

# Effects of Segregation on an Epidemic *Pseudomonas aeruginosa* Strain in a Cystic Fibrosis Clinic

Amanda L. Griffiths, Kris Jansen, John B. Carlin, Keith Grimwood, Rosemary Carzino, Philip J. Robinson, John Massie, and David S. Armstrong

Department of Respiratory Medicine, Royal Children's Hospital; Clinical Epidemiology and Biostatistics Unit, Murdoch Children's Research Institute; Department of Pediatrics, University of Melbourne, Parkville; Department of Pediatrics, Monash University; Department of Respiratory and Sleep Medicine, Monash Medical Centre, Clayton, Victoria, Australia; and Department of Pediatrics and Child Health, Wellington School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand

The detection of a clonal *Pseudomonas aeruginosa* strain in 21% of children attending a cystic fibrosis clinic during 1999, which may have led to a worse prognosis, prompted strict infection control measures, including cohort segregation. We determined whether these strategies interrupted cross-infection within the clinic. Patients from 1999 were observed and a cross-sectional study of the 2002 clinic was performed. By 2002, the epidemic strain prevalence had decreased from 21 to 14% ( $p = 0.03$ ), whereas the proportion of patients with nonepidemic *P. aeruginosa* strains was unchanged. The age- and sex-adjusted relative risk for epidemic strains among sputum producers in 2002 compared with 1999 was 0.64 (95% confidence interval, 0.47, 0.87;  $p = 0.004$ ). Increased mortality or transfer to another clinic did not explain this reduction. Although children with epidemic strains may have had increased mortality (adjusted odds ratio, 2.0; 95% confidence interval, 0.6–6.8), they did not demonstrate greater morbidity than those with other *P. aeruginosa* isolates. Successful infection control measures provided additional indirect evidence for person-to-person transmission of an epidemic strain within the clinic. Further studies are needed to resolve whether cohort segregation completely eliminates cross-infection and if acquisition of epidemic isolates is associated with worse outcomes.

**Keywords:** cross-infection; cystic fibrosis; infection control; *Pseudomonas aeruginosa*

The acquisition and persistence of *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis (CF) is associated with accelerated deterioration of pulmonary function, increased hospitalization, and reduced life expectancy (1, 2). Typically, early infecting strains of *P. aeruginosa* have a nonmucoid colonial appearance and are relatively susceptible to antibiotics. Eventually, they develop a mucoid phenotype, which marks the establishment of chronic infection. This stage usually occurs in older children and young adults who harbor the same isolate for many years (3, 4). It is presumed that most patients with CF acquire their own unique strains from the environment and, except for prolonged contact between siblings with CF, person-to-person transmission is unlikely (5). However, recent reports from Europe, Britain, and Australia have identified certain strains that appear capable of cross-infection both within and between CF centers (6–10).

Between 1991 and 1996, five unrelated children younger than

5 years, who attended the CF clinic at the Royal Children's Hospital, Melbourne, died of severe lung disease (11). All had recently acquired multiple antibiotic-resistant mucoid strains of *P. aeruginosa*, which, on testing by pulsed-field gel electrophoresis, proved to have an indistinguishable macrorestriction pattern. Ensuing surveillance of the CF clinic in 1999 discovered that 55% of those infected with *P. aeruginosa* (21% of all patients) had identical or closely related strains to isolates from these five children (12). Failure of two environmental surveys in 1995 and 1999 to identify a common source for this clonal strain raised the possibility of person-to-person transmission. Increased virulence was also suspected because infected patients had poorer pulmonary function and increased hospitalization compared with those infected with unrelated *P. aeruginosa* strains. The prospect of acquiring multiresistant strains that resisted attempts at early eradication and progressed to chronic infection was of additional concern.

Since January 2000, standard infection control measures in the clinic were reinforced, cohort segregation was introduced for outpatient clinics, and education seminars were arranged for staff and families (13, 14). Prospective molecular-based surveillance by pulsed-field gel electrophoresis genotyped current and new *P. aeruginosa* isolates. Strict individual segregation was continued for children with CF with *Burkholderia cepacia* complex or methicillin-resistant *Staphylococcus aureus* infection. Remaining patients were segregated into one of three cohorts as determined by their sputum microbiology: (1) epidemic *P. aeruginosa*, (2) nonepidemic *P. aeruginosa*, and (3) others. Within hospitals, patients were nursed in separate sections, attended physiotherapy sessions and lung function testing at different times, and avoided the hospital school.

We speculated that cohort segregation and standard infection control measures would interrupt cross-infection within the clinic by the epidemic *P. aeruginosa* strain. The current study determined the epidemic strain prevalence in 2002 compared with 1999, and measured clinical progress for all patients surveyed in 1999 over this period to further assess effects of the strain on patient health outcomes. Preliminary findings have been previously reported in abstract form (15).

## METHODS

The Royal Children's Hospital Ethics Committee approved this study as a retrospective clinical audit. Verbal consent was obtained from all participants and their caregivers.

### Cross-sectional Audit of the 2002 CF Clinic

The CF research database and laboratory records were surveyed for sputum culture and pulsed-field gel electrophoresis results. Clinical data, chest radiographs, and the best percent-predicted FEV<sub>1</sub> from the previous 6 months were recorded for patients with *P. aeruginosa* attending their first routine appointment in 2002. In those aged 5 years and older, the treating physician performed a National Institutes of Health (NIH) score (16). For each patient, medical chart and hospital pharmacy record

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Correspondence and requests for reprints should be addressed to David Armstrong, M.D., Department of Pediatrics, Monash University, Monash Medical Centre, Clayton Road, Clayton, Victoria 3168, Australia. E-mail: david.armstrong@southernhealth.org.au

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review by a single-blinded investigator (A.L.G.) determined for the previous 12 months the number of hospital days and if inhaled antibiotics or recombinant human DNase (rhDNase) had been prescribed.

### Follow-Up of the 1999 CF Clinic Cohort

The CF clinic managed 325 patients in 1999 and 291 in 2002, and included new patients diagnosed after newborn screening (17). Children were seen every 3 months, and sputum was collected from expectorating patients. Since 1992, patients have immediately received 2 weeks of intravenous antibiotics after the initial detection of *P. aeruginosa*. Typically, this was ticarcillin-clavulanic acid and tobramycin, then 3 months of oral ciprofloxacin and inhaled tobramycin (11). The 1999 patient cohort was traced using medical records, the CF research database, hospital laboratory reports, and correspondence concerning deceased and transferred patients.

### Microbiologic Methods

Sputum samples were plated onto selective media for *P. aeruginosa* and other pathogens by standard techniques (18). *P. aeruginosa* colony morphotypes were identified visually (19) and tested for antibiotic susceptibility by disk diffusion (20). As described previously, an independent laboratory scientist randomly selected a single mucoid and nonmucoid *P. aeruginosa* colony from each infected patient for molecular typing by pulsed-field gel electrophoresis (12, 21). Isolates showing identical restriction fragment profiles to the epidemic clone were considered to be the same strain; those differing by one to three bands were regarded as closely related and arising from the same clone, whereas isolates differing by four or more bands were considered unrelated and to be different strains (22).

### Statistical Analysis

Initial analyses compared the 1999 and 2002 clinics, treated as separate groups, to assess changes in prevalence of infection over time. Relative risks comparing proportions (prevalence values) in 2002 with 1999 were adjusted for age and sex using binomial regression with a log-link function. Further analyses focused on outcomes observed in 2002 for patients in the 1999 clinic cohort. Patients were categorized into one of the following subgroups according to sputum microbiology and *P. aeruginosa* genotyping results in 1999: (1) Melbourne epidemic *P. aeruginosa* strain, (2) nonepidemic *P. aeruginosa* strains, (3) no *P. aeruginosa*, and (4) non-sputum producers. Group mortality was analyzed as a percentage of the total cohort. Logistic regression was used to calculate odds ratios adjusted for age and sex for mortality or transfer to another clinic. Outcome variables measured in 2002 on the 1999 cohort (body mass index, inhaled antibiotic and rhDNase use, hospital days, NIH score, best recorded percent-predicted FEV<sub>1</sub>) were compared between cohort subgroups, adjusting for patient group, age, sex, and baseline value in 1999 by multiple regression. Statistical analysis was performed using Stata software (Stata Corporation, College Station, TX) (23).

## RESULTS

### Cross-sectional Comparisons of 1999 and 2002 Clinics

Ninety-one of 149 children able to produce sputum in 2002 had *P. aeruginosa* detected. Thirty-six had isolates with macrorestric-

tion bands indistinguishable from the epidemic strain, another four had isolates closely related to this particular strain, and all except one child had attended the 1999 clinic. As before, these isolates exhibited mainly a mucoid phenotype (mucoid in 16, mixed mucoid and nonmucoid colonies in 22, and nonmucoid in 2 children). Two of these patients also possessed distinct, unrelated second strains of *P. aeruginosa* in their sputum. Nonepidemic *P. aeruginosa* isolates were found in the remaining 51 children (mucoid in 18, mixed mucoid and nonmucoid in 24, and nonmucoid colonies in 9 children). Forty-seven were genotypically unique, and two sibling pairs each shared a distinct strain.

Although lacking a distinctive antibiotic susceptibility profile, epidemic strains were more resistant to antibiotics than nonepidemic *P. aeruginosa* isolates (ticarcillin-clavulanate, 57 vs. 17%; piperacillin, 85 vs. 25%; aztreonam, 72 vs. 32%; ceftazidime, 85 vs. 26%; imipenem, 93 vs. 32%; gentamicin, 98 vs. 71%; tobramycin, 85 vs. 41%; amikacin, 96 vs. 57%; ciprofloxacin, 76 vs. 46%;  $p < 0.001$  for all comparisons). Furthermore, resistance to all agents in two or more antibiotic classes ( $\beta$ -lactams, aminoglycosides, fluoroquinolones) (24) was more common among epidemic than nonepidemic strains (34 vs. 14%,  $p = 0.008$ ).

The overall proportion of clinic patients with the epidemic strain decreased from 21% (67 of 325) in 1999 to 14% (40 of 291) in 2002 ( $p = 0.03$ ). Table 1 shows that, among sputum producers attending the clinic in 2002, the adjusted risk of infection by the epidemic strain was 36% less than among the corresponding group in 1999 ( $p = 0.004$ ). In contrast, the risk of infection by sporadic strains remained basically unchanged. Furthermore, between 1999 and 2002, Table 2 shows a systematic decrease in the point prevalence of epidemic strains among sputum producers of all ages, which was statistically significant for those aged younger than 13 years.

### Follow-Up of the 1999 Cohort

Follow-up of the 325 patients with CF from the original 1999 cohort in 2002 is outlined in Figure 1. Seventy-eight patients remained non-sputum producers, whereas 66 transferred to other CF clinics. Of the latter, 31 were non-sputum producers and five did not have *P. aeruginosa* detected in their sputum. The remaining 30 children who transferred had *P. aeruginosa*, 19 with the epidemic strain. Table 3 shows that, by 2002, after adjustment for age and sex, patients with *P. aeruginosa* strains in 1999 did not have an increased rate of transfer to another CF clinic when compared with the non-sputum producers of 1999.

Ten patients (including one sibling pair) acquired the epidemic strain between the 1999 and 2002 surveys. Four were originally non-sputum producers and another had been infected by *S. aureus*. The epidemic strain was also found in five unrelated patients who had each been previously infected with unique *P. aeruginosa* isolates. For the 2002 audit, four patients with the

TABLE 1. PREVALENCE OF *PSEUDOMONAS AERUGINOSA* AMONG SPUTUM PRODUCERS IN 1999 AND 2002 CYSTIC FIBROSIS CLINICS

	1999 ( <i>n</i> = 153)	2002 ( <i>n</i> = 149)	Relative Risk*	Adjusted Relative Risk†	95% CI	<i>p</i> Value
<i>P. aeruginosa</i>	0.78	0.62	0.80	0.82	(0.73, 0.91)	< 0.001
Epidemic strain	0.44	0.27	0.61	0.64	(0.47, 0.87)	0.004
Nonepidemic strains	0.34	0.35	1.05	1.05	(0.77, 1.43)	0.744
Epidemic strain per total <i>P. aeruginosa</i> isolates	0.56	0.43	0.77	0.75	(0.57, 0.99)	0.04

Definition of abbreviation: CI = confidence interval.

\* Relative risk as proportion of sputum producers by 2002 compared with 1999.

† Adjusted for age and sex.

**TABLE 2. PREVALENCE OF THE EPIDEMIC *PSEUDOMONAS AERUGINOSA* STRAIN AMONG SPUTUM PRODUCERS BY AGE GROUP FOR THE 1999 AND 2002 CYSTIC FIBROSIS CLINICS**

Age group (yr)	Epidemic Strain		Crude Relative Risk*	95% CI	p Value
	Prevalence in 1999 (group size)	Prevalence in 2002 (group size)			
< 10	0.26 (39)	0.07 (41)	0.29	(0.08, 0.96)	0.03
10–12	0.47 (34)	0.18 (34)	0.38	(0.17, 0.84)	0.01
13–15	0.45 (42)	0.39 (36)	0.86	(0.51, 1.46)	0.57
≥ 16	0.58 (38)	0.45 (38)	0.77	(0.49, 1.21)	0.25

For definition of abbreviation, see Table 1.

\* Relative risk for 2002 compared with 1999.

epidemic strain in 1999 grew only nonepidemic *P. aeruginosa* isolates, whereas another four no longer produced sputum. Finally, a patient who received intensive antipseudomonal therapy immediately after acquisition of the epidemic strain in 1999 remained free of this organism in 2002, with serial negative sputum cultures for *P. aeruginosa*.

Within the original 1999 cohort, nonepidemic *P. aeruginosa* strains were found for the first time in 28 clinic patients. Eighteen were not sputum productive in 1999, whereas six had not grown *P. aeruginosa* in previous sputum cultures, and, as previously described, four patients with the epidemic strain in 1999 now had only nonepidemic isolates. Numbers were too small to reliably assess the difference in clearance of epidemic versus nonepidemic strains (1 of 34 vs. 5 of 33; Fisher's exact test,  $p = 0.11$ ).

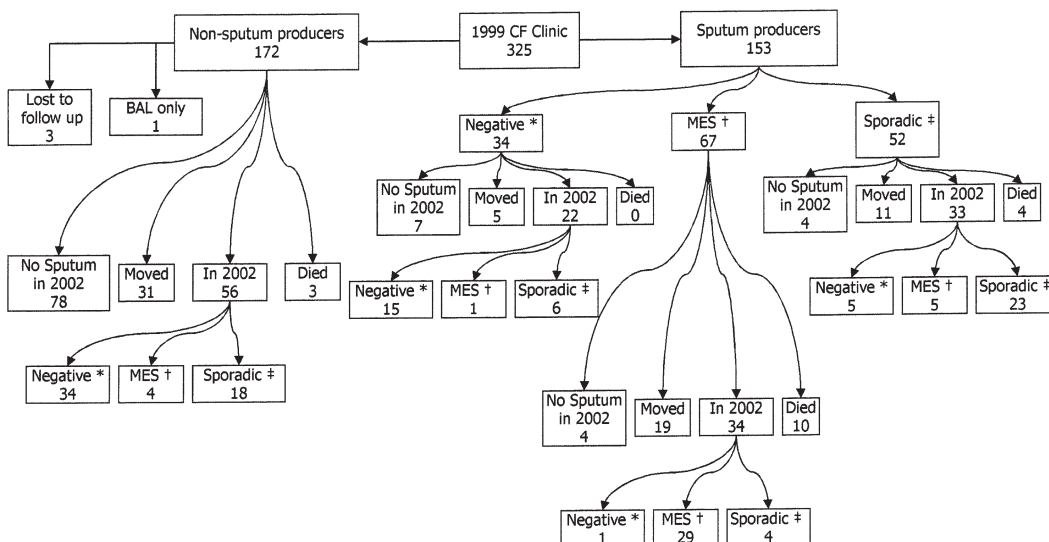
The odds of dying between 1999 and 2002 for those infected with the epidemic strain were almost twice those of children with nonepidemic strains, but again, numbers were small (10 of 67 vs. 4 of 52, respectively) and the increased mortality did not reach statistical significance (adjusted odds ratio, 1.97; 95% confidence interval, 0.57–6.82;  $p = 0.28$ ).

Table 4 shows little difference in morbidity in 2002 between those infected with epidemic or sporadic strains in 1999. Two additional analyses were performed, first using multiple regression to adjust for age, sex, and baseline values of outcomes in 1999, and second, including only those with either epidemic (29 patients) or nonepidemic (23 patients) isolates in both 1999 and 2002. Similar results were obtained. The baseline value for all parameters in 1999 was the best predictor of variation.

**DISCUSSION**

Within 3 years of enforcing standard infection control principles (25) and cohort segregation (13, 14), significantly fewer children in the Melbourne CF clinic grew epidemic *P. aeruginosa* strains from their sputum, whereas the prevalence of sporadic nonepidemic isolates remained unchanged. Infected patients transferring to other clinics or increased mortality could not explain this reduction. Instead, more patients had acquired nonepidemic than epidemic strains. Successful infection control and failure to identify epidemic isolates in environmental surveys further strengthen speculation that this strain spreads by person-to-person transmission rather than by independent acquisition from diverse sources (6, 12).

Although segregation policies are credited with limiting cross-infection by *B. cepacia* complex, their role in reducing *P. aeruginosa* within CF clinics is controversial (26, 27). Partly this is because most *P. aeruginosa* strains in patients with CF are likely to originate from the environment. In contrast, cross-infection has been convincingly demonstrated in relatively few clinics and a CF camp, with transmission apparently strain-dependent (6–10, 28, 29). Centers that have successfully introduced segregation of patients infected with *P. aeruginosa* have reported reduced prevalence and increased age of establishing chronic infection (13, 30, 31). However, using phenotypic rather than the more robust molecular-typing techniques as a basis for segregation complicates the interpretation of these findings. Moreover, other interventions were also introduced, most notably early and aggressive antibiotic treatment after the first isolation of *P. aeruginosa* from



**Figure 1.** Follow-up in 2002 of 325 children who attended the cystic fibrosis (CF) clinic in 1999. \**Pseudomonas aeruginosa* not detected in sputum; †Melbourne epidemic strain (MES); ‡unrelated nonepidemic strains. BAL = bronchoalveolar lavage.

**TABLE 3. RISK OF TRANSFER TO ANOTHER CLINIC BY 2002 ACCORDING TO SPUTUM PRODUCTION AND MICROBIOLOGY STATUS FOR 325 PATIENTS WITH CYSTIC FIBROSIS IN 1999**

	No. Patients	No. Patients Transferred	Adjusted Odds Ratio*	95% CI	p Value
Non-sputum producer	172	31 (18%)	—	—	—
No <i>P. aeruginosa</i>	34	5 (15%)	0.53	(0.17, 1.66)	0.26
<i>P. aeruginosa</i> isolates					
Epidemic	67	19 (28%)	0.46	(0.2, 1.03)	0.05
Nonepidemic	52	11 (21%)	0.43	(0.18, 1.06)	0.06

For definition of abbreviation, see Table 1.

\* Adjusted for age and sex, with non-sputum producers as the reference group.

respiratory secretions. Thus, although chronic infection was reduced, the annual incidence and mean age of first isolation of *P. aeruginosa* was unaffected (13, 30, 31).

This study has shown that cohort segregation determined by molecular typing of *P. aeruginosa* sputum isolates was accompanied by reduced numbers of patients with the epidemic strain. In particular, young children were less likely to be infected. However, there continued to be a small number of epidemic strain acquisitions, for which there are several potential explanations. Acquisition may have occurred outside the hospital for at least some cases, because one was a sibling of a patient already infected with the epidemic strain and three had been cared for at a nearby regional center. Unrecognized infection, especially among non-sputum producers, may have preceded the 1999 survey and subsequent segregation. Continuing low-level environmental exposure in the clinic remains possible but unlikely given the absence of more recent acquisitions.

Five children whose sporadic strains were replaced by the common epidemic strain may have had an unrecognized coinfection. Nevertheless, superinfection could have arisen between testing patients in 1999 and introducing cohort segregation shortly afterwards in 2000. During this transitional period, all those with *P. aeruginosa* were separated from others in the clinic and nursed together. In Britain, superinfection has been reported with another transmissible strain (32), and the Melbourne epidemic strain has also infected an adolescent with underlying non-CF lung disease (33). The risk of superinfection reinforces concerns about introducing a segregation policy based solely on antibiotic resistance (34). Implementation of cohort segregation

should be strain-specific and determined by genotyping, rather than antibiotic susceptibility patterns, because the latter cannot reliably differentiate between strains (12, 34).

In contrast to their effects on the epidemic strain, infection control measures did not alter acquisition of sporadic *P. aeruginosa* isolates. This finding is consistent with reports that found that most patients with CF acquire *P. aeruginosa* from the environment and that, outside of prolonged contact within families, person-to-person transmission is uncommon (5, 27). Considering the associated cost, stigmatization, and psychosocial stresses, cohort segregation should only be considered when there is strong epidemiologic and genotypic evidence for a transmissible *P. aeruginosa* strain in the clinic. Infection control information sessions and advice to limit social contact between individuals with CF outside the hospital led to broad acceptance of cohort segregation in Melbourne. Our recently published, questionnaire-based study showed that 86% of families of children with CF at the clinic had no contact with other CF families in the community (35).

Observations of patients with the epidemic strain in 1999 suggested that this organism was either more virulent than other strains or selectively infected sicker patients because of their increased exposure to the clinic environment (12). Furthermore, some clinics have reported worse outcomes for patients infected with transmissible strains. For example, over several years, the Liverpool epidemic strain appeared to confer a worse prognosis than infection with other isolates (36). The short duration of the present study meant there were few deaths, and it was not possible to determine if children with the Melbourne epidemic strain had a truly increased mortality rate. Furthermore, previously reported fatalities in young children with this strain (11) may have been caused by early establishment of chronic infection with *P. aeruginosa*, which itself carries a high mortality (37). The overall clinical status of children from Melbourne with either epidemic or sporadic isolates was similar. Nevertheless, epidemic isolates had increased antibiotic resistance and this can contribute to longer hospitalization for respiratory exacerbations and less frequent use of inhaled antibiotics compared with patients with other strains (38). We are currently analyzing longitudinal data from a cohort of children recruited at the time of diagnosis by newborn screening (11, 18, 21) to further examine over a much longer period whether the Melbourne epidemic strain is associated with increased mortality and morbidity.

A limitation of the present study was testing only one to two sputum isolates of *P. aeruginosa* per sample from each infected patient. This meant the prevalence of epidemic strains and incidence of superinfection might have been under- and overestimated, respectively, when coinfection with multiple strains occurred (39). Although sputum culture appears to be a reliable means for detecting *P. aeruginosa* in the lower airways, it remains controversial whether sputum is as sensitive as bronchial lavage

**TABLE 4. CHARACTERISTICS AT FOLLOW-UP OF CHILDREN FROM THE 1999 CYSTIC FIBROSIS CLINIC WHO WERE INFECTED WITH *PSEUDOMONAS AERUGINOSA* IN 1999 BY TYPE OF INFECTION IN 1999**

Outcome	Epidemic (n = 34)	Nonepidemic (n = 33)	p Value
Age, yr	14.8	14.3	0.54
Male, %	53	45	0.54
Body mass index, z-score	-0.69	-0.69	0.99
Inhaled antibiotics, %	56	79	0.05
rhDNase, %	50	42	0.53
Hospitalized days*	14.5	15.0	0.24
NIH score (16)	71	73	0.57
CXR score <sup>†</sup>	10.0	9.7	0.72
Best %predicted FEV <sub>1</sub> <sup>‡</sup>	76	80	0.43

Definition of abbreviations: NIH = National Institutes of Health; rhDNase = recombinant human DNase.

\* Median days in hospital for the previous 12 months assessed by the rank sum test.

<sup>†</sup> Derived from the NIH score (16).

<sup>‡</sup> During the previous 6 months.

for identifying different genotypes from the same patient (40, 41). For clinics wishing to implement segregation policies, several sputum isolates from each patient may need to be tested at different times.

In contrast, oropharyngeal cultures from nonexpectorating children were not collected in this study, because we have previously shown these specimens lacked sensitivity and predictive accuracy for identifying lower respiratory infection (21, 42). Reviewing the microbiology database for 138 CF clinic infants and young children recruited between 1992 and 1999 who underwent annual bronchial lavage and every-3-month oropharyngeal cultures, *P. aeruginosa* was identified in lower respiratory secretions from 27 patients. The epidemic strain was present in eight children, all of whom had persistent respiratory symptoms. Just three of these young patients had the epidemic strain detected in oropharyngeal cultures and only one survived. A further 16 asymptomatic infants had exclusive and transient colonization of their upper airways by sporadic, nonmucoid strains of *P. aeruginosa* (21). Thus, it is very unlikely that asymptomatic, nonexpectorating patients with CF were acting as a silent reservoir of the epidemic strain. Although performing serial bronchoscopies and taking bronchial lavage cultures from all 172 non-sputum producing patients with CF in the clinic might have detected early infection by the epidemic strain, this could not be justified given the low probability of detecting *P. aeruginosa* in asymptomatic infants and children with CF (21, 42). In light of this experience and consistent with clinical practice in other major Australian pediatric CF clinics, we relied on sputum microbiology, reserving bronchial lavage for unwell, nonexpectorating patients who failed empiric antibiotic therapy directed at *S. aureus* and *Haemophilus influenzae*.

In summary, at a time when the prevalence of children harboring a single clonal strain of *P. aeruginosa* within the CF clinic was 21% and in the range that maximizes epidemic spread (43), introduction of strict infection control policies and cohort segregation was followed by significantly reduced numbers of patients sharing this common strain. Although the epidemic isolates were mainly mucoid and had increased antibiotic resistance, these phenotypic features were highly variable and could not reliably discriminate between strains. Instead genotypic-based surveillance helped determine patient grouping for infection control. Given the difficulties of cohort segregation for large clinics with transmissible strains and the challenges of sampling from coinfecting patients, further studies are needed to determine if cross-infection can be reliably controlled. Additional data are also needed to learn whether the Melbourne epidemic strain is associated with a worse prognosis.

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