Shared *Pseudomonas aeruginosa* genotypes are common in Australian cystic fibrosis centres

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ABSTRACT: Recent molecular-typing studies suggest cross-infection as one of the potential acquisition pathways for *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF). In Australia, there is only limited evidence of unrelated patients sharing indistinguishable *P. aeruginosa* strains. We therefore examined the point-prevalence, distribution, diversity and clinical impact of *P. aeruginosa* strains in Australian CF patients nationally.

983 patients attending 18 Australian CF centres provided 2887 sputum *P. aeruginosa* isolates for genotyping by enterobacterial repetitive intergenic consensus-PCR assays with confirmation by multilocus sequence typing. Demographic and clinical details were recorded for each participant.

Overall, 610 (62%) patients harboured at least one of 38 shared genotypes. Most shared strains were in small patient clusters from a limited number of centres. However, the two predominant genotypes, AUST-01 and AUST-02, were widely dispersed, being detected in 220 (22%) and 173 (18%) patients attending 17 and 16 centres, respectively. AUST-01 was associated with significantly greater treatment requirements than unique *P. aeruginosa* strains.

Multiple clusters of shared *P. aeruginosa* strains are common in Australian CF centres. At least one of the predominant and widespread genotypes is associated with increased healthcare utilisation. Longitudinal studies are now needed to determine the infection control implications of these findings.

KEYWORDS: Cross-infection, molecular typing, PCR

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Chronic *Pseudomonas aeruginosa* infection is a leading cause of clinical deterioration in cystic fibrosis (CF) and preventive strategies are needed to assist patient management worldwide [1, 2]. Although most CF patients are thought to acquire their own unique environmental strains, recent typing studies from centres in several countries report unrelated patients sharing indistinguishable genotypes, suggesting cross-infection may also be occurring [3–7]. Some shared genotypes are associated with an accelerated decline in lung function, increased healthcare requirements, and a greater risk of death or lung transplantation [3, 8, 9]. Consequently, identifying shared strains across broader populations has important implications for patient management.

CF centres practising routine molecular surveillance and patient segregation have reduced the incidence of shared *P. aeruginosa* strains, providing further indirect evidence of healthcare-related transmission [10, 11]. However, these measures are controversial because of cost and complexity [12]. This is particularly relevant for adult centres caring for an ageing population where increasing numbers of patients are not infected with *P. aeruginosa* [13]. Before developing stricter, potentially complex and expensive infection control policies, it is therefore important to improve our understanding of *P. aeruginosa* genotype prevalence, distribution and diversity, and to determine the clinical impact upon patient wellbeing.

To date, all Australian investigations have found evidence for CF patients sharing one or more *P. aeruginosa* strains [4, 5, 14, 15]. Some reports also identified an association between shared genotypes and poorer clinical outcomes [4, 5, 10, 14, 15].

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However, these studies represent <25% of the Australian CF population, involving only six centres, four cities and three predominant genotypes from the south-eastern corner of Australia. Interestingly, these investigations also identified several small patient clusters sharing other novel genotypes. We aimed therefore to examine the point-prevalence, distribution, diversity and clinical impact of *P. aeruginosa* strains in Australian CF patients.

MATERIALS AND METHODS

Patients, data and isolate collection

Participants attended one of 18 Australian CF centres (Sydney Children's Hospital Cystic Fibrosis Clinic, Sydney; Royal Prince Alfred Hospital Cystic Fibrosis Clinic, Sydney; The Children's Hospital at Westmead Cystic Fibrosis Treatment Centre, Sydney; John Hunter Adult Cystic Fibrosis Clinic, Newcastle; John Hunter Children's Hospital Cystic Fibrosis Unit, Newcastle; The Royal Children's Hospital Melbourne Cystic Fibrosis Unit, Melbourne; The Alfred Hospital Cystic Fibrosis Service, Melbourne; Monash Medical Centre Cystic Fibrosis Unit, Melbourne; Royal Children's Hospital Cystic Fibrosis Unit, Brisbane; The Prince Charles Hospital Adult Cystic Fibrosis Centre, Brisbane; Mater Children's Cystic Fibrosis Unit, Brisbane; Mater Adult Cystic Fibrosis Unit, Brisbane; Gold Coast Hospital Adult Cystic Fibrosis Centre, Southport; Gold Coast Hospital Children's Cystic Fibrosis Centre, Southport; The Royal Adelaide Hospital Cystic Fibrosis Program, Adelaide; Women's and Children's Hospital Cystic Fibrosis Clinic, Adelaide; Sir Charles Gairdner Hospital Cystic Fibrosis and Bronchiectasis Unit, Perth; Tasmanian Adult Cystic Fibrosis Unit, Hobart), including 2677 patients (1300 aged ⩾18 years; centre size 25–294 patients), representing 91%, 89% and 90% of the paediatric, adult and total CF population, respectively [16].

Patients provided single sputum specimens during clinic visits or hospitalisation between September 2007 and June 2010 (table S1). Clinical details including age, sex, number of *i.v.* antibiotic courses and clinic visits during the previous 12-months were recorded. For logistic reasons, the best recorded forced expiratory volume in 1 s (FEV_1) in the calendar year of sample collection and its paired forced vital capacity (FVC) and body mass index (BMI) from the same day was obtained for each patient from the Australian CF Data Registry (ACFDR) [16]. The best spirometry was selected to adjust for between-group differences in clinical status at study entry (clinic visit *versus* hospitalisation). Age-adjusted pulmonary function prediction equations were used [17, 18], and standard deviation z-scores were calculated using United States National Centre for Health Statistics and Centres for Disease Control and Preventionnormalised growth reference values for BMI.

Sputum specimens were cultured by hospital diagnostic laboratories using standard techniques outlined previously [19]. When *P. aeruginosa* was identified, three colonies representing different morphotypes from each specimen (where possible) were selected by scientists independent of the study, isolated separately and transported to the research laboratory for storage at -80°C until further testing [19].

Genotyping

Following DNA extraction, isolates were confirmed as *P. aeruginosa* by a duplex real-time PCR assay before performing DNA fingerprinting using the enterobacterial repetitive intergenic consensus (ERIC)-PCR typing technique [19, 20]. Cluster analysis and genotype allocation of ERIC-PCR fingerprints were performed using FPQuest™ software (version 4.5; Bio-Rad Laboratories Pty Ltd, Hercules, CA, USA) as described previously [20]. Multilocus sequence typing (MLST) was also performed on all shared ERIC-PCR genotypes and in a randomly selected subset of unique ERIC-PCR genotypes following *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/) protocols. Representative isolates of previously described highly prevalent Australian and international strains were included as controls in ERIC-PCR and MLST assays (table 1). Geospatial relationships between genotypes and postcode were assessed using ArcGIS 10 software (Esri Australia Pty Ltd, Brisbane, Australia).

Statistical analysis

Residuals for FEV_1, FVC and FEV_1/FVC were estimated by linear regression on height, height squared, age, age squared, sex, and interaction of sex with height, height squared, age, and age squared. These residuals were used as dependent variables when analysing predictors of lung function.

Effects of ERIC-PCR genotype on lung function were assessed after adjusting for cystic fibrosis transmembrane conductance regulator (CFTR) status by ANCOVA. Least-square mean values for lung function variables were compared between patients with unique strains and five patient groups of the most highly prevalent strains. The significance of pairwise comparisons was adjusted by Dunnett's *post hoc* correction. The association of ERIC-PCR genotype with treatments (mucolytics, azithromycin and inhaled antibiotics) and treatment burden (*i.v.* antibiotic treatment-days, number of *i.v.* antibiotic courses and clinic visits) were estimated by logistic and negative binomial regression after adjustment for CFTR status and centre, and expressed as odds and rate ratios respectively with unique strains as the reference category. Yates-corrected Chi-squared (2×2) analysis with adjustment for multiple comparisons was used to assess the association

TABLE 1	Representative *Pseudomonas aeruginosa* control strains used in genotyping assays	
Reference strain	**Alias(es)**	**[Ref.]**
AUST-01	Melbourne strain, Pulsotype 1, M16, AES-I	[4]
AUST-02	Brisbane strain, Pulsotype 2, AES-II	[5]
AUST-03	AES-III	[15]
AUST-04	Tasmanian minor strain	[15]
AUST-06	Pulsotype 42	[5]
AUST-07	Pulsotype 5	[5]
AUST-11	Pulsotype 58	[5]
AUST-13	Pulsotype 3	[5]
LES	Liverpool epidemic strain H190	[6]
MAN	Manchester strain C3425	[6]
Clone C	Clone C strain CF128-1	[21]
Midlands 1	Midlands 1 strain C4114	[6]

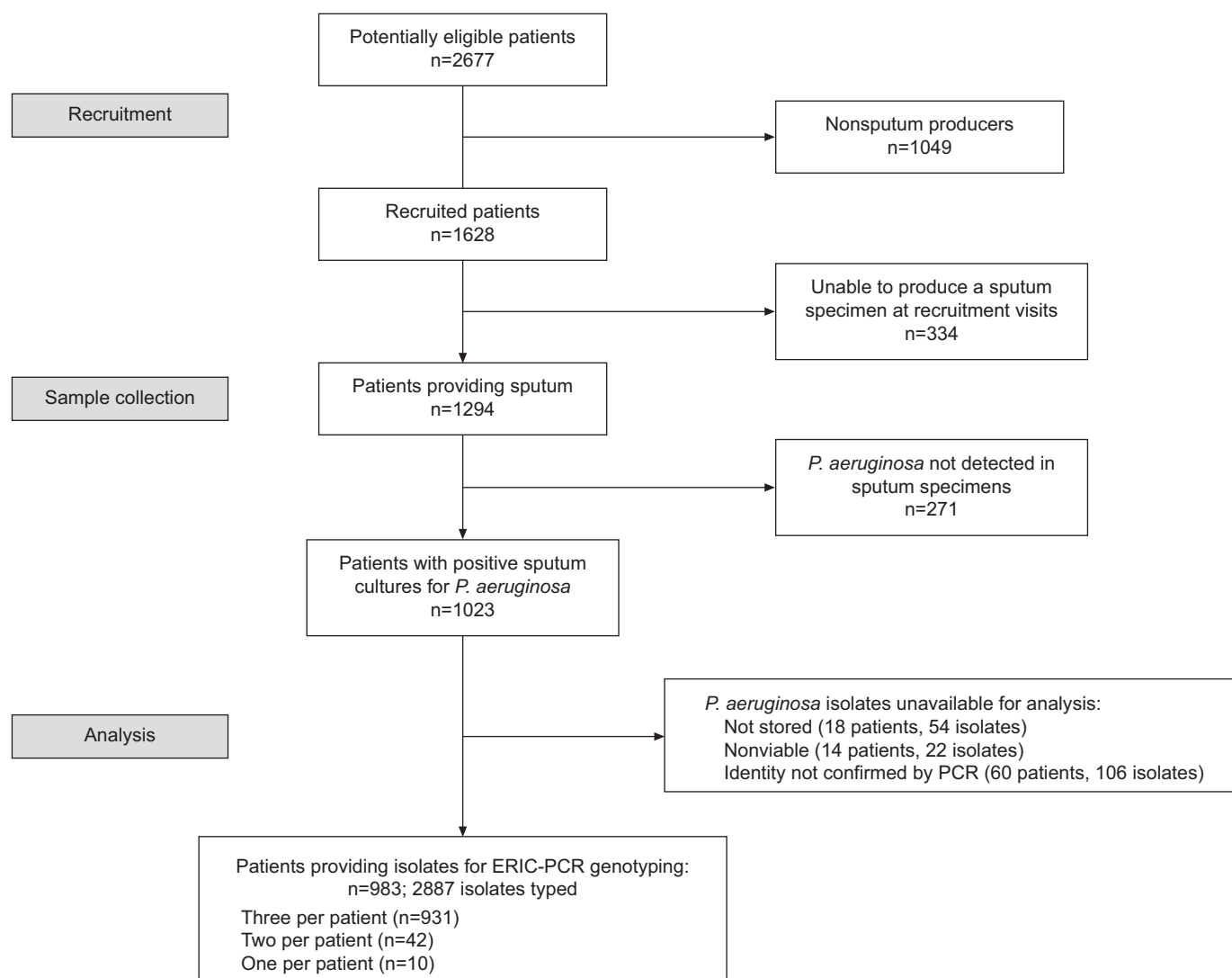AES: Australian Epidemic Strain.

FIGURE 1. Flowchart of patient recruitment, sample collection and isolates available for analysis. *P. aeruginosa*: *Pseudomonas aeruginosa*; ERIC: enterobacterial repetitive intergenic consensus.

between ERIC-PCR genotype and other microorganisms isolated from the study sputum specimens.

RESULTS
Patients
Of the 2677 patients attending 18 CF centres, 1294 (48%) provided a sputum sample for culture and *P. aeruginosa* was isolated from 1023 (79%) out of 1294 participants (fig. 1). One to three *P. aeruginosa* isolates were transported to the research laboratory where 2887 (96%) out of 2993 viable isolates from 983 patients were available for genotyping following confirmation as *P. aeruginosa* by real-time PCR.

Table 2 presents the characteristics of the 983 study participants and all *P. aeruginosa* positive patients listed in the ACFDR [16]. Participants were older, had worse BMI scores (children only) and spirometry than patients in the ACFDR. Accompanying co-pathogens isolated at the time of sample collection are listed in table S2.

The participant's median (interquartile range (IQR)) age at initial *P. aeruginosa* isolation and duration of infection was 8 (IQR 4–15) and 10 (IQR 6–15) years, respectively. They experienced a median of 14 (IQR 0–32) *i.v.* antibiotic treatment days, one (IQR 0–2) *i.v.* antibiotic course and five (IQR 3–8) clinic visits in the 12 months preceding sample collection. Overall, 807 (82%) out of 983 had a mucoid *P. aeruginosa* isolate cultured from their sputum, and 80% of samples were collected during clinic visits.

ERIC-PCR
All 2887 *P. aeruginosa* isolates were typeable by ERIC-PCR, yielding 531 distinct genotypes. Of the 931 patients who provided three isolates, 790 (85%), 130 (14%) and 11 (1%) had one, two and three genotypes, respectively.

Table 3 shows that 493 unique ERIC-PCR genotypes (or singleton strains) were identified, while a further 38 genotypes were shared by two or more (range 2–220) patients. Overall, 373 (38%) out of 983 patients had only unique strains and the

TABLE 2	Characteristics of patients with *Pseudomonas aeruginosa* recorded in the Australian Cystic Fibrosis Data Registry (ACFDR) and the study participants	
	P. aeruginosa-positive patients in ACFDR[#]	Current study participants[#]
Subjects n	1298	983
Age years	23.0±11.4	24.7±10.3
Adults aged ⩾18 years %	62	73
Males %	52	56
CFTR genotype %		
p.delF508 homozygotes	53	50
p.delF508 heterozygotes		33
Other/other		6
Unknown		11
BMI z-score (<18 years)[¶]	-0.05±0.86[+]	-0.35±0.9[+]
BMI (⩾18 years)[¶]	22.5±3.4 [+]	22.0±3.3[+]
FEV1 % pred	71.0±24.0[+,§]	64.4±22.3[+,§]
FVC % pred	84.6±20.2[+,ƒ]	80.1±19.7[+,ƒ]

Data are presented as mean±SD, unless otherwise stated. CFTR: cystic fibrosis transmembrane conductance regulator; BMI: body mass index; FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity. [#]: data presented in the ACFDR include the detection of *P. aeruginosa* at any time during the preceding calendar year, while for logistical reasons these data were recorded for participants in the current study only at the time of their sputum collection; [¶]: calculated using the United States National Centre for Health Statistics and Centres for Disease Control normalised growth reference values for BMI; [+]: ACFDR data represent the ''best'' recorded BMI, FEV1 % pred and FVC % pred values during the preceding calendar year, while for study participants the ''best'' FEV1 % pred value recorded in the calendar year of sample collection and its paired BMI and FVC % pred value recorded on the same day were used; [§]: values for the ACFDR and study participants in children aged <18 years were 87.8±19.6 and 81.0±20.3% pred, respectively, and adults aged ⩾18 years were 63.4±21.8 and 59.9±20.6% pred, respectively; [ƒ]: values for the ACFDR and study participants in children aged <18 years are 96.6±16.4 and 91.3±19.0% pred, and in adults aged ⩾18 years are 78.7±19.3 and 77.0±18.8% pred, respectively.

remaining 610 (62%) had at least one shared genotype, including 45 with multiple shared strains. Adults (476 (66%) out of 717) were significantly more likely than children (134 (50%) out of 266) to have shared *P. aeruginosa* genotypes (OR 1.95, 95% CI 1.45–2.62). Of those 461 (47%) patients in whom data about the age of first *P. aeruginosa* isolation were available, 23 had *P. aeruginosa* isolated within 12 months of the study sample collection date. 20 patients were each infected with unique ERIC-PCR genotypes, while the remaining three patients were each infected with AUST-01, AUST-04 and AUST-14, respectively.

Five shared ERIC-PCR genotypes were detected in clusters ranging from 31 to 220 patients, 14 involved clusters of 3–19 participants and a further 19 clusters involved two patients each (table 3). Overall, 220 (22%) patients from 17 centres had *P. aeruginosa* isolates indistinguishable from AUST-01, while 173 (18%) patients from 16 centres had isolates indistinguishable

from AUST-02. Other genotypes encountered frequently included AUST-04 (47 patients, 12 centres and six states), novel ERIC-PCR genotype AUST-05 (37 patients, six centres and four states) and AUST-06 (31 patients, four centres and three states) (tables 1 and 3). Smaller clusters involving <20 patients included previously characterised strains, such as AUST-03, AUST-07 and AUST-11, as well as several novel strains. A patient from the UK had the Liverpool epidemic strain (LES) in their sputum, but none of the other commonly shared genotypes from that region, including Midlands-1 or Manchester strains, were detected. Interestingly, three patients had Clone C, which is known to be distributed widely amongst CF patients and in natural environments throughout the northern hemisphere [21].

Sibling associations
Of 39 sibships, 31 (79%) shared at least one indistinguishable ERIC-PCR genotype. 21 sibling groups (20 pairs and one trio) shared AUST-01 (n=14), AUST-02 (n=5) and AUST-06 (n=1), and one sibling pair had both AUST-01 and AUST-05 genotypes. A further nine sibling pairs had genotypes detected in two-patient clusters only, and one sibling pair had a genotype that was observed in one other unrelated subject living interstate. Overall, sibships accounted for nine out of 19 genotypes shared by two patients.

Geographical distribution of genotypes
In contrast to the widely distributed unique ERIC-PCR genotypes, we observed various geographical relationships amongst highly-prevalent genotypes (fig. 2). Patients with AUST-01 were common in south-eastern Australia, whereas most AUST-02 isolates were observed in Queensland and Western Australia. Previously described Tasmanian strains (AUST-03 and AUST-04) were also detected frequently in patients in Victoria. AUST-06 was found almost exclusively in Queensland and most AUST-05 and AUST-08 isolates were from South Australia.

Correlation of clinical data with genotypes
Participants harbouring the five most common cluster groups (AUST-01, -02, -04, -05 and -06) were of similar age, sex and nutritional status to those with unique *P. aeruginosa* strains (table 4). Spirometry was also similar among these groups when compared with those with unique strains. However, patients with AUST-01 were significantly more likely to have used azithromycin and inhaled antibiotics in the previous 4 weeks, and to have had, in the last year, a greater number and duration of i.v. antibiotic courses and number of clinic visits, compared with those with unique strains (tables 4 and 5). In patients with AUST-02 and AUST-06 there was evidence of an increased number of i.v. antibiotic courses and clinic visits, but not of maintenance antibiotic treatment compared with unique *P. aeruginosa* genotypes. Differences in treatment burden were not seen with the other two most commonly shared strain clusters (AUST-04 and -05).

MLST
MLST was conducted on 139 isolates (138 patients) representing all shared ERIC-PCR genotypes and 70 isolates (69 patients) with unique ERIC-PCR genotypes. Analysis of the 209 isolates yielded 108 distinct sequence types (STs), which were highly concordant (85%) with the ERIC-PCR fingerprinting results

TABLE 3 Number of patients harbouring unique and shared enterobacterial repetitive intergenic consensus (ERIC)-PCR genotypes in each participating cystic fibrosis (CF) centre[#]

ERIC-PCR genotype	Patients[¶]	New South Wales					Victoria			Queensland						South Australia		Western Australia	Tasmania
		01	02	03	04	05	01	02	03	01	02	03	04	05	06	01	02	01	01
Total patients		18	113	61	20	14	64	124	50	57	171	20	38	15	5	72	24	92	25
Unique only[+]	373 (38)	10	54	40	5	8	45	41	16	19	47	6	4	2	1	25	4	37	9
AUST-01	220 (22)	2	35	5	8	2	11	58	16	1	21	8	17	5		17	3	7	4
AUST-02	173 (18)	1	9	1	2	2	1		1	20	69	1	11	9	4	5		36	1
AUST-04	47 (5)		1	5	1		1	16	14	1	2		1			1		3	1
AUST-05	37 (4)										1		1			18	15	1	1
AUST-06	31 (3)		1							12	17								1
AUST-07	19 (2)		2	1		1				3	10	2							
AUST-08	17 (2)	2	1										1			10		2	1
AUST-03	16 (2)				5			8	2										
AUST-09	12 (1)	1	4	2														1	5
AUST-10	8					1		3		2	2								
AUST-11	8							1	2		3								
AUST-12	5		3	1												1			
AUST-13	5						3				4		1						
AUST-14	4	1																2	
AUST-15[§]	3							3											
AUST-16	3		1										2						
AUST-17	3		1						1			1	1						
AUST-18	3							1	1	1		1							
AUST-19	3	1					1	1											
AUST-20 to AUST-38[ƒ]	38	2	3	6			2	1		2	8	3	1				2	6	2

Data are presented as n (%) or n. [#]: the four nonparticipating Australian cystic fibrosis centres included two paediatric, one adult and one combined paediatric and adult centre; [¶]: as 153 out of 983 patients had two or more ERIC-PCR genotypes in their sputum, the column totals may exceed the numbers of patients providing specimens; [+]: all ERIC-PCR genotypes in individual patients; [§]: ERIC-PCR genotype AUST-15 was indistinguishable from the Clone C strain; [ƒ]: ERIC-PCR genotypes AUST-20 to AUST-38 represent 19 different shared genotypes that were each detected in clusters of two patients.
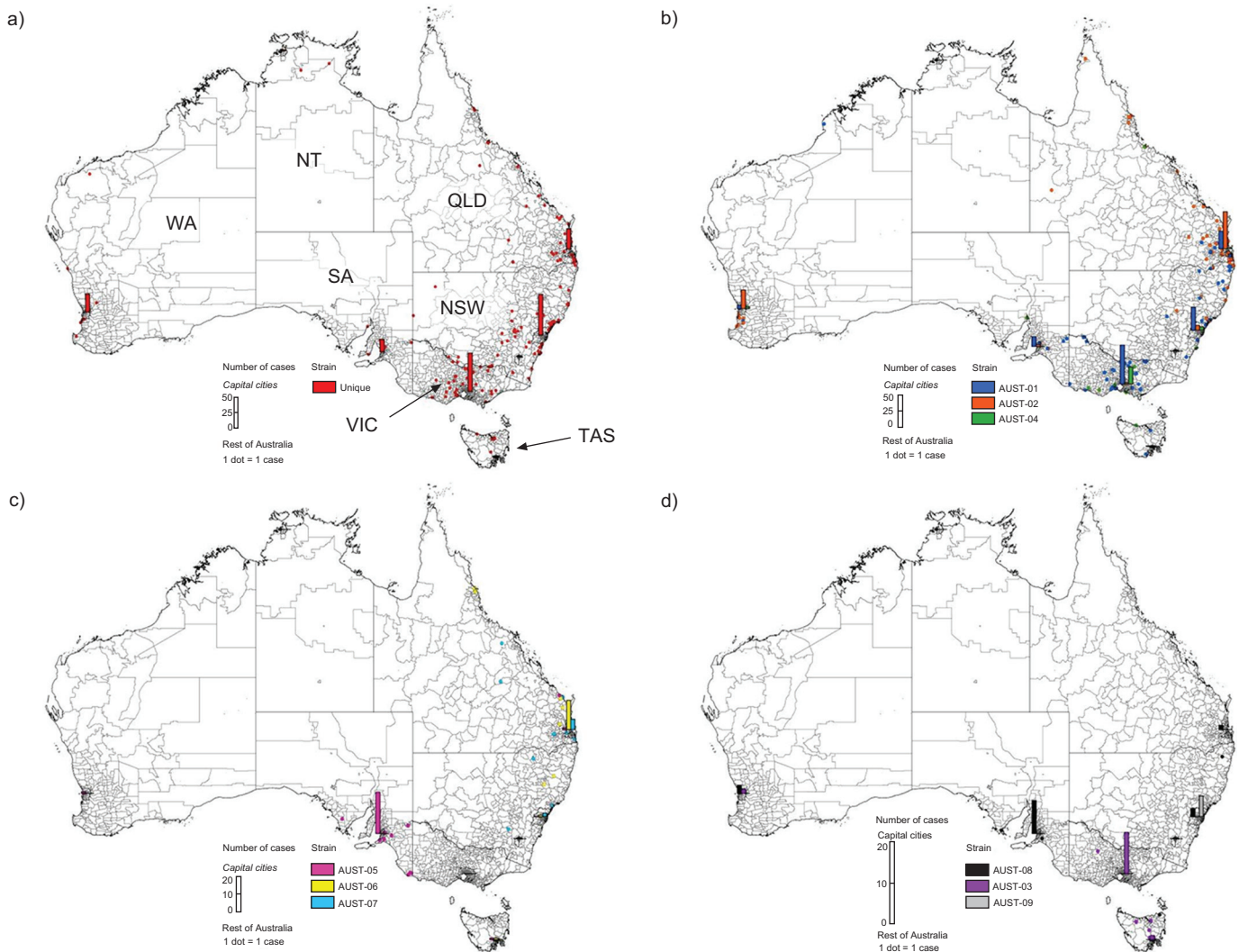
FIGURE 2. Geospatial relationships between enterobacterial repetitive intergenic consensus (ERIC)-PCR genotype and residential postcode for patients with: a) unique; b) AUST-01, -02 and -04; c) AUST-05, -06 and -07; and d) AUST-08, -03 and -09 strains. NSW: New South Wales; NT: Northern Territory; QLD: Queensland; SA: South Australia; TAS: Tasmania; VIC: Victoria; WA: Western Australia.

(tables S3 and S4). Discrepancies were observed between ERIC-PCR and MLST on nine occasions, where MLST indicated single- or double-locus variant STs for isolates categorised by ERIC-PCR as either AUST-05, -08, -09, -11, -19, -34 or -38 genotypes. There were also eight instances involving STs 12, 27, 155, 179, 274, 275, 822 and 905 where MLST identified indistinguishable strains, but where ERIC-PCR found several different genotypes. A further six isolates produced STs that were inconsistent with those assigned by the MLST website to AUST-02 (n=1), AUST-11 (n=4) and AUST-13 (n=1).

DISCUSSION

>60% of Australian CF patients with *P. aeruginosa* cultured from their sputum had strains indistinguishable from those of at least one other CF patient. Although multiple shared strains were detected, they were mainly in small clusters and from a limited number of centres and regions. In contrast, AUST-01 and -02 were highly prevalent and found in patients from geographically dispersed regions. One or both strains were present in all 18 CF centres surveyed, affecting >40% of study participants. Moreover, those harbouring AUST-01 in particular had greater healthcare utilisation than those with unique strains. These findings reinforce concerns that some *P. aeruginosa* genotypes are causing widespread cross-infection within and between CF centres across continental Australia.

Finding commonly shared strains across the Australian CF community is consistent with results from previous smaller studies. Patients attending CF centres in Victoria and Tasmania have high prevalence rates of AUST-01 and -03 [4, 15], whereas those in New South Wales and Queensland have clusters of AUST-01, -02 and several other less prevalent genotypes [5, 14]. Other *P. aeruginosa* strains are also widespread amongst patients attending CF centres in the northern hemisphere. In a survey of 849 patient isolates from 31 English and Welsh CF centres, the LES was detected in 11% of patients and 48% of centres [6]. Recently, the LES was also found in 15% of patients attending adult CF centres in Ontario, Canada [3]. Moreover, a study involving two large Dutch CF centres reported 70% of

TABLE 4	Comparison of clinical and microbiological data with enterobacterial repetitive intergenic consensus-PCR genotype					
	Unique	AUST-01	AUST-02	AUST-04	AUST-05	AUST-06
Patients n	373	220	173	47	37	31
Age years	24.6±12.4	24.8±7.3	25.3±8.8	28.1±10.6	22.5±5.8	21.4±8.7
BMI z-score <18 years[#]	-0.6±0.9	-0.2±0.9	-0.1±0.8*	-0.6±0.7	-1.2±0	-0.2±0.8
BMI ≥18 years[#]	22.5±3.4	21.6±2.7	22.0±4.0	22.6±3.1	21.9±2.8	22.7±2.9
Best FEV1 % pred	64.9±22.2	61.8±20.7	64.9±23.2	65.0±23.1	73.7±19.9	71.1±24.9
FVC % pred[#]	80.7±20.1	80.3±17.7	78.6±20.3	82.4±19.6	87.9±18.0	86.8±21.2
i.v. antibiotic courses	1 (0–2)	1 (1–3)*	1 (0–3)*	1 (0–2)	2 (0–3)	2 (1–3)*
i.v. antibiotic days	11 (0–23)	16 (3–40)*	14 (0–36)	9 (0–24)	30 (0–55)	30 (2–48)
Clinic visits	5 (2–7)	5 (3–9)*	7 (4–10)*	6 (3–9)	5 (2–6)	9 (3–11)*
Oral azithromycin[¶]	53.6	73.7*	58.5	65.9	64.7	72.7
Inhaled antibiotics[¶]	44.2	54.5*	40.9	50.0	50.0	27.3
Mucolytics[¶,+]	60.1	69.4	57.9	72.7	79.4	63.6

Data are presented as mean±SD, median (interquartile range) or %, unless otherwise stated. BMI: body mass index; FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity. [#]: measured on the same day as the best FEV1 % pred was recorded for the calendar year; [¶]: taken in the 4 weeks prior to sputum specimen collection; [+]: recombinant human DNase and/or hypertonic saline. *: $p < 0.05$ for comparison with unique strains.

patients harbouring *P. aeruginosa* shared genotypes with at least one other patient from the same centre [7]. However, not all studies have identified evidence of highly prevalent genotypes. Reports from Belgium, British Columbia (Canada) and New Zealand have only identified small clusters of genetically similar isolates in epidemiologically unrelated patients [22–24].

Common genotypes in CF centres and geographical regions indicate either healthcare-associated transmission or exposure to dominant environmental genotypes, as illustrated by Clone C [21]. Interestingly, we also detected Clone C in three patients. However, with large distances between participating CF centres, it seems unlikely that common source exposure can fully account for the widespread dispersal of the highly prevalent strains, AUST-01 and -02, which so far have not been found in environmental surveys [4, 25]. Instead, several studies raise the possibility of person-to-person transmission. During

coughing, patients with CF can produce respirable aerosols containing viable bacteria [26]. Shared *P. aeruginosa* strains have been found in air samples during spirometry, nebulisation and airway clearance in close proximity to infected patients [27]. Finally, some CF centres have reduced the incidence of shared strains by implementing strict patient segregation [10, 11]. Together, these observations suggest that major strain clusters, such as AUST-01 and -02, are most likely to be acquired by cross-infection.

A recent longitudinal Canadian study compared clinical outcomes of patients with LES to those harbouring unique strains [3]. No differences existed in nutritional status, pulmonary function decline, or rates of pulmonary exacerbation or hospitalisation. However, over 3 years, patients with LES had an associated increased risk of death or lung transplantation. In contrast, a study comparing patients with shared and unique strains at the Manchester Adult CF centre

TABLE 5	Mean changes in pulmonary function, and treatment burden rate and odds ratio estimates for patients with cystic fibrosis harbouring *Pseudomonas aeruginosa* AUST-01, -02 and -06 genotypes compared with those possessing unique strains					
	AUST-01	p-value	AUST-02	p-value	AUST-06	p-value
Best FEV1 % pred[#]	-2.1 (-8.1–3.9)	0.94	-0.9 (-6.9–5.1)	1.00	8.8 (-4.6–22.2)	0.42
FVC % pred[#]	0.6 (-4.9–6.2)	1.00	-2.9 (-8.3–2.6)	0.68	8.4 (-3.4–20.2)	0.32
i.v. antibiotic courses[¶,+]	1.4 (1.2–1.7)	<0.001	1.3 (1.0–1.6)	0.02	1.6 (1.1–2.5)	0.03
i.v. antibiotic days[¶,+]	1.5 (1.1–2.0)	0.01	1.4 (1.0–2.0)	0.09	1.6 (0.7–3.6)	0.22
Clinic visits[¶,+]	1.2 (1.0–1.3)	0.02	1.5 (1.3–1.7)	<0.001	1.5 (1.1–2.0)	0.005
Oral azithromycin[¶,§]	1.8 (1.2–2.7)	<0.001	1.2 (0.8–1.9)	0.42	2.4 (0.9–6.7)	0.09
Inhaled antibiotics[¶,§]	1.5 (1.0–2.3)	0.04	1.5 (0.9–2.3)	0.11	0.9 (0.3–2.6)	0.83
Mucolytics[¶,§,ƒ]	1.5 (1.0–2.2)	0.05	1.2 (0.7–1.8)	0.54	1.1 (0.4–2.9)	0.91

Data are presented as mean (95% CI), unless otherwise stated. FEV1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity. [#]: adjusted for cystic fibrosis transmembrane conductance regulator (CFTR) genotype; [¶]: adjusted for CFTR genotype and centre; [+]: rate ratios (95% CI); [§]: odds ratios (95% CI); [ƒ]: recombinant human DNase and/or hypertonic saline.

showed no differences in survival after 8 years, but those with shared strains exhibited increased treatment requirements [9]. Nevertheless, this latter finding could have been influenced by knowledge of strain genotype after routine molecular surveillance was introduced leading to altered treatment practices.

Patients with AUST-01 had more clinic visits and a greater treatment burden than those with unique genotypes. Only one centre performed regular molecular fingerprinting of *P. aeruginosa* isolates. With the exception of inhaled antibiotic use, repeating the analysis without this centre's data did not change these findings, suggesting that AUST-01 may be more pathogenic and not simply associated with increased treatment following recognition of strain type. Increased *i.v.* antibiotic courses and clinic visits were also observed in patients with AUST-02 and -06. Nevertheless, similar spirometry was seen between each of the major genotypic groups and patients with unique strains [4]. Our study poses important questions on infection control in Australian CF centres. However, other than for AUST-01, until longitudinally collected data clearly demonstrate adverse effects associated with specific shared *P. aeruginosa* strains, caution should be exercised. This is especially relevant in CF centres where multiple strains are shared, and where implementing complex and resource intensive cohort-segregation policies risk adverse psychological consequences for patients and their families without possible benefits [28]. Meanwhile, within Australian CF centres, it remains important to reinforce the current standard infection control measures, which include segregating CF patients with *P. aeruginosa* from all other CF patients [29].

This study has several limitations. We were unable to determine whether participants had intermittent or chronic *P. aeruginosa* infection. However, as most were adults with mucoid *P. aeruginosa*, it is likely that most were chronically infected. Similarly, we relied upon sputum specimens so that older patients with more severe disease were disproportionately recruited. Thus we undertook genotypic analyses of 205 *P. aeruginosa* isolates from upper airway and bronchoalveolar lavage samples from 81 children in an Australian pre-school CF patient cohort [30]. AUST-01 and -02 strains were detected in only one patient each, whereas common environmental strains (ST-27, ST-155 and ST-179) were identified frequently. Thus we are unlikely to have grossly underestimated the numbers of patients possessing one of the major genotypic clusters and the study should be broadly representative of CF patients with established *P. aeruginosa* infection. Importantly, only one patient from our study was identified to have been infected with AUST-01 as their first *P. aeruginosa* isolate, although this information was available in <50% of study participants.

The cross-sectional study design limited our ability to identify risk-factors for acquiring shared *P. aeruginosa* strains (including whether high healthcare utilisation is itself a risk-factor), and to fully assess the clinical impact of specific *P. aeruginosa* genotypes. Consequently, the patients now form a longitudinal cohort to determine acquisition risk factors and long-term effects of highly-prevalent *P. aeruginosa* strains in incident cases. Unfortunately, information on centre transfers and individual hospital and social interactions was outside the scope of this study.

Another possible limitation was employing ERIC-PCR as our primary genotyping tool, as pulsed-field gel electrophoresis and MLST were impractical for analysing almost 3000 isolates. Consistent with our earlier observations, the concordance and level of discrimination offered by ERIC-PCR compared to MLST was relatively high, and thus we are confident in our overall strain identification [20]. However, relatedness was occasionally overestimated by ERIC-PCR and reaffirms our previous recommendation of confirming relationships within and between ERIC-PCR types by categorical typing techniques [20]. We also found additional evidence of multiple ERIC-PCR genotypes bearing the same ST, which often involved small clusters within individual CF centres and sibling groups [20]. For example, isolates from 13 patients showing one unique and four shared ERIC-PCR genotypes were all assigned by MLST to ST-179. Likewise, ST-155 and ST-274 involved multiple ERIC-PCR genotypes. Interestingly, STs 155, 179 and 274 are distributed widely in European and North American CF patients and are frequently encountered in other ecological settings including the natural environment [25]. In contrast, predominant shared strains AUST-01 and -02 showed limited genotypic variation, were often found in large clusters in individual CF centres and, so far, have not been detected in other ecological niches, such as the natural environment and non-human animals [25].

Finally, we selected three colonies representing different morphotypes from each specimen for genotypic analysis. Different genotypes may have been underestimated using this strategy. Nevertheless, we are confident that adequate diversity has been captured, as 85% of patients with three isolates analysed showed the only one ERIC-PCR genotype and only 1% had three different genotypes identified. Similarly, *Sal*I fingerprint analysis of five isolates per patients in a recent New Zealand study revealed limited within patient diversity [23].

To our knowledge, this is the largest study yet to examine genotypic relationships between CF *P. aeruginosa* isolates and the most comprehensive epidemiological survey of *P. aeruginosa* infections in CF centres covering a large geographical land mass. Participants comprised ~75% of Australian CF patients recorded as being infected with *P. aeruginosa* on the national registry and included almost all CF centres for an entire continent. Shared *P. aeruginosa* strains are very common in Australian CF patients and, while many genotypes may have originated initially from the environment, cross-infection might also explain the most widely dispersed shared genotypes. The transmission pathways and clinical significance of these major strains in Australian CF patients remain unknown and urgently need identifying.

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