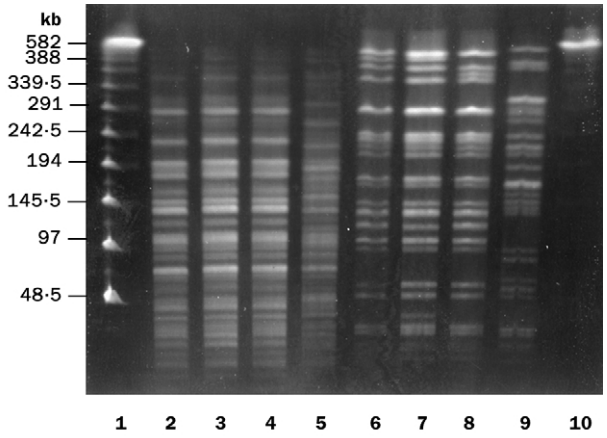

Genomic typing of *P aeruginosa*
Chromosomal DNA digested with *XbaI* (lanes 2–5) or *SpeI* (6–9) and separated by pulsed-field gel electrophoresis. Lanes 2, 3, 4, 6, 7, and 8 show profiles of the Manchester epidemic strain; lanes 5 and 9 show the Alder Hey epidemic strain; lanes 1 and 10 are DNA markers, λ concatemers (size range 48–582 kb).

problem of resistance in cystic fibrosis isolates of *P aeruginosa*, the remarkable genomic heterogeneity of *P aeruginosa*, and the unreliability of antibiotic susceptibility profiles to assess clonality or distinguish individual strains.

Our study raises several contentious issues. Is surveillance justified? How often should it be done? How much will add to the costs of patient care? What is the ideal typing system? Our finding that a multiresistant strain of *P aeruginosa* differing from that described previously[3] has spread among patients attending our adult cystic fibrosis centre highlights the need for appropriate microbiological surveillance as an essential component of infection control.[5] Surveillance would also determine the prevalence of other epidemic *P aeruginosa* in the cystic fibrosis community. Such strains may or may not exhibit multiresistance; thus reliance on antibiotic resistance patterns might not identify episodes of cross-infection.[5] Pulsed-field gel electrophoresis is timeconsuming and expensive but is presently the typing method of choice for *P aeruginosa*. Pyocin typing is also useful to provide additional information[2] and screen large numbers of isolates. Although surveillance has important resource implications, it also has the potential to prevent the acquisition and spread of emerging epidemic strains of *P aeruginosa*.

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Superinfection with a transmissible strain of *Pseudomonas aeruginosa* in adults with cystic fibrosis chronically colonised by *P aeruginosa*

Shona J McCallum, John Corkill, Malcolm Gallagher, Martin J Ledson, C Anthony Hart, Martin J Walshaw

Infection with transmissible strains of *Pseudomonas aeruginosa* can occur in uncolonised patients, but cross infection (superinfection) of patients already colonised with *P aeruginosa* has not been reported. With genotypic identification, we found superinfection by a multiresistant transmissible strain of *P aeruginosa* in four patients with cystic fibrosis (CF) who were already colonised by unique strains of *P aeruginosa*. No evidence of environmental contamination was found, but all patients became superinfected after contact with colonised individuals during inpatient stays. Inpatients with CF who are colonised with *P aeruginosa* should be separated by strain type. Such strain typing can only be reliably done by genomic methods, but this has resource implications.

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Chronic colonisation with *Pseudomonas aeruginosa* occurs in up to 80% of adults with cystic fibrosis (CF) and is associated with increased morbidity and mortality. Whilst it has been shown that unrelated patients attending the same clinic may be colonised by identical strains, suggesting colonisation from the same source or patient-to-patient spread, cross-infection (superinfection) between patients already colonised by different strains of *P aeruginosa* has not been shown. Many of the adult patients we see at the Cardiothoracic Centre, Liverpool, UK, are colonised by a transmissible strain, which predominates at the local paediatric clinic. Since 1998, we have longitudinally studied the genetic fingerprints of sputum *P aeruginosa* isolates of our patients with CF and looked for environmental contamination. In the environmental survey, despite repeated sampling, epidemic strains of *P aeruginosa* could not be isolated from sinks, drains, toilets, showers, or communal surfaces in areas frequented by patients with CF. In the longitudinal study, *P aeruginosa* morphotypes isolated from sputum samples collected at outpatient visits and inpatient stays were genomically analysed by pulsed-field gel electrophoresis (PFGE) and interpreted according to the protocol of Tenover.[1]

Here we present four adult patients with CF (all DF508/DF508), chronically colonised by unique strains of *P aeruginosa*, who became superinfected by a transmissible strain of *P aeruginosa*.

Patient 1 was colonised in 1984 at age 5 years by *P aeruginosa* while attending a peripheral paediatric clinic. At age 19 years she transferred to our unit and within 6 months had several admissions when she mixed freely with other patients with CF. Subsequent analysis of their sputa revealed that some were colonised with a multiresistant strain of *P aeruginosa* (strain AH). Analysis of isolates from the sputum of patient 1 during the first 3 months revealed three strains of *P aeruginosa* (M, N, and O). During the third month a further strain (AH) was isolated and after 6 months only strain AH was recoverable from her sputum, confirmed on repeat analysis 2 years later (figure 1A).

Patient 2 became colonised by *P aeruginosa* at age 11 years whilst attending a different remote paediatric

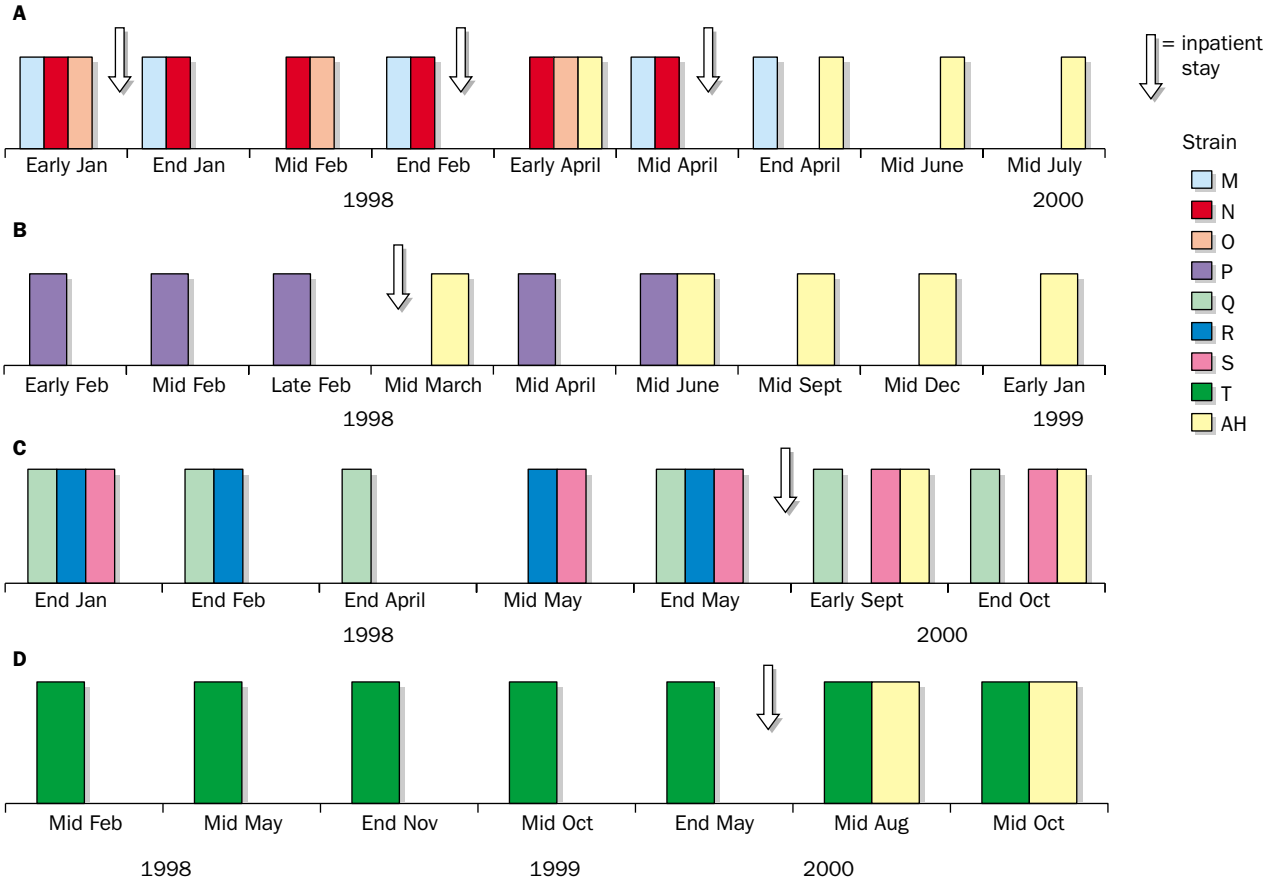

Figure 1: **Strains of *P aeruginosa* isolated from patients and occurrence of cross-infection by strain AH**

clinic. At the time of transfer to our unit (age 19 years), sputum analysis revealed a single *P aeruginosa* isolate (strain P). 2 months later, after an admission to hospital, analysis revealeda second isolate (strain AH). Subsequently, strain P disappeared and patient 2 was colonised solely by strain AH (figure 1B).

Patient 3 had been colonised by *P aeruginosa* for many years when she was transferred to our unit from a distant district general hospital clinic at age 33 years. In 1998, analysis of *P aeruginosa* isolates from outpatient sputum samples over 6 months revealed three unique strains of *P aeruginosa* (Q, R, and S). In October, 2000, after an inpatient stay, analysis revealed two of these strains (Q and R) plus strain AH (figure 1C).

Patient 4 was already colonised with *P aeruginosa* when transferred from the local paediatric hospital at age 16 years. Sputum analysis in 1998 revealed colonisation with a strain of *P aeruginosa* (strain T) which was sensitive to many antibiotics and was of varying morphology. In June, 2000 (at age 41 years), after hospital admission for 6 weeks, analysis revealed strain AH as well as strain T (figure 1D).

PFGE was carried out in 1% pulse-field agarose after cutting with *Spe1* and confirmed that strains M, N, O, P, Q, R, S, and T were distinct from each other and also from AH (figure 2). AH was compared with stored strains from the original paediatric outbreak and was found to be identical. In all four patients, superinfection by AH occurred after an inpatient stay. Furthermore, in patients 1 and 2, AH replaced the unique strains.

In 1996, while working in our local paediatric clinic, Cheng and colleagues[2] showed that many patients with CF were colonised by the same strain. They proposed that such colonisation was a result of cross-infection, or acquisition from a common environmental source. Our epidemic strain is the same transmissible strain of *P aeruginosa* and we have shown that such a strain can superinfect patients already colonised by unique strains. All four patients came from distant peripheral paediatric clinics or were referred from the local paediatric clinic many years earlier, and had not

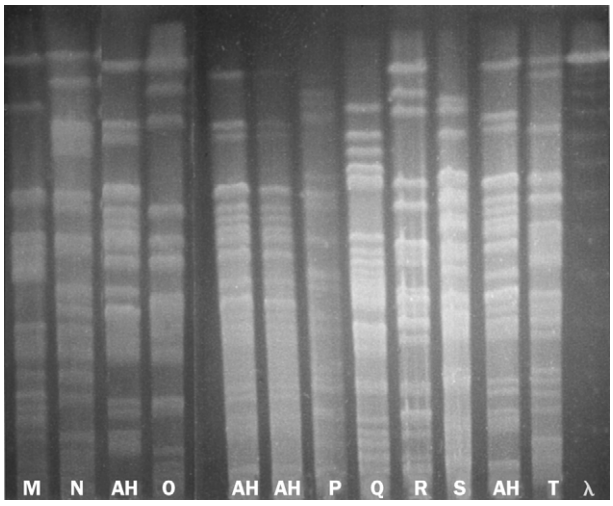

Figure 2: **Pulsed-field gel electrophoresis of *P aeruginosa* strains**
λ=reference lane.

previously been exposed to strain AH. Furthermore, cross-infection only occurred in those patients admitted for treatment: other patients with CF colonised by unique strains of *P aeruginosa*, who only attend as outpatients, were not superinfected, even though they were not separated from patients infected with AH strain. We have extensively investigated our inpatient CF facilities, and have not found the environment to be a source of epidemic *P aeruginosa*. In our unit, most patients are housed in separate siderooms on the same ward. Although contact between patients is discouraged, it is impossible to prevent social intermixing between young adult inpatients, who may be in hospital repeatedly for prolonged periods. We conclude that the four patients we describe acquired AH strain *P aeruginosa* through inpatient social contact with patients colonised with that strain.

Mindful of the poorer prognosis of patients colonised by *P aeruginosa*, some CF units separate uncolonised patients from the remainder. However, in those CF clinics in which epidemic strains exist, such an infection-control policy will not prevent superinfection and may even potentiate it by grouping at-risk patients together with those colonised by epidemic strains. Such grouping holds particular risk in adult CF centres, in which most patients are colonised by *P aeruginosa*. Furthermore, in those units without epidemic strains, such a policy is unnecessary since unique strains cannot, by definition, be transmitted between patients.

We believe that in those units that contain epidemic strains all patients with CF are at risk of superinfection and require frequent *P aeruginosa* strain typing. Unfortunately, a single *P aeruginosa* strain can exhibit many phenotypes and so identification techniques, which rely on phenotyping (for example, phage, pyocin, and serotyping), may not be sufficiently discriminatory.[2–4] Accurate strain identification is only possible by genotypic methods, which include PFGE.[5] We therefore chose PFGE as the gold standard to type all our *P aeruginosa* isolates. Now we regularly genotype all the *P aeruginosa* strains in our clinic and have adopted a policy of separating CF inpatients colonised with epidemic *P aeruginosa* strains from other CF inpatients and we recommend these measures to other centres. However, genotyping is expensive and time consuming, and will have serious funding consequences for CF units. Those units that rely on phenotypic methods to identify strains of *P aeruginosa* may need to separate all their patients colonised with *P aeruginosa* from each other and from uncolonised patients to prevent superinfection. Further work needs to be done to assess the prevalence and impact of epidemic strains of *P aeruginosa* in CF clinics, and cross-infection control policies must be refined accordingly.

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Length of gestation and birthweight in dizygotic twins

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Despite the longer gestation of girls, their birthweight is less than that of boys. Because unlike-sex twins provide a natural situation in which to investigate the influence of sex on gestation, we compared birthweight and gestation of 1929 same-sex and unlike-sex dizygotic pairs. Length of gestation in unlike-sex pairs was similar to that of female same-sex pairs, and significantly (0·4 weeks; p=0·02) longer than that of male same-sex pairs. Birthweight of girls from unlike-sex pairs was similar to that of girls from same-sex pairs, but boys from unlike-sex pairs weighed 78 g more than boys from same-sex pairs (p=0·001). These data show that in unlike-sex pairs it is the girl that prolongs gestation for her brother, resulting in a higher birthweight than that of same-sex boys.

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Despite the longer gestation of girls, their birthweight is less than that of boys. Anderson and Brown[1] noticed this phenomenon in 1943. In 1999 de Zegher and colleagues'[2] found that this gestation difference between boys and girls increases with decreasing birthweight—ie, to reach the same birthweight, gestation of boys is shorter than that of girls, and the more so the lower the birthweight. Unlike-sex twins provide a natural situation which allows the study of the influence of sex on gestation, since both a male and a female fetus share the womb. In our study, we compared birthweight and gestation of same-sex and unlike-sex dizygotic twins to examine which sex of the unlike-sex twins determines the length of gestation, and consequently the birthweight of the co-twin.

We studied data on 1929 dizygotic twin pairs of the East Flanders Prospective Twin Survey (EFPTS), which has prospectively registered all multiple births of the Belgian province of East Flanders since 1964. Birthweight and gestation, calculated as the number of completed weeks of pregnancy, were obtained from the obstetric records. Zygosity was established via sequential analysis based on sex, fetal membranes, blood groups, placental alkaline phosphatase, and DNA fingerprints (which allow a highly

	Same-sex	Unlike-sex	p
Boys			
n	936	1008	
Birthweight (g)	2515 (540·2)	2593 (527·8)	0·001
Birthweight standardised for gestational age (z score)	0·01 (0·99)	0·1 (0·98)	0·03
Girls			
n	906	1008	
Birthweight (g)	2469 (521·5)	2471 (508·4)	0·92
Birthweight standardised for gestational age (z score)	0·02 (0·99)	0·08 (0·97)	0·22

Mean (SD) birthweight of individual twins