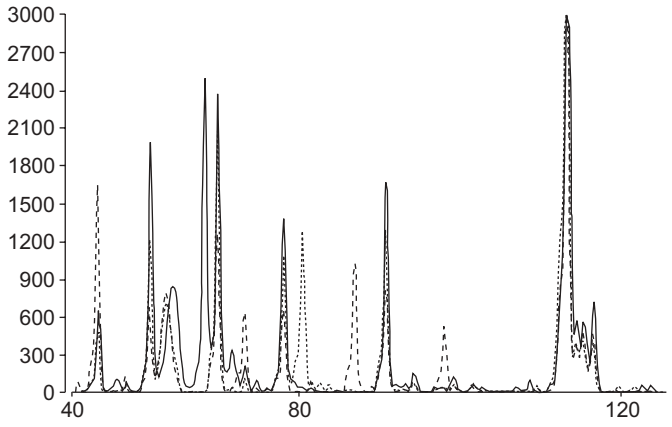

FIGURE 2. Fluorescent amplified fragment length polymorphism fingerprints belonging to three different clusters (—: cluster Y; ·····: cluster U; ----: cluster J) (only fragments between 60 and 120 bp shown).

The median stay during the study of the 68 patients who definitely did not acquire *P. aeruginosa* from another patient during their stay was 60.5 days. Eight patients acquired a new genotype during their stay. The eight patients with new acquisition of a cluster type during the study period (10.5%) had a median stay of 132 days (NS). The null hypothesis of no difference in length of stay between both groups cannot be formally rejected at the 5% significance level. To quantify the size of the difference, a 95% confidence interval was estimated for the difference in median stay between both groups with a bootstrap procedure. This confidence interval was equal to -14,192.5 and is rather wide, but lies largely above 0, indicating a longer stay for the group with possible patient-to-patient transmission.

One patient underwent two episodes of possible patient-to-patient transmission (with two different cluster types), during both study periods. For at least five of these eight patients, the newly acquired genotype, for which a strain with the same genotype was present in another patient during the stay, was transient, since it could no longer be isolated from the patients' sample taken on departure (table 3). Of these eight newly infected patients, three at most were persistently colonised with the newly acquired strain (4%). One patient continued to carry the newly acquired strain 1 yr later, as determined from a nasopharyngeal aspirate taken after lung transplantation. In the other two patients, the newly acquired strain was cultured just before leaving the rehabilitation centre and, unfortunately, no further samples could be obtained thereafter. The patient-to-patient transmission took place twice in the first period and seven times in the second (and longest) study period.

Only four *P. aeruginosa* isolates could be cultured from 13 environmental sampling sites: one from a bedroom sink and three from the recreation rooms. However, these isolates belonged to distinct genotypes and were also different from the patients' genotypes.

DISCUSSION

Debate continues as to whether regular stay in a CF rehabilitation centre is beneficial to CF patients. Benefits

TABLE 3 Numbers of patients with shared genotypes and number of possible patient-to-patient transmissions during the study period

Genotype designation	Patients with this genotype n	Patients with this genotype on arrival n	Patients with shared genotypes with overlapping stay n	Possible patient-to-patient transmission during stay
K	2 (1 S)	2	0	0
M	5	5	0	0
J	2 (1 S)	2	0	0
O	7	5	2	2 T
P	6	5	1	1 P or T
Q	3	2	1	1 P or T
R	2	1	1	1 T
T	4	4	0	0
U	5	3	5	1 P
W	2	0	2	2 T
X	2	2	0	0
Y	10 (1 S)	9	0	1 T
Z	2	2	0	0

S: sibling pair; P: permanent, *i.e.* the same genotype acquired during the stay was still present on departure; T: transient, *i.e.* the same genotype could not be isolated from the patient upon departure.

include opportunity for sport, and intensified and optimised physiotherapeutic techniques. More attention is paid to feeding habits, and the social and psychological benefits of peer contact are obvious. However, some reports have indicated the opportunities for cross-infection of important CF pathogens, in particular *P. aeruginosa* and *B. cepacia* complex species. The aim of this study was to assess the risk of transmission of *P. aeruginosa* in the Belgian rehabilitation centre in De Haan. In Belgium, most CF patients are seen on a regular basis in one of seven hospital reference centres and are sporadically referred to the rehabilitation centre in De Haan, where adult patients live together in a home for several weeks, with separate bedrooms but with shared living and dining facilities. Younger patients live together as in a boarding school. From 1992, segregation has been practiced. Patients are cohorted in a *P. aeruginosa*-negative or a *P. aeruginosa*-positive group during physiotherapeutic sessions and, since 1995, the sinks and water closets are decontaminated daily by alternatively rinsing with vinegar and liquid bleach.

The risk and frequency of *P. aeruginosa* cross-infection among CF patients remains controversial. Genotyping techniques, such as macro-restriction analysis in combination with pulsed-field gel electrophoresis, RAPD and fAFLP have enabled reasonably accurate determination of the clonal relationship of *P. aeruginosa* isolates from patients within a CF centre or region [26–29].

Nevertheless, different conclusions on the transmissibility of this pathogen have been drawn from two recent molecular epidemiological studies from two large centres without segregation policies in Australia [16] and Canada [20]. The Australian cross-sectional study found a widespread clone of *P. aeruginosa* in 55% of 118 infected patients in a paediatric CF clinic [16]. In contrast, the Canadian longitudinal study, run over two decades, showed a low risk of patient-to-patient spread among 174 patients, except for patients with prolonged and close contacts, such as siblings [20]. Previous studies have supported the position of both groups, and indicate the difficulty of making general statements about this highly diverse and adaptable pathogen. Cross-sectional and longitudinal studies in Liverpool [12, 14], Manchester [13, 30, 31] and Sheffield [32] have provided compelling evidence for transmission of highly transmissible strains. In a Norwegian CF centre, 45% of the patients colonised with *P. aeruginosa* carried the same strain [15]; these patients had previous contacts at summer camps and training courses. This once more raised the issue of the risk of cross-infection associated with CF holiday camps. OYENIYI *et al*. [33] previously demonstrated that the five *P. aeruginosa*-negative patients who attended a winter camp in Spain together with 17 patients who were already colonised with *P. aeruginosa* all acquired *P. aeruginosa* strains identical to those carried by the colonised patients. The findings of HOOGKAMP–KORSTANJE *et al*. [34] were completely different. Ninety-one CF patients who attended a CF camp had respiratory cultures performed on arrival, after 2 weeks, after 2 months and regularly thereafter. The incidence of cross-infection was 7% in previously *P. aeruginosa*-negative individuals. The incidence of new and persistent *P. aeruginosa* colonisations was ~2%. The authors concluded that the overall risk of acquisition was comparable to that occurring in the community, and that it was trivial compared with the obvious joy and social benefit derived from a holiday camp. A Brazilian study [35] in an outpatient CF clinic also concluded that the risk of cross-infection is low.

In this study, the genotypic diversity of *P. aeruginosa* isolates was identified initially by RAPD analysis and then with fAFLP in the case of representative isolates of different RAPD types cultured from individual patients. By including multiple isolates from individual patients, the findings of a previous report [26], which concluded that the discriminatory power of RAPD and fAFLP were similar, was confirmed. In all cases studied here, isolates with identical RAPD fingerprints also

had identical fAFLP fingerprints, ensuring that no unrelated isolates were grouped into the same RAPD genotype. For each patient, all of the RAPD products were obtained during the same thermal cycling and electrophoresis run, to avoid differences due to the limited reproducibility of the technique. fAFLP is generally known to be more reproducible and, due to automated digitisation of the fingerprints, it makes large-scale comparison of hundreds of fingerprints possible, an endeavour that is impossible with RAPD analysis. This combined use of a rapid and cheap initial screening technique (RAPD) and a more sophisticated, reproducible and digitised, but also more expensive and laborious technique (fAFLP), enabled the authors to genotype a large number of isolates in an affordable, reasonably convenient, and a highly reliable and discriminatory manner. Moreover, the established library of fAFLP fingerprints of CF *P. aeruginosa* strains can be used for further comparisons and long-term studies.

To the authors' knowledge, this is the first study to examine the risk of cross-infection in a CF rehabilitation centre, where patients live together closely and for long periods of time. Among the 749 *P. aeruginosa* isolates examined, which deliberately included different colony morphology types from an individual patient, only 71 different genotypes were found. In most chronically colonised patients, different colony morphology types were observed on the primary isolation plate, but in most cases these belonged to the same genotype. HOOGKAMP-KORSTANJE *et al*. [34] also observed that isolates dissimilar in macroscopic appearance and of different serotype, pyocin type and phage type, could be of the same, unique genotype. This conclusion was supported by DA SILVA FILHO *et al*. [35].

Forty-nine of the 76 patients (64%) carried only a single genotype, 20 carried two genotypes (26%) and seven carried three types (10%). This confirms the data by MAHENTHIRALINGAM *et al*. [27], who reported that 15 out of 20 patients were colonised by a single strain and that five out of 20 were colonised with two or more strains. This was also in agreement with the findings of HOOGKAMP-KORSTANJE *et al*. [34].

All three sibling pairs in the present study harboured at least one strain in common. These findings are also supported by the data of SPEERT *et al*. [17], who found that 10 out of 12 sibling pairs carried the same strain. GROTHUES *et al*. [36] stated that cross-infection between siblings is common and showed that in three out of the five cases where only one sibling harboured *P. aeruginosa,* the siblings lived in separate homes.

Of the 44 patients that carried a strain that was also present in other patients, 38 already carried this strain on arrival at the CF centre. Therefore, the strain could have been acquired from a common source or from another patient during one of the previous stays in the CF centre, before more stringent infection control measures were introduced. For example, when considering the Y cluster, nine out of ten patients were already colonised with the same strain at the beginning of the study period. Although the 10th patient could have newly acquired his Y-cluster strain (since it could not be cultured from the sample taken at arrival), this strain was isolated sporadically and intermittently from five of the 36 isolates, taken from 16 samples of this patient over a period of 8 months. It is possible that this patient carried the Y-cluster strain at arrival, but only in low numbers. Eight out of the 10 patients with genotype Y isolates attended the centre before the study period and these previous stays overlapped for at least 53 and, at most, 242 days, with a total of 1,583 days of overlap. Therefore, cross-infection could have occurred in this centre during previous stays. However, two children carrying this cluster strain had never stayed in the rehabilitation centre before. It is possible that they acquired this strain at their own CF centres through contacts with other Y-cluster patients.

During the study period, nine episodes in eight patients were noted in which the patient was newly infected by a genotype already carried by another patient during overlapping stay. In such cases it is difficult to avoid the conclusion that patient-to-patient transmission occurred.

The role of the environment as a source of *P. aeruginosa* acquisition in CF-patients is difficult to prove and remains a matter of debate. Most previous studies [16, 37–39] have not been able to identify *P. aeruginosa* in hospital wards or have found only a small number of isolates, which were different from the CF strains. However, DÖRING *et al*. [40] linked several strains from hospital sinks to those carried by patients in sputum, on their hands, throat, nose and anus, and on the hands of staff members. Due to the stringent antiseptic measures, the presence of *P. aeruginosa* in environmental samples at the centre in this study centre may be low. In addition, genotyping showed that those isolates were distinct from those found in patients.

For most patients carrying isolates with the same genotype, it is difficult to assess whether this is due to direct patient-to-patient transmission, to a persistent source of infection in the environment or to continuous recontamination of the environment by colonised patients, which increases the risk of infection from an environmental source. In the De Haan centre, the environment seemed an unlikely source, because the few *P. aeruginosa* environmental isolates that were cultured were different from patient isolates. Furthermore, the large genotypic diversity that one can expect among environmental *P. aeruginosa* isolates would not predict the occurrence of several patients with identical isolates when these isolates had been acquired independently from the environment. Therefore, it seems likely that an important reservoir responsible for *P. aeruginosa* acquisition is the infected CF patient, a hypothesis strengthened by the high number of identical strains found in sibling studies, including this report.

In summary, these findings confirm that different colonial morphotypes of *Pseudomonas aeruginosa* from the same cystic fibrosis patient usually belonged to the same genotype. Since 38 out of the 44 patients with shared genotypes already carried their genotype on arrival, patient-to-patient transmission could have happened in the past, during previous stays. The risk of patient-to-patient-transmission during the study period (with a total stay of 8,218 days) was relatively low (10%), and the risk of persisting colonisation with a newly acquired strain during the study period was also low (4%). The influence of strain-specific differences in *Pseudomonas aeruginosa* transmissibility, infection control practices or acquisition from environmental

reservoirs (natural or contaminated) on the data collected in this study remains unclear. However, it seems reasonable to conclude that each of these factors should be taken into account in debating the controversy that surrounds the prevalence, management and risks of *Pseudomonas aeruginosa* cross-infection.

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