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Comparison of Three Molecular Techniques for Typing *Pseudomonas aeruginosa* Isolates in Sputum Samples from Patients with Cystic Fibrosis▽†

Timothy J. Kidd,[1,2]* Keith Grimwood,[1,2] Kay A. Ramsay,[1,2] Paul B. Rainey,[3] and Scott C. Bell[1,2,4]

Queensland Paediatric Infectious Diseases Laboratory, Department of Infectious Diseases, Royal Children's Hospital, Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, QLD, Australia[1]; School of Medicine, The University of Queensland, Brisbane, QLD, Australia[2]; New Zealand Institute for Advanced Study and Allan Wilson Centre for Molecular Ecology and Evolution, Massey University at Albany, Auckland, New Zealand[3]; and Adult Cystic Fibrosis Centre, Department of Thoracic Medicine, The Prince Charles Hospital, Brisbane, QLD, Australia[4]

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Monitoring the emergence and transmission of *Pseudomonas aeruginosa* strains among cystic fibrosis (CF) patients is important for infection control in CF centers internationally. A recently developed multilocus sequence typing (MLST) scheme is used for epidemiologic analyses of *P. aeruginosa* outbreaks; however, little is known about its suitability for isolates from CF patients compared with that of pulsed-field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). As part of a prevalence study of *P. aeruginosa* strains in Australian CF clinics, we compared the discriminatory power and concordance of ERIC-PCR, PFGE, and MLST among 93 CF sputum and 11 control *P. aeruginosa* isolates. PFGE and MLST analyses were also performed on 30 paired isolates collected 85 to 354 days apart from 30 patients attending two CF centers separated by 3,600 kilometers in order to detect within-host evolution. Each of the three methods displayed high levels of concordance and discrimination; however, overall lower discrimination was seen with ERIC-PCR than with MLST and PFGE. Analysis of the 50 ERIC-PCR types yielded 54 PFGE types, which were related by ≤6 band differences, and 59 sequence types, which were classified into 7 BURST groups and 42 singletons. MLST also proved useful for detecting novel and known strains and for inferring relatedness among unique PFGE types. However, 47% of the paired isolates produced PFGE patterns that within 1 year differed by one to five bands, whereas with MLST all paired isolates remained identical. MLST thus represents a categorical analysis tool with resolving power similar to that of PFGE for typing *P. aeruginosa*. Its focus on highly conserved housekeeping genes is particularly suited for long-term clinical monitoring and detecting novel strains.

Pseudomonas aeruginosa is a major determinant of morbidity and mortality in cystic fibrosis (CF) (9, 13, 28). While many CF patients acquire unique strains from the environment, there are reports of patients sharing genotypically related strains in CF centers in the United Kingdom, Europe, Canada, and Australia, suggesting that person-to-person transmission is also a factor (1, 3, 29, 33, 36). Furthermore, some strains are associated with poorer clinical outcomes and increased treatment requirements (2, 3, 20). Some strains, such as clone C and PA14, are distributed globally and found in non-CF human infections, animals, and the natural environment (31, 34, 45). Consequently, molecular typing techniques have become important tools in helping to control cross-infection in CF centers and identifying transmission pathways.

Several factors require consideration when selecting a molecular typing strategy. These include discriminatory power, reproducibility, and typeability, as well as the biological basis for grouping similar strains, cost, and logistics (7, 40). For large epidemiologic studies and CF reference laboratories, cost, simplicity, and effectiveness have become major factors in the choice of method. Rapid and inexpensive PCR-based typing techniques, such as enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR), can be used to screen for genetic relatedness, while the potentially more discriminatory techniques of pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) help confirm results (7, 32).

The high discriminatory power of PFGE makes it the "gold standard" for DNA fingerprinting techniques. PFGE was intended originally for outbreak situations involving epidemiologically related isolates collected over a short time period (41). However, because single mutations can destroy or create restriction sites, minor evolutionary events can cause substantive changes in PFGE restriction profiles and generate spurious genotyping results (12). In contrast, MLST is based on allelic variation in housekeeping genes, and while it monitors change over just a small portion of the genome, it is highly discriminating and provides insight into genetic structure (7). MLST may therefore be more suitable than PFGE for strain

* Corresponding author. Mailing address: Qpid Laboratory, Level 7, Block 28, Queensland Children's Medical Research Institute, Royal Children's Hospital, Herston, QLD 4029, Australia. Phone: 61 7 3636 1400. Fax: 61 7 3636 1401. E-mail: Tim_Kidd@health.qld.gov.au.

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surveillance in CF centers (7, 26, 43). While both PCR-based typing techniques (ERIC-PCR) and PFGE have been compared using *P. aeruginosa* isolates from CF patients (39), comparisons of MLST and PFGE have been limited to VIM-1-like metallo-β-lactamase-producing strains and isolates collected from an intensive care unit (14, 18). Consequently, little is known about the relative performances of all three tests when typing *P. aeruginosa* sputum isolates from chronically infected CF patients.

In this study we compared ERIC-PCR, PFGE, and MLST using isolates obtained during the first year of a national point prevalence study of clonal *P. aeruginosa* strains in Australian CF centers (cross-sectional study) (25). We also report the results of a temporal study in which we collected isolates 3 to 12 months apart from individual patients attending two CF centers separated from one another by 3,600 kilometers (replicate study). Together our results demonstrate close concordance among the three methods but draw attention to particular—and complementary—strengths associated with the combined use of both PFGE and MLST.

MATERIALS AND METHODS

Bacterial isolates. *P. aeruginosa* isolates from sputum were collected as part of a national multicenter prevalence study of clonal strains within Australian CF centers between September 2007 and September 2008 and were identified and stored as described previously (25). Human research ethics committees at each of the 11 participating centers approved the study.

Controls. The Australian epidemic strains AES-I (3), AES-II (29), and AES-III (4), the previously described Australian minor clonal strains P42, P3, P5, P58 (29), and the Tasmanian minor strain (TMS) (4) were included as controls and supplemented with the Liverpool epidemic strain H190 (LES) (6), Manchester strain C3425 (MAN) (21), and clone C strain CF128-1 (34).

ERIC-PCR. Initially, each isolate underwent ERIC-PCR typing as described previously (39). Thermal cycling was performed in a Bio-Rad Mycycler personal thermal cycler (Bio-Rad Laboratories Pty Ltd., Gladesville, Australia), and 7 μl of the amplified products was subjected to electrophoresis (80 V) in 1.2% agarose for 180 min. Amplified products were detected by ethidium bromide staining and visualized in a Gel Doc XR molecular imager (Bio-Rad Laboratories Pty Ltd.). Comparison of ERIC-PCR banding patterns was performed using FPQuest cluster analysis software, version 4.5 (Bio-Rad Laboratories Pty Ltd.). Isolates producing fingerprints related by 100% (Dice coefficient/unweighted pair-group method with arithmetic mean [UPGMA]) were allocated to the same ERIC-PCR type.

PFGE. DNA extraction and SpeI digestion were performed using the GenePath Group 3 reagent kit (Bio-Rad Laboratories Pty Ltd.) following the manufacturer's instructions. Fragment separation was achieved using a CHEF DR III system (Bio-Rad Laboratories Pty Ltd.), with a switch time ramped from 5.3 to 34.9 s over 19.5 h at 14°C. The relatedness of each PFGE fingerprint was determined using both FPQuest cluster analysis (Dice coefficient/UPGMA) and the Tenover criteria (41). Isolates producing PFGE fingerprints related by ≥80% and with ≤6 band differences were categorized as the same PFGE type (PT).

MLST. The reagents, primers, and reaction conditions used for MLST were identical to those described on the *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/). Thermal cycling was performed in a Bio-Rad Mycycler personal thermal cycler, and cycle sequencing was undertaken by the Australian Genome Research Facility using the BigDye Terminator v3.1 cycle sequencing kit and an AB3730xl genetic analyzer (Applied Biosystems Inc., Foster City, CA). Sequence verification and editing were performed using Sequence Scanner v1.0 (Applied Biosystems Inc.) and Vector NTI Advance 11.0 (Invitrogen Australia Pty Ltd.) software. Sequence type (ST) assignment was performed on the *P. aeruginosa* MLST website, START2 software generated a UPGMA dendrogram of the allelic profiles, and the eBURST algorithm inferred clonal relationships between each ST (10, 19). STs sharing ≥6 alleles were allocated to the same BURST group (BG).

Statistical analysis. The discriminatory power and the congruence between ERIC-PCR, MLST (STs and BGs), and PFGE (PTs) were calculated with EpiCompare v1.0 software (Ridom GmbH, Wurzburg, Germany), using Simpson's index of diversity (with 95% confidence intervals), the adjusted Rand index, and the Wallace coefficient (5, 15, 16, 17, 44). The adjusted Rand index provides quantification of congruence between methods, and the Wallace coefficient provides, for a given typing method, an estimate of how much new information would be provided by another method (5). Thus, for the Wallace coefficient, bidirectional values are provided. For MLST analyses, both ST and BG were used to examine for concordance.

RESULTS

Cross-sectional study. (i) ERIC-PCR. Initially, 1,120 *P. aeruginosa* sputum isolates from 346 CF patients (277 adults ≥18 years of age) and the 11 control strains were typed by ERIC-PCR, and 117 discrete ERIC-PCR types were identified. Subsequently, 93 isolates from 91 Australian CF patients (78 adults) and 11 control strains were selected for PFGE and MLST analyses. These represented 50 different ERIC-PCR types (Table 1; see Fig. S1 in the supplemental material) and included (i) 20 isolates categorized by ERIC-PCR as either AES-I or AES-II strains and selected randomly from the two most geographically separated adult CF centers, (ii) 20 isolates from five centers with ERIC-PCR types matching one of eight clones (P3, P5, P42, P58, AES-III, TMS, LES, and clone C) described in Materials and Methods, (iii) 31 isolates from 10 centers representing 13 novel ERIC-PCR strains detected in clusters involving more than one patient, (iv) 22 unrelated strains selected randomly from 4 CF centers, and (v) 11 control isolates.

(ii) PFGE. SpeI digestion was performed successfully on all but one of the 104 selected isolates, resulting in 80 different banding patterns, which were grouped into 54 PTs (see Fig. S2 in the supplemental material). Table 1 shows that PFGE and ERIC-PCR gave concordant typing results for 92% (95/103) of the clinical and control isolates. All eight discordant typing results involved clinical isolates. These included one each of the epidemic strains LES (E15) and AES-II (E25) and multiple unrelated PTs for ERIC-PCR types E26, E31, and E33.

(iii) MLST and eBURST analysis. Table 1 shows that MLST and ERIC-PCR produced concordant typing results for 83% (86/104) of the tested isolates. Of the 59 STs detected (Table 1; see Fig. S3 in the supplemental material), 37 (63%) represented novel STs not listed previously on the *P. aeruginosa* MLST website. One ST (STΔ119) had a 2-base-pair deletion within the *mutL* locus (nucleotides [nt] 119/120; this was a potential mutator strain [30]), which could not be analyzed by the MLST website or eBURST. BURST analysis of the remaining 103 isolates identified seven different BGs (A to G) and 42 singletons (Table 2). Eighty-eight isolates (85%) had concordant ERIC-PCR and eBURST analyses. As with PFGE, one of the clinical E25 isolates was not typed as an AES-II (ST775) strain, E26 was also divided into BG-B and ST389, and E31 was separated into four unrelated STs (ST803, ST508, ST804, and ST822). Other discrepancies between ERIC-PCR and MLST were noted among ERIC-PCR types E32 to E35, which were grouped into BG-E and ST179, and six unrelated ERIC-PCR types were clustered into BG-D (E01 and E31), BG-C (E25 and E40), and BG-A (E43 and E44) (Tables 1 and 2; see Fig. S3 in the supplemental material).

(iv) Comparison of ERIC-PCR, PFGE, and MLST. Comparison of the typing results generated by the three techniques revealed few discrepancies (Table 1). The LES E15 isolates were identical by MLST (ST146), and discordance involving

TABLE 1. *P. aeruginosa* strain typing results obtained using ERIC-PCR, PFGE, and MLST

Corresponding control strain[a]	ERIC-PCR type (*n*)	PFGE type(s) (*n*)	MLST ST(s) (*n*)
	E01 (1)	PT01 (1)	791 (1)
	E02 (1)	PT02 (1)	792 (1)
	E03 (1)	PT03 (1)	793 (1)
	E04 (1)	PT04 (1)	794 (1)
	E05 (1)	PT05 (1)	795 (1)
	E06 (1)	PT06 (1)	377 (1)
	E07 (1)	PT07 (1)	796 (1)
	E08 (1)	PT08 (1)	797 (1)
	E09 (1)	PT09 (1)	798 (1)
	E10 (1)	PT10 (1)	655 (1)
	E11 (1)	PT11 (1)	821 (1)
	E12 (1)	PT12 (1)	259 (1)
	E13 (1)	PT13 (1)	776 (1)
	E14 (1)	PT14 (1)	777 (1)
LES	E15 (2)	PT15 (1), PT54 (1)	146 (2)
	E16 (1)	PT16 (1)	778 (1)
	E17 (1)	PT17 (1)	789 (1)
	E18 (1)	PT18 (1)	790 (1)
	E19 (1)	PT19 (1)	494 (1)
	E20 (1)	Nontypeable (1)	805 (1)
	E21 (1)	PT20 (1)	780 (1)
	E22 (1)	PT21 (1)	802 (1)
	E23 (1)	PT22 (1)	253 (1)
AES-I	E24 (11)	PT23 (11)	649 (11)
AES-II	E25 (11)	PT24 (10), PT25 (1)	775 (10), 266 (1)
P3	E26 (5)	PT26 (4), PT27 (1)	799 (1), 800 (3), 389 (1)
P5	E27 (3)	PT28 (3)	262 (3)
AES-III	E28 (2)	PT29 (2)	242 (2)
TMS	E29 (4)	PT30 (4)	787 (1), 788 (3)
P42	E30 (5)	PT31 (5)	801 (5)
P58	E31 (5)	PT32 (2), PT33 (1), PT34 (1), PT35 (1)	803 (2), 508 (1), 804 (1), 822 (1)
	E32 (2)	PT36 (2)	179 (2)
	E33 (3)	PT36 (1), PT37 (1), PT39 (1)	179 (1), 155 (2)
	E34 (2)	PT37 (2)	155 (1), 786 (1)
	E35 (1)	PT38 (1)	155 (1)
	E36 (6)	PT40 (6)	274 (5), 781 (1)
	E37 (1)	PT40 (1)	274 (1)
	E38 (6)	PT41 (6)	782 (3), 783 (1), 784 (1), 785 (1)
	E39 (1)	PT42 (1)	807 (1)
	E40 (1)	PT43 (1)	808 (1)
	E41 (1)	PT44 (1)	809 (1)
Clone C	E42 (2)	PT45 (2)	17 (2)
	E43 (1)	PT46 (1)	261 (1)
	E44 (1)	PT47 (1)	810 (1)
	E45 (1)	PT48 (1)	236 (1)
	E46 (1)	PT49 (1)	277 (1)
	E47 (1)	PT50 (1)	260 (1)
	E48 (1)	PT51 (1)	254 (1)
	E49 (1)	PT52 (1)	Δ119 (1)[b]
MAN	E50 (1)	PT53 (1)	217 (1)

[a] A selection of previously known epidemic and minor clonal CF *P. aeruginosa* strains (3, 4, 6, 21, 29, 34).

[b] STΔ119 represents a clinical isolate with a 2-base-pair deletion within the *mutL* locus (nt 119/120) which could not be assigned a formal sequence type using the MLST website.

E32 to E36 isolates was also observed. eBURST analysis allowed clonal relationships between six unrelated PTs (PT01, PT32, PT24, PT43, PT46, PT47) clustered into BG-D, BG-C, and BG-A to be inferred.

Simpson's index of diversity was used to determine the discriminatory power of ERIC-PCR, PFGE, MLST, and MLST following eBURST analysis. Diversity indices and their 95% confidence intervals are presented in Table 3. The discriminatory powers of the typing methods were equivalent. However, to compare the congruency between type assignments, the adjusted Rand index and the Wallace coefficient provided additional information (Table 3). Overall, there was strong correlation between the information provided by all typing methods. Using the adjusted Rand index, the highest level of agreement was observed between PFGE and MLST, whether analyzed as STs or following eBURST analysis (0.897 and 0.921). Similarly, Wallace coefficients for PFGE and MLST indicate strong bidirectional correspondence (0.847 to 0.965) between types generated by these methods. However, despite similar Simpson's diversity indices, ERIC-PCR was less concordant with PFGE and MLST than PFGE and MLST were with each other. Both MLST and PFGE were better predictors of ERIC-PCR type than ERIC-PCR was of MLST and/or PFGE type.

TABLE 2. Summary of eBURST analysis

eBURST group	MLST STs	No. of isolates
A	261, 810	2
B	799, 800	4
C	775, 808	11
D	791, 803	3
E	155, 786	5
F	787, 788	4
G	782, 783, 784, 785	6
Singletons	$n = 42$	68

Replicate study. Thirty random isolate pairs obtained several months apart from 30 patients attending two Australian adult CF centers participating in the national clonal *P. aeruginosa* prevalence study (26) and separated by 3,600 kilometers were selected to undergo PFGE and MLST genotyping. At the baseline sputum collection, adult participants had been infected with *P. aeruginosa* for 6 to 33 (mean, 14.7; standard deviation [SD], 6.4) years. The second isolate came from sputum collected 85 to 354 (mean, 203; SD, 52) days after the baseline sample. According to ERIC-PCR typing, 10 paired isolates were AES-I, 10 were AES-II, and 10 represented unique, unrelated strains. However, PFGE and MLST both showed that one AES-II isolate pair belonged to an unrelated strain (see "Cross-sectional study" above). MLST identified 13 STs among the 30 paired isolates, while among these same isolate pairs, PFGE yielded 31 different band patterns, which were grouped into 13 distinct PTs. Although the isolates represented by the 13 different PTs and 13 different STs were the same, 14 (47%) of the 30 isolate pairs diverged by one to five PFGE bands between the first and second sputum collections (Table 4), whereas the ST remained stable for all 30 isolate pairs.

DISCUSSION

ERIC-PCR, PFGE, and MLST provide measures of genetic diversity, but they are not equivalent. ERIC-PCR reveals a profile of DNA fragments of different sizes based, in principle

TABLE 3. Number of types, Simpson's index of diversity, adjusted Rand indices, and Wallace coefficients for ERIC-PCR, PFGE, MLST sequence types, and BURST groups

Method	No. of types	Simpson's index of diversity (95% confidence interval)	Adjusted Rand's coefficient				Wallace coefficient			
			ERIC-PCR	PFGE	MLST ST	MLST BG	ERIC-PCR	PFGE	MLST ST	MLST BG
ERIC-PCR	50	0.965 (0.951–0.979)	1.000	0.893	0.813	0.838	1.000	0.856	0.738	0.840
PFGE	54	0.968 (0.954–0.982)		1.000	0.897	0.921	0.941	1.000	0.847	0.965
MLST ST	59	0.972 (0.958–0.986)			1.000	0.892	0.920	0.960	1.000	1.000
MLST BG	50	0.965 (0.951–0.980)				1.000	0.849	0.886	0.811	1.000

(but see reference 46), on the genomic locations of specific repetitive sequences; PFGE reveals restriction site polymorphisms arising from the genomic locations of SpeI sites; and MLST provides a measure of nucleotide polymorphism based on the DNA sequences of seven housekeeping genes. The first two approaches capture a genome-wide perspective on diversity, with differences between strains arising from a potentially wide range of genetic changes, from point mutations through large-scale inter- and intragenomic recombinational events, although the specific causes of any differences can only be presumed. MLST reveals highly detailed information on genetic changes in specific housekeeping genes and thus provides direct insight into evolutionary changes of the core genome. While MLST is highly portable and provides direct access to the tools and approaches of population genetics, it sheds little light on specific genetic changes beyond the targeted loci.

Despite the differences in underlying genetic "currency," the three different techniques demonstrate a remarkably high level of concordance, discrimination, and typeability. The limited variability of MLST combined with its ready portability suggests that it is better suited than PFGE for long-term strain surveillance within CF centers and for identification of specific evolutionary lineages. In addition, MLST proved useful for detecting novel and previously known strains and for inferring relatedness among isolates that appeared to be unrelated by PFGE.

Several other studies also have reported temporal variation in CF *P. aeruginosa* isolate PFGE band patterns (22, 32, 38) and point to evidence of within-host evolution. In contrast, MLST, as shown here and elsewhere (43), reveals evidence of long-term stability. Such findings, while seemingly contradictory, are readily reconciled by the fact that MLST reveals the identity of the evolving lineage, while PFGE, by virtue of its potential to sample a greater range of genetic changes (large-scale insertions and deletions as well as point mutations that create or destroy restriction sites), stands to shed light on the dynamic nature of the *P. aeruginosa* genome. The variation in PTs that we observed among some STs (e.g., ST146, ST155, and ST179) likely reflects genetic events including intra- and intergenomic recombination within variable regions of the genome or the accessory gene pool. Such changes could have significant implications for the evolution of *P. aeruginosa*.

TABLE 4. PFGE band differences in 30 paired sputum *P. aeruginosa* isolate sets collected 85 to 354 days apart from chronically infected adults with CF

Strain (no. of isolates)	Total no. of PFGE band differences vs baseline isolate	No. of individuals
AES-1 (20)	0	7
	1	1
	2	2
AES-2 (18)	0	5
	1	0
	2	3
	3	1
Unrelated strains (22)	0	4
	1	1
	2	1
	3	3
	4	1
	5	1
Total		30

Simpson's index was used to assess each method's ability to assign a different type to two randomly sampled strains (17), although caution in interpreting the results is required because our sample was not randomly collected. Nonetheless, overall, the discriminatory power of each method was high (>0.96) (7), with each method showing equivalent levels of discrimination. Despite reservations regarding the appropriateness of Simpson's index, two other statistical approaches (the adjusted Rand index and the Wallace coefficient) also revealed a high level of congruence between ERIC-PCR, PFGE, and MLST. PFGE and eBURST analyses showed the strongest correlation, suggesting that using both techniques together may be redundant (i.e., additional information provided by performing the second typing method was minimal). We note that our finding of MLST having the greatest discriminatory power is at odds with one previous study, which found PFGE to be the superior methodology for detecting genetic relatedness between *P. aeruginosa* strains in perirectal surveillance swabs from patients nursed in intensive care units at a single center (18). Differences in the nature and origins of the strains and/or the interpretative criteria used for defining PTs may help explain our contradictory findings with CF sputum isolates.

In the current study, the level of congruence between the techniques was lower for ERIC-PCR than for MLST and PFGE (Table 3). Nevertheless, ERIC-PCR did perform well and in our hands and appears to be a more reliable typing strategy for *P. aeruginosa* than are other novel PCR-based typing methodologies (11). While clonality of *P. aeruginosa* may be overestimated by ERIC-PCR, suggesting that an additional typing method should be used to confirm relationships between isolates displaying indistinguishable types, we also observed that compared to MLST, both ERIC-PCR and PFGE may occasionally overly discriminate for some STs (e.g., ST155, ST179, and ST274). Taken together, these results draw

further attention to the fact that different typing methods, which are each based on different attributes of genetic variation, are unlikely to demonstrate 100% concordance.

During this study we confirmed the presence of several novel and known strains that were previously undetected in Australian CF patients. Among these strains were LES (ST146), clone C (ST17), PA14 (ST253), ST179, and ST155. These strains are distributed widely throughout the Northern Hemisphere and have been found in the natural environment, in animals, and in different patient groups (1, 8, 23, 36, 42, 43, 45). Using the *P. aeruginosa* MLST website, we have also established that several Australian CF strains (e.g., AES-I [ST649], ST274, ST260, and ST254) are in CF and non-CF settings elsewhere in the world (24, 27). While most of these strains are distinguishable by PFGE and ERIC-PCR, isolates belonging to ST155 and ST179 are less readily distinguished. Variations in RAPD and PFGE patterns in ST155 and ST179 isolates from a Canadian CF study spanning 19 years have been published recently on the *P. aeruginosa* MLST website (37). The detection international strains within Australian CF patients provides further evidence for the global distribution of several major *P. aeruginosa* clones (31, 35, 42, 45) and for the portability of MLST (7). These observations are important for understanding local and global epidemiology and identifying transmission pathways. Had MLST not been used in the current study, then the presence of PA14, ST155, and ST179 in Australia is likely to have gone undetected.

To our knowledge this study represents the most extensive evaluation of ERIC-PCR, PFGE, and MLST with *P. aeruginosa* CF isolates. Generally, ERIC-PCR, PFGE, and MLST were concordant, provided that isolates were grouped into PFGE types related by ≤6 bands and into BGs sharing at least 6 alleles. Advantages of MLST are its (i) utility and unambiguous, categorical approach to interpretation, (ii) stability over time and in various CF center settings, and (iii) capacity to identify novel strains and to infer clonal relatedness in epidemiologically unrelated isolates. The increasing availability of affordable high-throughput sequencing methods draws attention to the potential for MLST and other sequence-based typing methods to complement PFGE as a typing strategy.

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REFERENCES

1. **Aaron, S., et al.** 2008. Epidemic strains of *P. aeruginosa* in adult CF patients in Ontario, Canada—prevalence and epidemiology. Paediatr. Pulmonol. **43:** 327.
2. **Al-Aloul, M., et al.** 2004. Increased morbidity associated with chronic infection by an epidemic *Pseudomonas aeruginosa* strain in CF patients. Thorax **59:**334–336.
3. **Armstrong, D. S., et al.** 2002. Detection of a widespread clone of *Pseudomonas aeruginosa* in a pediatric cystic fibrosis clinic. Am. J. Respir. Crit. Care Med. **166:**983–987.
4. **Bradbury, R., A. Champion, and D. W. Reid.** 2008. Poor clinical outcomes associated with a multi-drug resistant clonal strain of *Pseudomonas aeruginosa* in the Tasmanian cystic fibrosis population. Respirology **13:**886–892.
5. **Carrico, J. A., et al.** 2006. Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. J. Clin. Microbiol. **44:**2524–2532.
6. **Cheng, K., et al.** 1996. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. Lancet **348:**639–642.
7. **Clinical and Laboratory Standards Institute.** 2007. Molecular methods for bacterial strain typing; approved guideline. CLSI document MM11-A. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Curran, B., D. Jonas, H. Grundmann, T. Pitt, and C. G. Dowson.** 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. **42:**5644–5649.
9. **Emerson, J., M. Rosenfeld, S. McNamara, B. Ramsey, and R. L. Gibson.** 2002. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr. Pulmonol. **34:**91–100.
10. **Feil, E. J., B. C. Li, D. M. Aanensen, W. P. Hanage, and B. G. Spratt.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. **186:**1518–1530.
11. **Fluit, A. C., et al.** 2010. Evaluation of the DiversiLab system for detection of hospital outbreaks of infections by different bacterial species. J. Clin. Microbiol. **48:**3979–3989.
12. **Fothergill, J. L., et al.** 2010. Impact of *Pseudomonas aeruginosa* genomic instability on the application of typing methods for chronic cystic fibrosis infections. J. Clin. Microbiol. **48:**2053–2059.
13. **Gibson, R. L., J. L. Burns, and B. W. Ramsey.** 2003. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am. J. Respir. Crit. Care Med. **168:**918–951.
14. **Giske, C. G., et al.** 2006. Establishing clonal relationships between VIM-1-like metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains from four European countries by multilocus sequence typing. J. Clin. Microbiol. **44:**4309–4315.
15. **Grundmann, H., S. Hori, and G. Tanner.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192.
16. **Hubert, L., and P. Arabie.** 1985. Comparing partitions. J. Classification **2:**193–218.
17. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
18. **Johnson, J. K., S. M. Arduino, O. C. Stine, J. A. Johnson, and A. D. Harris.** 2007. Multilocus sequence typing compared to pulsed-field gel electrophoresis for molecular typing of *Pseudomonas aeruginosa*. J. Clin. Microbiol. **45:** 3707–3712.
19. **Jolley, K. A., E. J. Feil, M. S. Chan, and M. C. Maiden.** 2001. Sequence type analysis and recombinational tests (START). Bioinformatics **17:**1230–1231.
20. **Jones, A. M., et al.** 2010. Clinical outcome for cystic fibrosis patients infected with transmissible *P. aeruginosa*: an 8 year prospective study. Chest **137:** 1405–1409.
21. **Jones, A. M., et al.** 2001. Spread of a multiresistant strain of *Pseudomonas aeruginosa* in an adult cystic fibrosis clinic. Lancet **358:**557–558.
22. **Jung, A., et al.** 2002. Sequential genotyping of *Pseudomonas aeruginosa* from upper and lower airways of cystic fibrosis patients. Eur. Respir. J. **20:**1457–1463.
23. **Khan, N. H., et al.** 2008. Multilocus sequence typing and phylogenetic analyses of *Pseudomonas aeruginosa* isolated from the ocean. Appl. Environ. Microbiol. **74:**6194–6205.
24. **Kidd, T. J., et al.** 2010. Multilocus sequence typing (MLST) of *Pseudomonas aeruginosa* collected from different ecological niches. J. Cyst. Fibros. **9**(Suppl. 1)**:**S30.

25. **Kidd, T. J., et al.** 2009. Low rates of *Pseudomonas aeruginosa* misidentification in isolates from cystic fibrosis patients. J. Clin. Microbiol. **47:**1503–1509.
26. **Maiden, M. C., et al.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140–3145.
27. **Nemec, A., L. Krizova, M. Maixnerova, and M. Musilek.** 2010. Multidrug-resistant epidemic clones among bloodstream isolates of *Pseudomonas aeruginosa* in the Czech Republic. Res. Microbiol. **161:**234–242.
28. **Nixon, G. M., et al.** 2001. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. J. Pediatr. **138:**699–704.
29. **O'Carroll, M. R., et al.** 2004. Clonal strains of *Pseudomonas aeruginosa* in paediatric and adult cystic fibrosis units. Eur. Respir. J. **24:**101–106.
30. **Oliver, A., R. Canton, P. Campo, F. Baquero, and J. Blazquez.** 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science **288:**1251–1254.
31. **Pirnay, J. P., et al.** 2009. *Pseudomonas aeruginosa* population structure revisited. PLoS One **4:**e7740.
32. **Renders, N., Y. Romling, H. Verbrugh, and A. van Belkum.** 1996. Comparative typing of *Pseudomonas aeruginosa* by random amplification of polymorphic DNA or pulsed-field gel electrophoresis of DNA macrorestriction fragments. J. Clin. Microbiol. **34:**3190–3195.
33. **Romling, U., A. Kader, D. D. Sriramulu, R. Simm, and G. Kronvall.** 2005. Worldwide distribution of *Pseudomonas aeruginosa* clone C strains in the aquatic environment and cystic fibrosis patients. Environ. Microbiol. **7:**1029–1038.
34. **Romling, U., J. Wingender, H. Muller, and B. Tummler.** 1994. A major *Pseudomonas aeruginosa* clone common to patients and aquatic habitats. Appl. Environ. Microbiol. **60:**1734–1738.
35. **Schmid, J., et al.** 2008. *Pseudomonas aeruginosa* transmission is infrequent in New Zealand cystic fibrosis clinics. Eur. Respir. J. **32:**1583–1590.
36. **Scott, F. W., and T. L. Pitt.** 2004. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. J. Med. Microbiol. **53:**609–615.
37. **Speert, D. P., et al.** 2002. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. Am. J. Respir. Crit. Care Med. **166:**988–993.
38. **Struelens, M. J., V. Schwam, A. Deplano, and D. Baran.** 1993. Genome macrorestriction analysis of diversity and variability of *Pseudomonas aeruginosa* strains infecting cystic fibrosis patients. J. Clin. Microbiol. **31:**2320–2326.
39. **Syrmis, M. W., et al.** 2004. Rapid genotyping of *Pseudomonas aeruginosa* isolates harboured by adult and paediatric patients with cystic fibrosis using repetitive-element-based PCR assays. J. Med. Microbiol. **53:**1089–1096.
40. **Tenover, F. C., R. D. Arbeit, and R. V. Goering.** 1997. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. Infect. Control Hosp. Epidemiol. **18:**426–439.
41. **Tenover, F. C., et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
42. **van Mansfeld, R., et al.** 2009. *Pseudomonas aeruginosa* genotype prevalence in Dutch cystic fibrosis patients and age dependency of colonization by various *P. aeruginosa* sequence types. J. Clin. Microbiol. **47:**4096–4101.
43. **Waine, D. J., D. Honeybourne, E. G. Smith, J. L. Whitehouse, and C. G. Dowson.** 2009. Cross-sectional and longitudinal multilocus sequence typing of *Pseudomonas aeruginosa* in cystic fibrosis sputum samples. J. Clin. Microbiol. **47:**3444–3448.
44. **Wallace, D. L.** 1983. A method for comparing two hierarchical clusterings: comment. J. Am. Stat. Assoc. **78:**569–576.
45. **Wiehlmann, L., et al.** 2007. Population structure of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A. **104:**8101–8106.
46. **Wilson, L. A., and P. M. Sharp.** 2006. Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: Evolution and implications for ERIC-PCR. Mol. Biol. Evol. **23:**1156–1168.