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SHARED *PSEUDOMONAS AERUGINOSA* GENOTYPES ARE COMMON IN AUSTRALIAN CYSTIC FIBROSIS CENTRES.

Timothy J. Kidd[1], Kay A. Ramsay[1], Honghua Hu[2,3], Guy B. Marks[4], Claire E. Wainwright[1,5], Peter T. Bye[6], Mark R. Elkins[6], Philip J. Robinson[7], Barbara R. Rose[2], John W Wilson[8], Keith Grimwood[1], Scott C. Bell[1,9], and the ACPinCF Investigator Group.

Author affiliations:

[1] Queensland Paediatric Infectious Diseases Laboratory, Queensland Children's Medical Research Institute, Royal Children's Hospital, The University of Queensland, Brisbane, Australia.

[2] Department of Infectious Diseases and Immunology, The University of Sydney, Sydney, Australia.

[3] Australian School of Advanced Medicine, Macquarie University, Sydney, Australia.

[4] The Woolcock Institute of Medical Research, Sydney, Australia.

[5] Queensland Children's Respiratory Centre, Royal Children's Hospital, Brisbane, Australia.

[6] Department of Respiratory Medicine, Royal Prince Alfred Hospital, Sydney, Australia.

[7] Department of Respiratory Medicine, Royal Children's Hospital, Melbourne, Australia.

[8] Department of Allergy, Immunology & Respiratory Medicine, The Alfred Hospital, Melbourne, Australia.

[9] Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, Australia.

Corresponding author:

Timothy J. Kidd

Qpid Laboratory, Queensland Children's Medical Research Institute

Level 7, Block C28, Royal Children's Hospital

Back Road, off Bramston Tce,

Herston, QLD, Australia 4029

Phone: 61 7 3636 1400

Fax: 61 7 3636 1401

Email: Tim_Kidd@health.qld.gov.au

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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.

Nine-hundred and eighty-three patients attending 18 Australian CF centres provided 2,887 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.

INTRODUCTION

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]. However, these studies represent

fewer than 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, including 2,677 patients (1,300 aged ≥18-years; centre size: 25-294), 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 (online Table S1). Clinical details including age, sex, number of intravenous antibiotic courses and clinic visits during the previous 12-months were recorded. For logistic reasons, the best recorded forced expiratory volume in one-second (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 normalised 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^{0}C until further testing [19].

Genotyping

Following DNA extraction, isolates were confirmed as *P. aeruginosa* by a duplex real-time polymerase-chain reaction (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) 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).

Statistical analysis

Residuals for FEV_1, FVC and FEV_1/FVC were estimated by linear regression on height, height^2, age, age^2, sex, and interaction of sex with height, height^2, age, and age^2. 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 analysis of covariance. 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/inhaled antibiotics) and treatment burden (intravenous antibiotic treatment-days; number of intravenous 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-square (2 x 2) analysis with adjustment for multiple comparisons was used to assess the association between ERIC-PCR genotype and other microorganisms isolated from the study sputum specimens.

RESULTS

Patients

Of the 2,677 patients attending 18 CF centres, 1,294 (48%) provided a sputum sample for culture and *P. aeruginosa* was isolated from 1,023/1,294 (79%) participants (Figure 1). One-to-three *P. aeruginosa* isolates were transported to the research laboratory where 2,887/2,993 (96%) 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 the online Table S2.

The participant's median age at initial *P. aeruginosa* isolation and duration of infection was 8 (interquartile range [IQR]: 4, 15) and 10 (IQR: 6, 15) years respectively. They experienced a median of 14 (IQR: 0, 32) intravenous antibiotic treatment-days, 1 (IQR: 0, 2) intravenous antibiotic course, and 5 (IQR: 3, 8) clinic visits in the 12-months preceding sample collection. Overall, 807/983 (82%) had a mucoid *P. aeruginosa* isolate cultured from their sputum, and 80% of samples were collected during clinic visits.

ERIC-PCR

All 2,887 *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 1, 2, and 3 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, 373/983 (38%) patients had only unique strains and the remaining 610 (62%) had at least one shared genotype, including 45 with multiple shared strains. Adults (476/717; 66%) were significantly more likely than children (134/266; 50%) to have shared *P. aeruginosa* genotypes (odds ratio 1.95, 95% confidence interval 1.45, 2.62). Of those 461 patients (47%) where data about the age of first *P. aeruginosa* isolation was available, 23 had *P .aeruginosa* isolated within 12 months of the study sample collection date. Twenty patients were each infected with unique ERIC-PCR

genotypes, while the remaining 3 patients were each infected with AUST-01, AUST-04, and AUST-14, respectively.

Five shared ERIC-PCR genotypes were detected in clusters ranging from 31-220 patients, 14 involved clusters of 3-19 participants, and a further 19 clusters involved 2 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, 6 states), novel ERIC-PCR genotype AUST-05 (37 patients, 6 centres, 4 states), and AUST-06 (31 patients, 4 centres, 3 states) (Tables 1 and 3). Smaller clusters involving fewer than 20 patients included previously characterised strains such as AUST-03, AUST-07and AUST-11, as well as several novel strains. A patient from the United Kingdom 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. Twenty-one sibling groups (20 pairs, 1 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 9/19 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 (Figure 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 Victorian patients. 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, gender and nutritional status to those with unique *P. aeruginosa* strains (Table 4). Spirometry was also similar amongst these groups when compared to 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 in the last year to have had a greater number and duration of intravenous 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 intravenous 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, AUST-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 (online 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

More than 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 AUST-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 more than 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 AUST-03 [4, 15], whereas those in New South Wales and Queensland have clusters of AUST-01, AUST-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 patients harbouring *P. aeruginosa* shared genotypes with at least one other centre patient [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 AUST-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 AUST-02, are most likely 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 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 on *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 intravenous antibiotic courses and clinic visits were also observed in patients with AUST-02 and

AUST-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 if 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 AUST-02 strains were detected in only one patient each, whereas common environmental strains (ST-27, ST-155, ST-179) were identified frequently. Thus we are unlikely to have grossly under-estimated 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 was identified in this study to have been infected with AUST-01 as their first *P. aeruginosa* isolate, though this information was available only in less than 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 3,000 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, occasionally relatedness was 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 AUST-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 animals [25].

Finally, we selected 3 colonies representing different morphotypes from each specimen for genotypic analysis. Different genotypes may have been under-estimated using this strategy. Nevertheless, we are confident that adequate diversity has been captured as 85% of patients with 3 isolates analysed showed the only one ERIC-PCR genotype and only 1% had 3 different genotypes identified. Similarly, *Sal*I fingerprint analysis of 5 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 approximately 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 need identifying urgently.

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ACPinCF INVESTIGATOR GROUP

Scott C Bell* (Queensland Children's Medical Research Institute, The Prince Charles Hospital, Brisbane), Timothy J Kidd* (Queensland Children's Medical Research Institute, Brisbane), Keith Grimwood* (Queensland Children's Medical Research Institute, Brisbane), David S Armstrong (Monash Medical Centre, Melbourne), Peter T Bye* (Royal Prince Alfred Hospital, Sydney), Peter J Cooper (The Children's Hospital at Westmead, Sydney), Carolyn J. Dakin (Mater Children's Hospital, Brisbane), Mark R Elkins* (Royal Prince Alfred Hospital, Sydney), Iain H Feather (Gold Coast Hospital, Southport), Hugh Greville (Royal Adelaide Hospital, Adelaide), Colin Harbour (University of Sydney, Sydney), Honghua Hu* (The University of Sydney, Macquarie University, Sydney), Adam Jaffé (Sydney Children's Hospital, Sydney), A James Martin (Women's and Children's Hospital, Adelaide), Karen O McKay (The Children's Hospital at Westmead, Sydney), Guy B Marks* (The Woolcock Institute of Medical Research), Judith M Morton (Monash Medical Centre, Melbourne), Michael D Nissen (Pathology Queensland and Queensland Children's Medical Research Institute, Brisbane), Darrell Price (Gold Coast Hospital, Southport), Kay Ramsay* (Queensland Children's Medical Research Institute, Brisbane), David W Reid (Royal Hobart Hospital, Hobart and The Prince Charles Hospital, Brisbane), Philip J Robinson* (Royal Children's Hospital, Melbourne), Barbara R

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AUTHOR CONTRIBUTIONS

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STATEMENT OF INTERESTS None declared

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Table 1 Representative *P. aeruginosa* control strains used in genotyping assays

Reference strain	Alias(es)	Reference
AUST-01	Melbourne strain, Pulsotype 1, M16, Australian Epidemic Strain (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]

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[†] (n=1,298)	Current study participants[†] (n=983)
Mean age (SD), years	23.0 (11.4)	24.7 (10.3)
Adults aged ≥18-yrs, %	62	73
Sex (male), %	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)[‡]
Mean (SD) FEV_1% predicted [17, 18]	71.0 (24.0)[‡ ‖]	64.4 (22.3)[‡ ‖]
Mean (SD) FVC % predicted [17, 18]	84.6 (20.2)[‡§]	80.1 (19.7)[‡§]

Definition of abbreviations: ACFDR, Australian Cystic Fibrosis Data Registry of all paediatric and adults CF patients [16]; BMI, body-mass index; CFTR, cystic fibrosis transmembrane conductance regulator; FEV_1 forced expiratory volume in one second; FVC, forced vital capacity; SD, standard deviation.

* Calculated using the United States National Centre for Health Statistics and Centres for Disease Control normalised growth reference values for BMI.

[†] Data presented in the ACFDR includes 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.

‡ ACFDR data represent the "best" recorded BMI, FEV_1% and FVC% predicted values during the preceding calendar year, while for study participants the "best" FEV_1% predicted value recorded in the calendar year of sample collection and its paired BMI and FVC% predicted value recorded on the same day were used.

‖ Mean (SD) FEV_1% predicted values for the ACFDR and study participants in children aged <18-years were 87.8 (19.6) and 81.0 (20.3), and adults aged ≥18-years were 63.4 (21.8) and 59.9 (20.6), respectively.

§ Mean (SD) FVC% predicted in children aged <18-years is 96.6 (16.4) and 91.3 (19.0), adults aged ≥18-years is 78.7 (19.3), 77.0 (18.8), respectively.

Table 3 Number of patients harbouring unique and shared enterobacterial repetitive intergenic consensus (ERIC)-PCR genotypes in each participating CF centre*

ERIC-PCR genotype	No. of patients (%)†	State/CF centre code (number of patients analysed)																	
		NSW/01	NSW02	NSW/03	NSW/04	NSW/05	VIC/01	VIC/02	VIC/03	QLD/01	QLD/02	QLD/03	QLD/04	QLD/05	QLD/06	SA/01	SA/02	WA/01	TAS/01
		(18)	(113)	(61)	(20)	(14)	(64)	(124)	(50)	(57)	(171)	(20)	(38)	(15)	(5)	(72)	(24)	(92)	(25)
Unique(s) 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)	-	-	-	-	-	-	8	2	-	-	-	-	-	-	-	-	1	5
AUST-09	12 (1)	1	4	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AUST-10	8	-	-	-	-	1	-	3	-	2	2	-	-	-	-	-	-	-	-
AUST-11	8	-	-	-	-	-	-	1	2	-	3	-	-	-	-	-	-	2	-
AUST-12	5	-	3	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
AUST-13	5	-	-	-	-	-	-	-	-	-	4	-	1	-	-	-	-	-	-
AUST-14	4	1	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
AUST-15§	3	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
AUST-16	3	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AUST-17	3	-	1	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
AUST-18	3	-	-	-	-	-	-	-	1	-	-	1	1	-	-	-	-	-	-
AUST-19	3	-	-	-	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-
AUST-20 to AUST-38‖	38	2	3	6	-	-	2	1	-	2	8	3	1	-	-	-	2	6	2

* The four non-participating Australian cystic fibrosis centres included two paediatric, one adult and one combined paediatric and adult centre. † As 153/983 patients had ≥2 ERIC-PCR genotypes in their sputum, the column totals may exceed the numbers of patients providing specimens. ‡ Refers to all ERIC-PCR genotypes in individual patients. § ERIC-PCR genotype AUST-15 was indistinguishable from the Clone C strain. ‖ ERIC-PCR genotypes AUST-20 to AUST-38 represent 19 different shared genotypes that were each detected in clusters of two patients.

Table 4 Comparison of clinical and microbiological data with enterobacterial repetitive intergenic consensus (ERIC)-PCR genotype.

	Unique	AUST-01*	AUST-02*	AUST-04	AUST-05	AUST-06*
Number of patients	373	220	173	47	37	31
Mean (SD) age	24.6 (12.4)	24.8 (7.3)	25.3 (8.8)	28.1 (10.6)	22.5 (5.8)	21.4 (8.7)
Mean (SD) BMI *z*-score (<18-yrs)[†]	-0.6 (0.9)	-0.2 (0.9)	**-0.1 (0.8)**	-0.6 (0.7)	-1.2 (0)	-0.2 (0.8)
Mean (SD) BMI (≥18-yrs)[†]	22.5 (3.4)	21.6 (2.7)	22.0 (4.0)	22.6 (3.1)	21.9 (2.8)	22.7 (2.9)
Mean (SD) Best FEV_1% predicted	64.9 (22.2)	61.8 (20.7)	64.9 (23.2)	65.0 (23.1)	73.7 (19.9)	71.1 (24.9)
Mean (SD) FVC% predicted[†]	80.7 (20.1)	80.3 (17.7)	78.6 (20.3)	82.4 (19.6)	87.9 (18.0)	86.8 (21.2)
Median (IQR) IV antibiotic (courses)	1 (0, 2)	**1 (1, 3)**	**1 (0, 3)**	1 (0, 2)	2 (0, 3)	**2 (1, 3)**
Median (IQR) IV antibiotic (days)	11 (0, 23)	**16 (3, 40)**	14 (0, 36)	9 (0, 24)	30 (0, 55)	30 (2, 48)
Median (IQR) 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

Definition of abbreviations: BMI, body-mass index; FEV_1 forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range; IV, intravenous; SD, standard deviation.

***** Bold type indicates *P*-values <0.05 for comparison with unique strains.

[†] Measured on the same day as the best FEV_1% predicted was recorded for the calendar year.

[‡] Taken in the 4-weeks prior to sputum specimen collection.

[§] Recombinant human DNase and/or hypertonic saline.

Table 5 Mean changes in pulmonary function, and treatment burden rate and odds ratio estimates, with accompanying 95th percentile confidence intervals (CI) for patients with cystic fibrosis harbouring *Pseudomonas aeruginosa* AUST-01, AUST-02 and AUST-06 genotypes compared with those possessing unique strains.

	AUST-01	AUST-02	AUST-06
Best FEV1% predicted*	-2.1 (-8.1, 3.9); p = 0.94	-0.9 (-6.9, 5.1); p = 1.00	8.8 (-4.6, 22.2); p = 0.42
FVC % predicted*	0.6 (-4.9, 6.2); p = 1.00	-2.9 (-8.3, 2.6); p = 0.68	8.4 (-3.4, 20.2); p = 0.32
IV antibiotic (courses) †‡	1.4 (1.2, 1.7); p < 0.001	1.3 (1.0, 1.6); p = 0.02	1.6 (1.1, 2.5); p = 0.03
IV antibiotic (days) †‡	1.5 (1.1, 2.0); p = 0.01	1.4 (1.0, 2.0); p = 0.09	1.6 (0.7, 3.6); p = 0.22
Clinic visits †‡	1.2 (1.0, 1.3); p = 0.02	1.5 (1.3, 1.7); p < 0.001	1.5 (1.1, 2.0); p = 0.005
Oral azithromycin †§	1.8 (1.2, 2.7); p < 0.001	1.2 (0.8, 1.9); p = 0.42	2.4 (0.9, 6.7); p = 0.09
Inhaled antibiotics †§	1.5 (1.0, 2.3); p = 0.04	1.5 (0.9, 2.3); p = 0.11	0.9 (0.3, 2.6); p = 0.83
Mucolytics †§I	1.5 (1.0, 2.2); p = 0.05	1.2 (0.7, 1.8); p = 0.54	1.1 (0.4, 2.9); p = 0.91

Definition of abbreviations: FEV_1 forced expiratory volume in one second; FVC, forced vital capacity; IV, intravenous.

*Adjusted for cystic fibrosis transmembrane conductance regulator (CFTR) genotype.

† Adjusted for CFTR genotype and centre.

‡ Rate ratios (95% CI).

§ Odds ratios (95% CI).

I Recombinant human DNase and/or hypertonic saline.

FIGURE LEGENDS

Figure 1 Flowchart of patient recruitment, sample collection and isolates available for analysis

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. *Definition of Australian state and territory abbreviations:* NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia

Figure 1

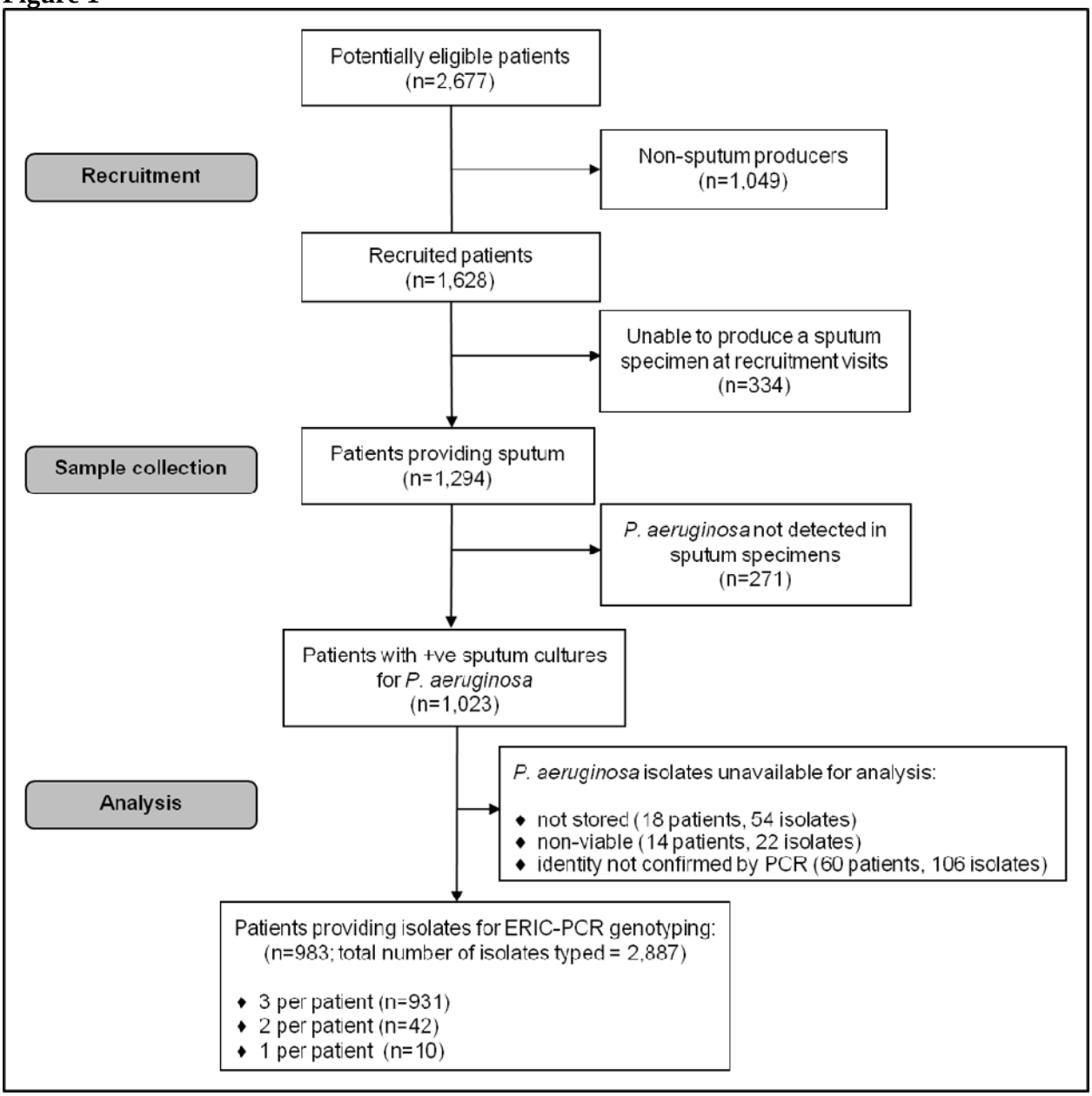
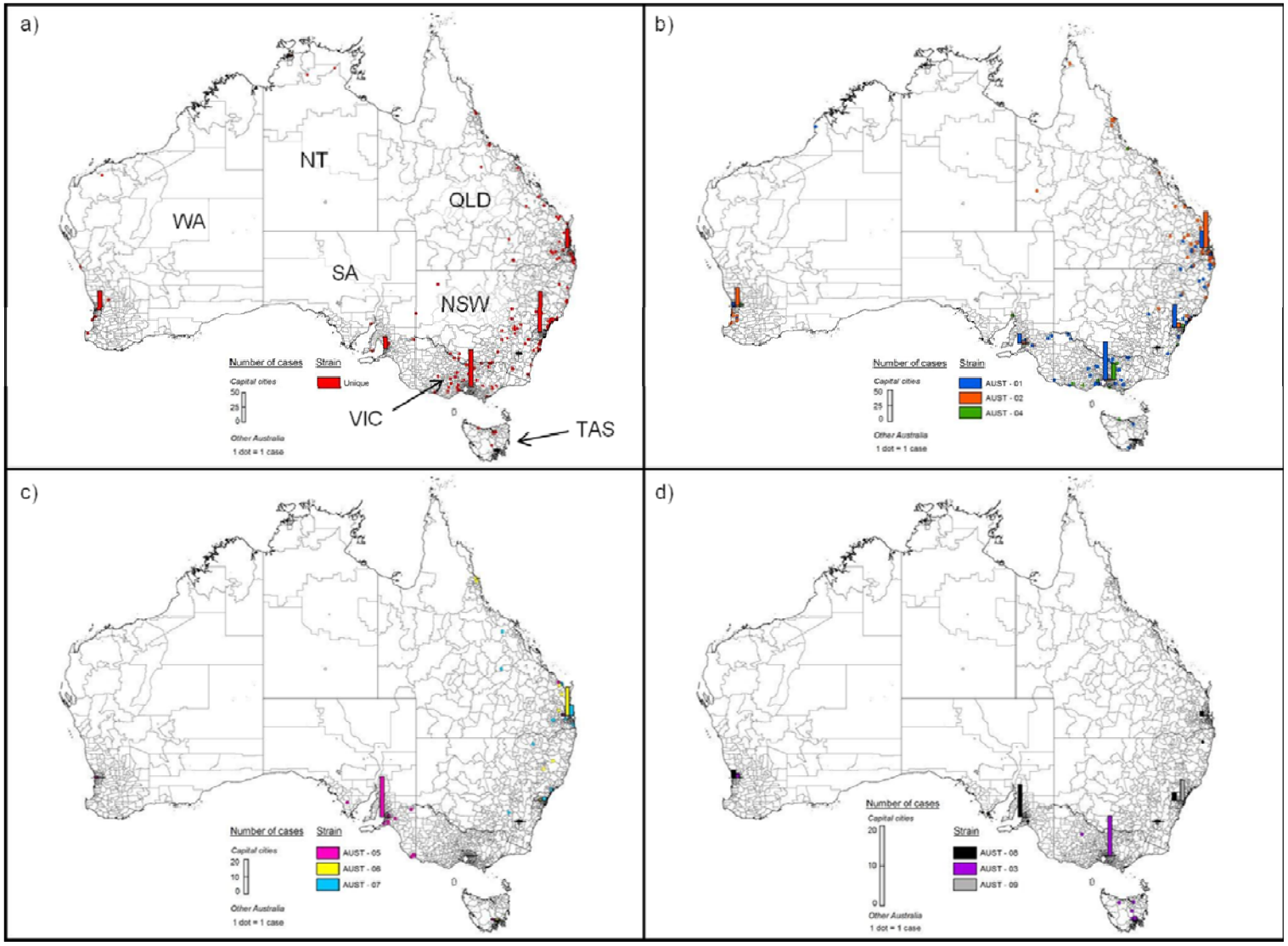

Potentially eligible patients (n=2,677)
Recruitment
Non-sputum producers (n=1,049)
Recruited patients (n=1,628)
Unable to produce a sputum specimen at recruitment visits (n=334)
Sample collection
Patients providing sputum (n=1,294)
P. aeruginosa not detected in sputum specimens (n=271)
Patients with +ve sputum cultures for P. aeruginosa (n=1,023)
Analysis
P. aeruginosa isolates unavailable for analysis:
not stored (18 patients, 54 isolates)
non-viable (14 patients, 22 isolates)
identity not confirmed by PCR (60 patients, 106 isolates)
Patients providing isolates for ERIC-PCR genotyping: (n=983; total number of isolates typed = 2,887)
3 per patient (n=931)
2 per patient (n=42)
1 per patient (n=10)

Figure 2


a)
NT
QLD
WA
SA
NSW
VIC
TAS
Number of cases
Capital cities
50
25
0
Other Australia
1 dot = 1 case
Strain
Unique
b)
Number of cases
Capital cities
50
25
0
Other Australia
1 dot = 1 case
Strain
AUST - 01
AUST - 02
AUST - 04
c)
Number of cases
Capital cities
20
10
0
Other Australia
1 dot = 1 case
Strain
AUST - 05
AUST - 06
AUST - 07
d)
Number of cases
Capital cities
20
10
0
Other Australia
1 dot = 1 case
Strain
AUST - 08
AUST - 03
AUST - 09