Pseudomonas aeruginosa in the home environment of newly infected cystic fibrosis patients

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ABSTRACT: The source of acquisition of *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients remains unknown. Patient-to-patient transmission has been well documented but the role of the environment as a source of initial infection is as yet unclear.

In the present study, the origin of the first *P. aeruginosa* isolate in CF patients was investigated by comparing the *P. aeruginosa* genotype(s) from newly infected patients with genotypes of *P. aeruginosa* isolates from the home environment and from other patients from the same CF centre.

A total of 50 newly infected patients were studied. *P. aeruginosa* could be cultured from 5.9% of the environmental samples, corresponding to 18 patients. For nine of these, the genotype of the environmental *P. aeruginosa* isolate was identical to the patient's isolate. In total, 72% of the environmental *P. aeruginosa* isolates were encountered in the bathroom. Patient-to-patient transmission within the CF centre could not be ruled out for three patients.

In summary, a low prevalence of *Pseudomonas aeruginosa* was found in the home environment of the newly infected cystic fibrosis patients. The bathroom should be targeted in any preventive cleaning procedures. An environmental source of the new infection could not be ruled out in nine patients.

KEYWORDS: Cystic fibrosis, environment, genotyping, *Pseudomonas aeruginosa*

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The major pulmonary pathogen in patients with cystic fibrosis (CF) is *Pseudomonas aeruginosa*. Dehydration of mucus during exacerbations and a defective host defence make CF airways prone to chronic infection with *Pseudomonas aeruginosa* [1, 2]. By adulthood, >80% of patients are infected with this pathogen, which adversely affects lung function and survival [3, 4].

To date, the source of initial infection of CF patients with *P. aeruginosa* remains unknown. Possible sources include the environment, person-to-person spread (especially from another person with CF), or through contact with contaminated objects. Patient-to-patient transmission has been well documented. It generally results from prolonged social contact, such as that between siblings [5], close friends or people staying at holiday camps [6, 7] and CF rehabilitation centres [7, 8].

Epidemiological studies have not confirmed the acquisition of *P. aeruginosa* from the environment. As *P. aeruginosa* is widely present in soil, plants and water [9, 10], the environment could be the initial source of infection. The lack of knowledge about the exact role of the environment in the acquisition of *P. aeruginosa* by the CF patient is of concern to parents and physicians, and sometimes leads to questionable preventive measures, such as not drinking tap water unless it has been boiled or not visiting swimming pools [11, 12].

In an attempt to elucidate the source of new strain acquisitions, the current authors carried out a Belgian multicentre study. For all patients who were not initially infected with *P. aeruginosa*, but for whom a recent new infection with *P. aeruginosa* could be documented, cultures of wet surfaces were taken at their homes as soon as possible after detection of the infection. DNA fingerprints of the *P. aeruginosa* isolates from the newly infected patients were compared with fingerprints of *P. aeruginosa* strains recovered from the home environment. Using the Belgian inventory of the DNA fingerprints of most patient *P. aeruginosa* isolates [7], the DNA fingerprints of the *P. aeruginosa* isolates from the newly infected patients were compared with the fingerprints of *P. aeruginosa* isolates from chronically infected CF patients attending the same CF centre as the newly infected patients.

TABLE 1 Patient and sampling characteristics

Patient	Centre	Sex	CFTR genotype	Age yrs m	Time interval sampling#	Environmental samples n	Sampling remarks¶	Genotypes of patient and environment Identical	Different
1	1	M	p.[delPhe508]+[delPhe508]	19 01	<1 w	3	No bathroom		
2	1	M	p.[delPhe508]+[Glu656X]	07 04	1 m	5			
3	1	M	p.[delPhe508]+c.[1703delT]	25 05	<1 w	4	No kitchen		
4	1	F	p.[delPhe508]+[Arg1070Trp]	01 00	<1 w	3	No kitchen		
5	1	M	p.[delPhe508]+[delPhe508]	15 03	6 m#	5		Kitchen washbasin drain	
6	1	F	p.[delPhe508]+[delPhe508]	20 07	<1 w	5	No kitchen		
7	1	F	p.[delPhe508]+[delPhe508]	23 08	6 m	4	No kitchen		
8	2	F	p.[delPhe508]+c.[1585G>A]	04 00	<1 w	11			
9	2	M	c.[3528delC]+[3140-26A>G]	02 07	<1 w	11			
10	2	M	p.[delPhe508]+c.[1585G>A]	00 09	2 m	10			
11	2	F	p.[delPhe508]+[delPhe508]	01 00	<1 w	10		Bathroom bath drain = bathroom bath tap	
12	2	F	p.[delPhe508]+[delPhe508]	28 00	<1 w	9			Bathroom bath drain <> bathroom washbasin drain
13	2	M	p.[delPhe508]+[delPhe508]	23 09	2 w	10	No toilet		
14	2	M	p.[delPhe508]+[delPhe508]	02 04	<1 w	9	No aerosol		
15	2	F	p.[delPhe508]+[delPhe508]	04 05	3 w	10			
16	2	M	p.[delPhe508]+[Ser1251Asn]	17 03	1 m	10			
17	2	M	p.[delPhe508]+[delPhe508]	03 05	<1 w	10		Bathroom shower drain	
18	2	F	p.[delPhe508]+[delPhe508]	04 07	<1 w	8+			Bathroom shower drain. See patient 21
19	2	M	p.[delPhe508]+[Ala455Glu]	04 04	<1 w	10			
20	2	F	p.[delPhe508]+[delPhe508]	03 07	2 w	7		Bathroom washbasin drain = kitchen sink drain	
21	2	M	p.[delPhe508]+[delPhe508]	02 10	<1 w	8+			Bathroom shower drain. See patient 18
22	2	F	p.[delPhe508]+[Arg553X]	02 11	<1 w	10			
23	2	F	p.[delPhe508]+[delPhe508]	00 11	<1 w	10	No bathroom		
24	2	M	p.[delPhe508]+[Iledel507]	06 01	2 w	8			
25	3	F	p.[delPhe508]+[delPhe508]	05 00	<1 w	6			
26	3	M	p.[delPhe508]+[delPhe508]	15 02	4 m#	6		Kitchen sink drain	
27	3	F	p.[delPhe508]+[delPhe508]	08 02	<1 w	9			
28	3	M	p.[delPhe508]+[Ser1251Asn]	07 11	7 w	5			
29	3	F	p.[delPhe508]+[Arg553X]	10 04	3 m	10			Bathroom bath drain

TABLE 1 continued

Patient	Centre	Sex	CFTR genotype	Age yrs m	Time interval sampling[#]	Environmental samples n	Sampling remarks[¶]	Genotypes of patient and environment	
								Identical	Different
30	3	F	p.[delPhe508]+[delPhe508]	06 01	<1 w and 5 m	16+9			Bathroom shower drain <> 2 × bathroom bath drain$
31	3	M	p.[delPhe508]+[delPhe508]	31 04	<1 w	5			
32	3	M	p.[delPhe508]+[Ser1251Asn]	01 04	6 w	7			
33	3	F	p.[delPhe508]+[delPhe508]	11 00	<1 w	10			
34	3	F	p. [delPhe508]+[Cys276X]	09 05	3 w	11	No nebuliser		
35	3	M	p.[delPhe508]+[Gly542X]	03 07	<1 w	7			
36	3	F	p.[delPhe508]+[delPhe508]	14 01	<1 w and 5 w	5			
37	3	M	p.delPhe508]+[delPhe508] [Ser1251Asn]	16 00	2 w	10			
38	3	F	p.[delPhe508]+[delPhe508]	11 11	<1 w	12		Toilet first floor = toilet second floor	
39	3	M	p.[delPhe508]+[delPhe508]	07 06	<1 w	6		Nebuliser	
40	3	M	p.[delPhe508]+[delPhe508]	08 05	7 m[#] and 7 w	10+4		Bathroom bath drain = bathroom washbasin	
41	3	M	p.[delPhe508]+[delPhe508]	13 04	4 m	13			
42	3	M	p.[delPhe508]+[delPhe508]	20 04	<1 w	5	No kitchen		
43	3	F	c.[1585-1G>A]+[?]	02 00	<1 w	8			Bathroom toilet
44	3	M	p.[delPhe508]+[Asn1303Lys]	05 01	14 m[#]	7			Bathroom washbasin tap
45	4	M	NA	13 01	2.5 m	10			
46	4	NA	NA	08 08	<1 w	8			
47	4	NA	NA	09 06	<1 w	7	No toilet		
48	5	M	p.[delPhe508]+[Asn1303Lys]	12 10	<1 w	12			
49	5	F	p.[delPhe508]+[delPhe508]	09 07	<1 w	16			Bathroom washbasin drain = kitchen sink tap
50	5	F	p.[delPhe508]+[delPhe508]	02 09	<1 w	9		Bathroom bath drain	

CFTR: cystic fibrosis transmembrane conductance regulator gene; M: male; F: female; m: months; w: weeks; =: the genotype of *Pseudomonas aeruginosa* was the same in both locations; <>: different genotypes of *P. aeruginosa* were present in the locations; NA: not available. [#]: in all patients, environmental sampling took place later than patient sampling, except for patients 5, 26, 40 and 44, where environmental sampling took place prior to patient sampling; [¶]: "no bathroom", "no kitchen", "no toilet", "no aerosol", "no nebuliser" means that no sample was collected from that location; [+]: six samples were shared for patients 18 and 21, and for each patient, an additional two samples were obtained from the nebuliser; [$]: bathroom bath drain samples were taken with an interval of 4 months.

METHODS

Study population and sampling

Between January 2003 and December 2005, five Belgian CF centres sent sputum, nasopharyngeal aspirate or an isolate of newly *P. aeruginosa*-infected patients to the microbiology laboratory of Ghent University Hospital (GUH; Ghent, Belgium) for culture, confirmation of the identification and genotyping of the *P. aeruginosa* isolates. Approval of the Ethics Committee of the GUH was obtained. In each centre, *P. aeruginosa*-negative CF patients were seen at 3-month intervals in segregated consultation rooms or on different consultation days. All newly infected patients were treated for 3 months with oral ciprofloxacin (15 mg·kg^{-1} *b.i.d.*) and inhaled colistin (2×10^6 U *b.i.d.*) according to the international guidelines [13]. As soon as possible after the diagnosis of a first *P. aeruginosa* infection, the CF centre nurse visited the home of the patient and collected environmental samples. Samples were taken from the nebuliser, the taps and sink in the kitchen, the taps and drains of the bath, the shower and washbasin in the bathroom and the toilet(s). Samples from other wet surfaces, *e.g.* swimming pools and aquaria, were taken at the discretion of the nurses. A sterile swab (Nuova Aptaca, Canelli, Italy) was used to sample wet surfaces. Surface water (5 mL) was collected from toilets, swimming pools and aquaria by means of a sterile syringe. The medication container of the nebuliser was rinsed with 5 mL of sterile saline, which was poured into a sterile sample container.

Microbiology

Sputum and environmental samples were inoculated onto McConkey agar (BBL Becton Dickinson, Cockeysville, MD, USA). After 2 days of incubation at 37°C, morphologically different lactose-negative colonies were picked, subcultured on 5% sheep blood agar (BBL Becton Dickinson) and tested for oxidase. Only oxidase-positive colonies were further identified using tDNA-PCR [14].

Genotyping

For each patient, all *P. aeruginosa* isolates exhibiting different colonial morphology on McConkey were first genotyped using alkaline cell lysis for DNA extraction and the random amplified polymorphic DNA fingerprinting technique with Ready-to-Go beads (Amersham Biosciences AB, Uppsala, Sweden) and primer ERIC2 (AAGTAAGTGACTGGGGTG AGCG) at an annealing temperature of 35°C, as described previously [8]. This approach is designated ERIC2-PCR in the present study. This allowed a reduction in the number of isolates that were subsequently genotyped by the more laborious fluorescent amplified fragment length polymorphism analysis technique [7], since only single representatives of each ERIC2-PCR type were further genotyped by this procedure.

RESULTS

Table 1 presents the epidemiological and sampling data of the patients included.

Genotyping of *P. aeruginosa* was performed for 50 patients (26 male, sex not recorded for two; median age (range) 7 yrs and 11 months (9 months–31 yrs and 4 months)), including one sibling pair. A total of 427 environmental samples were obtained and genotyped with a median number of eight samples per patient (mean (range) samples 8.5 (4–23)). Nurses were asked to collect a minimum of four samples per home, with at least one sample from the kitchen, bathroom, toilet and the patient's nebuliser. Complete sampling was carried out for 39 out of 50 patients. Overall, one-third and one-fifth of the samples originated from the bathroom and the kitchen, respectively (table 2), for which 70.8% originated from the drains. The environmental samples were taken within 1 month of the positive sputum culture for *P. aeruginosa* in 37 of the 50 patients and within 1 week for 29 of these 37 patients (table 1). Environmental samples were also taken and processed for three patients for whom isolation of *P. aeruginosa* by the referring centre could not be confirmed by the reference laboratory. For one of these patients, an identical *P. aeruginosa* genotype was encountered in the sink drain 6 months before the first confirmed isolation of *P. aeruginosa* in the patient. A total of 25 environmental samples from 17 houses harboured *P. aeruginosa*. This corresponded to 18 patients, including one sibling pair. The median number of samples taken from houses with *P. aeruginosa* (nine samples per house) was not statistically different from the median number taken from all houses (eight samples per house) and the locations from which the samples were taken (*i.e.* bathroom kitchen, drain or tap) did not differ globally either. The vast majority of *P. aeruginosa*-positive samples were recovered from the bathroom (*i.e.* 18 out of 25, 72%). For 12 out of the 25 patients for which *P. aeruginosa* could be recovered from the house, the house isolate was different from the patient isolate. These 12 samples came from nine patients (including one sibling pair) at eight different houses. A total of 13 of 25 environmental samples harboured *P. aeruginosa* with an identical genotype to that of the patient. The 13 samples came from nine patients at nine different houses. *P. aeruginosa* was found in only one of the 75 samples taken from the 48 nebulisers of the 50 patients. The latter *P. aeruginosa* isolate had an identical genotype to that of the patient. Another six nebulisers from six different patients harboured other bacteria, with one nebuliser contaminated with *Stenotrophomonas maltophilia*. The *S. maltophilia* genotype was different from the genotypes of the two positive samples for *S. maltophilia* found in the home environment of this patient. However, this patient had no positive sputum culture for *S. maltophilia*. The other bacteria cultured from the nebulisers are not usually considered to be of pathological significance for CF patients. It should be noted that in the present study, the nebuliser samples were only processed to recover gram-negative organisms. *P. aeruginosa* could not be recovered from any of the three private swimming pools of the presently studied CF patients. The sibling pair in the study (patients 18 and 21) was apparently simultaneously newly infected by the same strain of *P. aeruginosa*. The source of infection could not be identified, as the environmental *P. aeruginosa* recovered had a different genotype. By consulting the previously published national Belgian database of *P. aeruginosa* genotypes [7], the *P. aeruginosa* genotypes of the newly infected patients were also compared with the genotypes of *P. aeruginosa* cultured from chronically infected CF patients followed at the same centre. In three patients, followed at two different centres, the genotypes of the newly acquired *P. aeruginosa* strain were identical to the genotype of already chronically infected patients from their own centres.

TABLE 2	Sampling details			
Sampling site	**Samples**	**Positive samples**		
		Identical[#]	Different[¶]	%
Bathroom	146 (34.2)	8	10	12.4
Bath drain	39	3	4	17.9
Bath showerhead	2	0	0	0
Bath tap	7	1	0	14.3
Shower drain	17	1	2	17.6
Shower tap	3	0	0	0
Showerhead	8	0	0	0
Toilet	5	0	1	20.0
Toothbrush	1	0	0	0
Washbasin drain	49	3	2	10.2
Washbasin tap	15	0	1	6.7
Kitchen	87 (20.4)	2	2	4.9
Sink drain	60	2	1	5.0
Sink tap	22	0	1	4.5
Other	5	0	0	0
Toilet room	68 (15.9)	2	0	2.9
Toilet	56	2	0	3.6
Washbasin drain	11	0	0	0
Washbasin tap	1	0	0	0
Nebuliser	75 (17.6)	1	0	13.3
Diverse	51 (11.9)	0	0	0
Animal	1	0	0	0
Aquarium	7	0	0	0
Bedroom	3	0	0	0
Plant	3	0	0	0
Swimming pool	3	0	0	0
Tumble dryer	2	0	0	0
Washing machine	3	0	0	0
Washroom	6	0	0	0
Other inside house	19	0	0	0
Other outside house	4	0	0	0
Total	427	13	12	5.9

Data are presented as n or n (%), unless otherwise indicated. [#]: genotype of the environmental isolate identical to the genotype from the patient genotype; [¶]: genotype of the environmental isolate different to the genotype from the patient genotype.

DISCUSSION

P. aeruginosa is the most important pathogen in patients with CF. The origin of *P. aeruginosa* infection in patients with CF has not yet been clearly established, and the role of the environment as a source of *P. aeruginosa* acquisition in CF patients is still difficult to ascertain and remains a matter of debate [11, 12]. Patients with CF rarely appear to share genotypes, unless they are siblings or close friends, suggesting that patient-to-patient transmission is rare and requires intense and longstanding contact. *P. aeruginosa* is a typical water organism and has been isolated from a number of environmental reservoirs [9, 10]. The wide distribution of *P. aeruginosa* genotypes established in young CF children suggests acquisition from environmental reservoirs [15]. *P. aeruginosa* has been recovered from environmental sources in both in- and outpatient

healthcare settings, *e.g.* sinks, tap water in a paediatric ward [16], toys, baths, hand soaps [17], pulmonary function equipment, hospital drains [18], whirlpool baths [19], dental equipment [20], and the hands of healthcare workers and CF patients [16–18]. The possibility of environment-to-patient transmission of these strains has been studied by DORING *et al.* [16], who described the possibility of environment-to-patient transmission of *P. aeruginosa* in a hospital setting. DORING *et al.* [16] suggested that the ability of CF sputum to facilitate survival of *P. aeruginosa* may be an important issue for strain transmission. During a cross-infection outbreak, JONES *et al.* [21] looked for the presence of epidemic strains in the environment of a CF centre and concluded that aerosol dissemination may be the most important factor in patient-to-patient spread of epidemic strains of *P. aeruginosa*.

In contrast to the extensive studies on the presence of *P. aeruginosa* in the hospital environment [16–20, 22–24] and on patient-to-patient transmission, there are only a few reports on the prevalence of *P. aeruginosa* in the home environment of both CF and non-CF patients (table 3) [22, 25–28]. These studies provide conflicting results. None of them studied the possibility of environment-to-patient transmission, neither did they compare genotypes of *P. aeruginosa* strains recovered from either patients or from their home environment.

To the best of the present authors' knowledge, the current study is the first to look for the presence of *P. aeruginosa* in the home environment of newly *P. aeruginosa*-infected CF patients and to compare genotypes of the environmental strains with the genotypes found in the patients.

In the present study, *P. aeruginosa* could be cultured from 5.9% of the home environmental samples in 34% of the houses. The data are comparable to data found in non-CF houses, although comparison is difficult because of different methodologies; however, a lower environmental contamination with *P. aeruginosa* is observed in CF houses compared with the other studies. The different findings might be explained by the following differences in procedures: 1) different sampling sites were tested, *i.e.* the present study did not investigate vegetables, which were an important source of *P. aeruginosa* in the study of MORTENSEN *et al.* [27]; 2) a different sampling methodology was used, *i.e.* 100-mL aliquots of tap water were collected in the study by BARBEN *et al.* [26] *versus* 5-mL aliquots in the present study and cultures of standing water before the taps were used in the mornings in the studies of BARBEN *et al.* [26] and REGNATH *et al.* [25]; 3) different cleaning procedures were carried out by the patients; and 4) perhaps most importantly, the different colonisation status of the CF patients investigated, *i.e.* noncolonised patients in the present study, and *P. aeruginosa*-colonised patients in the other studies. However, the colonisation status is not always explicitly described in all other studies.

Although previous studies occasionally detected strains with the same (geno)type in the healthcare environment and in patients [16, 17, 24, 29], it remains unclear whether patients were the initial source of environmental contamination or whether the strains from the contaminated environmental source infected the patients. In the present study, for nine of the patients, the genotype of the environmental *P. aeruginosa*

TABLE 3	Studies on the prevalence of *Pseudomonas aeruginosa* in cystic fibrosis (CF) and non-CF houses				
First author [ref.]	**Study population**	**Sampling method**	**Sampling sites**	**Culture medium[#]**	**Positive/total samples n/n (%)**
REGNATH [25][¶]	102 houses of 118 CF patients	Sterile swab	Bathroom washbasin drain	Cetrimid agar and acetamide broth	35/101 (34.7)
		Water sample	Bathroom washbasin tap	Cetrimid agar and acetamide broth	1/102 (1)
		Sterile swab	Bathroom shower drain	Cetrimid agar and acetamide broth	40/101 (39.6)
		Sterile swab	Bathroom showerhead	Cetrimid agar and acetamide broth	6/100 (6)
		Direct agar contact plate method	Bathroom bath	Rodac plate	5/102 (4.9)
		Sterile swab	Toilet washbasin drain	Cetrimid agar and acetamide broth	3/66 (4.5)
		Water sample	Toilet drain	Cetrimid agar and acetamide broth	27/102 (26.5)
		Sterile swab	Kitchen sink drain	Cetrimid agar and acetamide broth	35/100 (35)
		Water sample	Kitchen sink tap	Cetrimid agar and acetamide broth	7/102 (6.9)
BARBEN [26][+]	50 houses of 50 CF patients		Bathroom washbasin tap		
		Water (100 mL)	Standing water winter	Cetrimid agar	0/51 (0)
		Water (100 mL)	Running water winter	Cetrimid agar	0/51 (0)
		Water (100 mL)	Standing water summer	Cetrimid agar	2/50 (4)
		Water (100 mL)	Running water summer	Cetrimid agar	0/50 (0)
MORTENSEN [27][§]	14 houses of 14 CF patients	Sterile swab	All drains[ƒ]	McConkey agar	10/106 (9.4)
	13 houses in same area as CF houses	Sterile swab	All drains[ƒ]	McConkey agar	10/106 (9.4)
WHITBY [22][##]	33 houses of 33 hospital laboratory staff members	Sterile swab	Bath, sink and washbasin drains	Modified King's medium B	7/114 (6.1)
		Sterile swab	Bath, sink and washbasin drains	Modified King's medium B	1/21 (4.8)
OJIMA [28]	86 Japanese houses	Direct agar contact plate method	Kitchen sink tap	NAC agar	6/83 (7.2)[¶¶]
		Direct agar contact plate method	Kitchen sink drain	NAC agar	23/85 (27.1)[¶¶]
		Direct agar contact plate method	Kitchen sink tap	NAC agar	11/85 (12.9)[¶¶]
		Direct agar contact plate method	Bathroom bath	NAC agar	11/86 (1.2)[¶¶]
		Direct agar contact plate method	Bathroom showerhead	NAC agar	0/5 (0)[¶¶]
		Direct agar contact plate method	Toilet room sink	NAC agar	0/79 (0)[¶¶]
		Direct agar contact plate method	Washroom sink	NAC agar	1/43 (2.3)[¶¶]

[#]: acetamide broth (Fluka AG, Buchs, Switzerland); cetrimid agar, Rodac plate, McConkey agar and King's medium B (Becton Dickinson, Franklin Lakes, NJ, USA); and nalidixic acid cetrimide (NAC) agar (Nissui Pharmaceuticals, Tokyo, Japan). [¶]: *P. aeruginosa* colonisation status of patients known for 88 patients, of whom 28 were colonised. Age ranged from 7 months to 41 yrs (mean 11 yrs). *P. aeruginosa* was detected in 71.6% of the CF-houses. [+]: ages and colonisation status for *P. aeruginosa* of patients unknown. [§]: patients colonised with *Burkholderia cepacia*. Ages and colonisation status for *P. aeruginosa* of patients unknown. [ƒ]: all drains = kitchen sink drains + shower heads + refrigerator drain pan + bathtub drains. [##]: *P. aeruginosa* detected in four out of 33 houses. [¶¶]: number of houses with *P. aeruginosa* in the specified location/total number of houses examined for this location (percentages of households in which *P. aeruginosa* is detected).

isolate was identical to that of *P. aeruginosa* isolated from the patient's sputum or nasopharyngeal aspirate. Therefore, an environmental strain could have been a possible source of infection for only nine of 50 (18%) of the newly *P. aeruginosa*-infected CF patients. As regular prospective home environmental cultures before the first positive *P. aeruginosa* infection in the patient are lacking from the present study, the authors cannot definitely conclude that the environmental strains were the source of infection or conversely that contamination of the environment was caused by the patient. However, the present study indicates that there is no overwhelming *P. aeruginosa* contamination of the home environment of newly infected CF patients. As the vast majority of environmental *P. aeruginosa* isolates were found in the bathroom of the patients, it would be advisable to pay special attention to the cleaning and disinfecting procedures for bathrooms. Compared with data from other studies [30, 31], contamination of the nebulisers with *P. aeruginosa* in the present study was low. These findings suggest that the specific disinfecting procedures of the nebuliser equipment, as advised by the medical committee of the Belgian CF patient association [32], are effective.

In only three (6%) of the 50 newly infected patients could there have been patient-to-patient transmission within the same CF centre. As far as was traceable, this transmission remains unexplained, as there was no close relationship between the patients, they were seen in segregated consultations, they were never hospitalised at the same time and they never attended the same CF rehabilitation centre or CF summer camp. None of these genotypes belonged to a cluster of genotypes established previously for Belgian colonised CF patients [7]. These results confirm the low patient-to-patient transmission percentages after the introduction of segregation measures in the CF centres [33].

Since the present study results suggest that neither home environment nor other patients are important sources of *P. aeruginosa* infection in CF patients, the question remains regarding the actual source of an initial infection. Apart from wet surfaces, the potential sources of *P. aeruginosa* in the home environment mentioned in literature are washing cloths, sponges for cleaning [28] and vegetables [27, 34]. As *P. aeruginosa* is widespread in soil and water, acquisition from the environment outside the house is probable. More studies are needed to elucidate the role of these potential infecting sources of *P. aeruginosa* in CF patients.

In conclusion, to the best of the present authors' knowledge, the current study is the first to look for the presence of *Pseudomonas aeruginosa* in the home environment of cystic fibrosis patients newly infected with *Pseudomonas aeruginosa* and to compare genotypes of environmental *Pseudomonas aeruginosa* with the genotypes of the strains recovered from these patients. Low percentages of possible home environment-to-patient transmission (18%) and of patient-to-patient transmission within the same CF centre (6%) are reported. In addition, the present study clearly shows that there is no overwhelming contamination level with *Pseudomonas aeruginosa* in the home environment of this population of newly *Pseudomonas aeruginosa*-infected patients. Contamination seems to occur mainly in the bathroom of the patient, suggesting that this place should be targeted for more rigorous cleaning procedures. More studies are needed to

elucidate the role of other potential infecting sources of *Pseudomonas aeruginosa* in cystic fibrosis patients.

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