Intraclonal genetic diversity amongst cystic fibrosis and keratitis isolates of *Pseudomonas aeruginosa*

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Given the emergence of transmissible strains of *Pseudomonas aeruginosa*, such as the Liverpool epidemic strain (LES), in cystic fibrosis (CF) centres, it is important to carry out regular surveillance of isolates. In a survey of 22 *P. aeruginosa* isolates, each from a different CF patient identified as negative for LES in a paediatric centre in Liverpool, six (23 %) were identified as being the same clone type (clone D) using array-tube genotyping. Using a series of alternative genotyping approaches [PFGE, random amplification of polymorphic DNA (RAPD), variable number of tandem repeats (VNTR) and multilocus sequence typing (MLST)], the six CF clone D isolates and eight previously identified clone D isolates associated with infections leading to keratitis were compared. All but two of the clone D isolates (both keratitis-associated) were assigned by MLST to sequence type 235 and were highly similar using VNTR analysis. However, there was considerable variation found among the isolates when using PFGE or RAPD, highlighting the limitations of these methods. The discordance with respect to two of the isolates identified by array-tube genotyping as clone D, when using all the other typing methods, emphasizes the need to use more than one method for reliable identification of strains.

**INTRODUCTION**

*Pseudomonas aeruginosa* is a versatile opportunistic pathogen capable of causing a wide range of infections, including chronic respiratory infections in patients with cystic fibrosis (CF), the most common inherited life-threatening disorder in the UK. Mutations in the CF transmembrane conductance regulator (*CFTR*) gene result in the production of thick, viscous lung secretions and reduced mucociliary clearance of the airways, leaving patients susceptible to chronic bacterial lung infections, leading to progressive lung damage and death from respiratory failure.

Although it is thought that CF patients generally acquire unique *P. aeruginosa* strains from the environment, it is now widely accepted that some strains exist that can transmit between patients, raising serious concerns with respect to cross-infection in a hospital setting (Fothergill et al., 2012). Cheng et al. (1996) reported the emergence of a multidrug-resistant strain of *P. aeruginosa* in the CF unit of Alder Hey Children’s Hospital in Liverpool, UK. This was termed the Liverpool epidemic strain (LES) and was identified in 71 % of the children studied (Cheng et al., 1996). Further analysis identified this strain in 79 % of patients in the adult CF unit in Liverpool (Panagea et al., 2003) and it was found to be widespread across the UK (Scott & Pitt, 2004). LES has been associated with increased morbidity and mortality (Aaron et al., 2010; Al-Aloul et al., 2005), increased resistance to antibiotics (Ashish et al., 2012), the ability to superinfect patients already harbouring their unique strains of *P. aeruginosa* (McCallum et al., 2001) and overproduction of important virulence factors (Fothergill et al., 2007). As a consequence of segregation measures based on rapid PCR tests to identify LES (Fothergill et al., 2008; Parsons et al., 2002), new cases of LES-infected patients are extremely rare in Liverpool, and the prevalence in the paediatric CF unit has declined sharply (Gaillard et al., 2004). One clear lesson from these and similar experiences elsewhere with transmissible strains (Fothergill et al., 2012) is the need to identify such strains as they emerge in order to limit cross-infection.
A number of different approaches have been applied to the genotyping of *P. aeruginosa* isolates. These include PFGE (Fothergill et al., 2010b; Ledson et al., 1998), random amplification of polymorphic DNA (RAPD) (Mahanthiralingam et al., 1996), variable number of tandem repeats (VNTR) (Turton et al., 2010) and multilocus sequence typing (MLST) (Curran et al., 2004; Kidd et al., 2011). The array-tube (AT) method (Wielhmann et al., 2007) has been used to study *P. aeruginosa* populations from various sources (Rakhimova et al., 2009; Selezka et al., 2012), including a UK-wide collection of isolates associated with keratitis (Stewart et al., 2011). AT genotyping is a rapid, inexpensive method, and an electronic database is available for comparison with a large number of isolates (Wielhmann et al., 2007).

Here, we have reported the genotyping of a collection of non-LES *P. aeruginosa* isolates from the paediatric CF unit in Liverpool. Having identified a dominant clone type that matched the most common clone type among our previously reported collection of keratitis isolates (Stewart et al., 2011), we used a range of other commonly used genotyping approaches to compare the two collections and assess intraclonal diversity.

**METHODS**

**Bacterial strains used in this study.** Twenty-two *P. aeruginosa* isolates from CF children attending the Alder Hey paediatric unit in 2006 were analysed, each from a different patient. All isolates were confirmed as *P. aeruginosa* using a PCR assay for amplification of the outer-membrane lipoprotein gene oprL (De Vos et al., 1997) and as non-LES using published PCR assays for two LES-specific markers: PS21 and LESF9 (Fothergill et al., 2008; Smart et al., 2006). A previously described collection of eight isolates associated with bacterial keratitis and identified as clone D were also analysed (Stewart et al., 2011). The oligonucleotide primers used for PCR amplification are listed in Table 1.

**Genotyping using CLONDIAG ArrayTubes.** An AT genotyping system (CLONDIAG ArrayTubes; Alere Technologies) was used to assign clone types, as described previously (Stewart et al., 2011; Wielhmann et al., 2007). The AT microarray chip detects: (i) 13 single-nucleotide polymorphisms (SNPs) for analysis of the conserved genome; and (ii) 38 variable genetic markers for analysis of the accessory genome, including markers for virulence factors and previously reported genomic islands (Arora et al., 2001; de Chial et al., 2003; Klockgether et al., 2004; Larbig et al., 2002; Lee et al., 2006; Liang et al., 2001; Spencer et al., 2003; Stover et al., 2000). Data from the 13 SNPs, flagellin type (a/b) and the presence of the mutually exclusive type III secretion exotoxins (S or U) were converted into a ‘hexadecimal code’ represented by four digits, allowing the published database to be searched. In the database, genotypes identified more than once in the original study were assigned a clone ‘type’ name. Genotypes occurring only once (referred to as singletons) were listed using only the four-digit hexadecimal code (for example, 882A) (Wielhmann et al., 2007). For the purpose of this study, we have retained these definitions of clone type and singleton. Any isolates with four-digit codes not matching the database were recorded as unique.

**RAPD.** RAPD analysis was adapted from the method described by Mahenthiralingam et al. (1996). PCR amplifications were carried out in 25 μl volumes. For each reaction, a master mix comprising 1× Flexi Taq Buffer (Promega), 200 μM dNTPs, 3 μM oligonucleotide primer 272 (Table 1; Sigma-Genosys), 2.5 mM MgCl₂ (Promega) and 1.25 U Taq DNA polymerase (Promega) was prepared. Subsequently, 1 μl DNA in Chelex-100 (Bio-Rad) adjusted to 40 ng μl⁻¹ was added to 24 μl of the master mix. Amplifications were carried out in an Eppendorf Gradient Mastercycler as follows: 5 min at 94 °C; four cycles of 5 min at 36 °C, 5 min at 72 °C and 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C; and a 10 min hold at 72 °C.

**PFGE.** PFGE using analysis of SpeI-digested DNA was carried out as described previously (Fothergill et al., 2010b; Ledson et al., 1998). Electrophoresis was performed on a 2 % (w/v) gel using a CHEF DR III apparatus (Bio-Rad Laboratories) alongside a size marker (pulse marker, 50–1000 kb; Sigma). Conditions were as follows: a 120° angle, 6 V cm⁻¹ and a pulse ramp of 1–50 s for 25 h. Thiourea at a concentration of 100 μM was added to improve band definition.

**VNTR.** For VNTR analysis, repeat numbers at nine VNTR loci (Table 2) were determined as described previously (Onteniente et al., 2003; Turton et al., 2010; Vu-Thien et al., 2007). All reactions were performed in 25 μl volumes consisting of 2 μl crude DNA extract, 1× Flexi Taq buffer (Promega), 200 μM dNTPs, 4 μM oligonucleotide primer, 2.5 mM MgCl₂ (Promega) and 1.25 U Taq DNA polymerase (Promega). For primers for loci 172, 211, 213, 214, 217 and 222 (primer set 1), amplification conditions consisted of initial denaturation for 3 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 60 °C and 45 s at 72 °C; and a 10 min hold at 72 °C, followed by separation on a 1.5 % (w/v) agarose gel. For primers for loci 061, 207 and 209 (primer set 2), amplification conditions were: initial denaturation for 15 min at 95 °C; 35 cycles of 30 s at 94 °C, 90 s at 58 °C and 90 s at 72 °C; and a 10 min hold at 72 °C, followed by separation on a 3 % (w/v) agarose gel.

**MLST.** For MLST typing, regions of the acsA, aroE, guaA, mutL, nuoD, pspA and trpE genes were amplified and sequenced (Source Biosciences) using the recommended primers (Table 1) (Curran et al., 2004). Amplifications were carried out in 50 μl volumes using the following PCR conditions: 1 min at 96 °C; 30 cycles of 1 min at 96 °C, 1 min at 55 °C and 1 min at 72 °C; and final extension of 10 min at 72 °C. Sequence types (STs) were assigned using the available database (http://pubmlst.org/paeruginosa/). For amplification of trpE, the elongation time was adjusted to 30 s and the annealing temperature was increased to 60 °C for improved product yield.

**Relationship analysis.** The banding patterns for PFGE and RAPD were analysed using GelCompar II software (Applied Maths). Dendrograms were produced using a dice similarity coefficient with 0.5 % tolerance and UPGMA.

A cut-off value of 80 % was used to define clusters. The discriminatory power of each method was determined using Simpson’s index of diversity, which is based on the probability that two unrelated strains sampled at random from the test population will be placed into different typing groups (Hunter & Gaston, 1988).

**RESULTS**

**AT clone types identified among the paediatric CF isolates**

Amongst the 22 isolates genotyped, eight separate clone types, two singletons and two unique isolates, as defined in...
Methods, were identified (Fig. 1). Six of the 22 isolates were identified as the same clone (clone D; hexadecimal code F469). This clone was identified previously as the most common among a UK-wide collection of *P. aeruginosa* isolates associated with eye infections leading to keratitis (Stewart et al., 2011).

**PFGE, RAPD and VNTR analysis of clone D isolates associated with CF or keratitis infection**

We analysed further the six CF-associated clone D isolates and eight keratitis-associated clone D isolates using four alternative genotyping methods. Using PFGE, the CF isolates could be subdivided into four different genotypes, with only three of the strains (34, 36 and 38) clustering at >80% similarity. The variation was even greater among the eight keratitis isolates, where no clusters were identified (Fig. 2a).

RAPD analysis of the 14 isolates revealed three separate profiles for the CF isolates, with a range of 80–86.5% similarity, and eight profiles for the keratitis isolates (Fig. 2b). There were no cases where the same strains were clustered using both PFGE and RAPD. In order for such clustering to occur, the percentage similarity cut-off would have had to been reduced to 70%, and even then only two isolates (34 and 36) would have clustered using both methods.

Using VNTR, the six CF clone D isolates were identical at eight loci, but differences were observed at the ninth locus (061), dividing the isolates into two profiles (Table 2). The eight clone D keratitis isolates showed much more variation, with six profiles being identified. Four of the eight isolates were identical at eight loci, but two of the isolates, 39267 and 39131, were considerably different from the others, whilst sharing four loci with each other.

**Table 1. Oligonucleotide primers used in this study**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Amplicon (bp)</th>
<th>Target</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS21F</td>
<td>AAGCAACGCCAGCGGTGCTCA</td>
<td>364</td>
<td>LES PS21 Marker</td>
<td>Parsons et al. (2002)</td>
</tr>
<tr>
<td>PS21 R</td>
<td>AAAACGTAGCAAGCAGTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LES F9F</td>
<td>AACACCTGCTCAGCTACGTGC</td>
<td>431</td>
<td>LES F9 Marker</td>
<td>Smart et al. (2006)</td>
</tr>
<tr>
<td>LES F9R</td>
<td>CAGGATATCCGCAAGAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL1</td>
<td>ATGGGAATTGCTGAAATTTCCG</td>
<td>504</td>
<td>oprL gene</td>
<td>De Vos et al. (1997)</td>
</tr>
<tr>
<td>PAL2</td>
<td>CTTCTCGACGCACCGCACG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>272</td>
<td>AGCGGGCACA</td>
<td></td>
<td>Random</td>
<td>Mahenthiralingam et al. (1996)</td>
</tr>
<tr>
<td>acsA-F</td>
<td>ACCTGGTGTTACGCCCTGCTGAC</td>
<td>842</td>
<td>Acetyl coenzyme A synthase PA0887</td>
<td>Curran et al. (2004)</td>
</tr>
<tr>
<td>acsA-R</td>
<td>GACATAGATGCCCTGGCCCTTGAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aroE-F</td>
<td>TGGGGCTATGACTGGAAC</td>
<td>825</td>
<td>Shikimate dehydrogenase PA0023</td>
<td>Curran et al. (2004)</td>
</tr>
<tr>
<td>aroE-R</td>
<td>TAACCCGGTTTTGTGATTCCCTACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>guaA-R</td>
<td>GAACCCTGCGTCGGTTGGTGTAG</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>mutL-F</td>
<td>CCAGATCGCCGCAGGTGGAGTG</td>
<td>940</td>
<td>DNA mismatch repair protein PA4946</td>
<td>Curran et al. (2004)</td>
</tr>
<tr>
<td>mutL-R</td>
<td>CAGGTTGCATAGAGGAAATC</td>
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<td></td>
</tr>
<tr>
<td>nuoD-F</td>
<td>ACCGCCACCCGTACTG</td>
<td>1042</td>
<td>NADH dehydrogenase I chain C,D. PA2639</td>
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<td>nuoD-R</td>
<td>TCTGCCACCTTCGACCA</td>
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</tr>
<tr>
<td>ppsA-F</td>
<td>GTCCGCTCGGTCAAAGGTAGTG</td>
<td>989</td>
<td>Phosphoenolpyruvate synthase PA1770</td>
<td>Curran et al. (2004)</td>
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<td>GGTTTCTCTTCTCAGGCTCGTAG</td>
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<tr>
<td>trpE-F</td>
<td>GCCGCCAGGTCGTGAG</td>
<td>811</td>
<td>Anthralite synthetase component 1 PA0609</td>
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<td>trpE-R</td>
<td>CCCGCCGCTTTGTGGATGGTT</td>
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</table>
(Table 2). For isolate 39131, it was not possible to produce a PCR product for locus 214, despite repeated testing.

**MLST of clone D isolates from CF and keratitis**

Using MLST, the CF isolates and all but two of the keratitis isolates were confirmed as ST-235. Isolates 39131 and 39267 were markedly different from the others and each other, with allele variations from the majority of clone D isolates at six and seven loci, respectively (Table 3).

**Simpson’s index of diversity**

The Simpson’s indices were 0.967, 0.967, 0.846 and 0.275, respectively, for PFGE, RAPD, VNTR and MLST. Hence, the discriminatory powers of PFGE, RAPD and VNTR were relatively high in comparison with MLST, which resulted in 86% of the isolates belonging to one cluster.

**Accessory genome variations among clone D isolates**

As well as identifying SNP patterns to enable the assignment of clone types, the AT method contains a number of markers for variable genes and genomic islands to enable a comparison of accessory genomes. Variations in the accessory genome data derived from the clone D isolates are shown in Fig. 3. It is interesting to note that, although all of the clone D isolates were identified as positive for the exotoxin U gene (rather than exotoxin S gene) and were type a flagellin strains, a number of other variations were apparent. Notably, the keratitis isolates 39131 and 39267 were pyoverdine receptor types I and III, whereas all CF isolates were type II, and the other keratitis isolates were either identified as type II or had no clear identity (Fig. 3).

All of the isolates in this study tested positive for the marker designed to identify the common genomic island PAGI-1. Carriage of PAGI-2/3-like islands was more variable, but all of the CF isolates tested positive for at least three of the ten markers included on the chip, whereas the keratitis isolates were almost entirely negative. Markers for the PAPI-1/pKLC102 group islands were also more frequently positive amongst the CF isolates, but with some interstrain variations apparent (Fig. 3). The AT markers pKL-1 and pKL-3, which target the conserved region of this family of genomic islands, were absent from all keratitis isolates. These markers were both present in only two of the six CF isolates, and were entirely absent from two others. The clone D isolates only occasionally tested positive for any of the individual variable genes included on the AT chip. The two keratitis isolates that were

![Fig. 1. Distribution of clone types for 22 paediatric isolates using AT genotyping. Profiles with hexadecimal codes that were not represented in the database were recorded as unique. Those classified as unique were not the same as each other.](http://jmm.sgmjournals.org)

Table 2. Allelic profiles using VNTR at nine loci

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>VNTR locus</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>172</td>
</tr>
<tr>
<td>34</td>
<td>CF</td>
<td>13</td>
</tr>
<tr>
<td>36</td>
<td>CF</td>
<td>13</td>
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</tr>
<tr>
<td>50</td>
<td>CF</td>
<td>13</td>
</tr>
<tr>
<td>39212</td>
<td>Keratitis</td>
<td>13</td>
</tr>
<tr>
<td>27</td>
<td>CF</td>
<td>13</td>
</tr>
<tr>
<td>38</td>
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<tr>
<td>39375</td>
<td>Keratitis</td>
<td>13</td>
</tr>
<tr>
<td>39394</td>
<td>Keratitis</td>
<td>13</td>
</tr>
<tr>
<td>39087</td>
<td>Keratitis</td>
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</tr>
<tr>
<td>39304</td>
<td>Keratitis</td>
<td>13</td>
</tr>
<tr>
<td>39016</td>
<td>Keratitis</td>
<td>13</td>
</tr>
<tr>
<td>39131</td>
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<td>10</td>
</tr>
<tr>
<td>39267</td>
<td>Keratitis</td>
<td>10</td>
</tr>
</tbody>
</table>
consistently different from the majority using the other genotyping methods were the only two testing positive for some of these variable genes (Fig. 3).

**DISCUSSION**

Chronic *P. aeruginosa* infections are impossible to eradicate from the CF airways, and delay of onset of chronic infections is seen as a key therapeutic target (Hansen *et al.*, 2008; Lee, 2009). The emergence of transmissible strains of *P. aeruginosa* is well documented (Fothergill *et al.*, 2012), and it is important that CF units have clear strategies to identify the emergence of epidemic strains that may increase the risk of chronic *P. aeruginosa* infection in their patients. It has been demonstrated that segregation policies can be effective (Fothergill *et al.*, 2012; Govan *et al.*, 2007), but in order to implement these effectively, clinicians first need to know which patients carry potential problem strains.

**Table 3.** MLST analysis of *P. aeruginosa* isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>MLST locus</th>
<th>ST</th>
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<td></td>
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<tr>
<td>39267</td>
<td>Keratitis</td>
<td>35</td>
<td>8</td>
</tr>
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</table>
As a first step towards carrying out such surveillance in the Liverpool paediatric CF unit where the LES was first identified, we used a portable genotyping method (AT) to assign isolates to clone types. In so doing, we identified a potential new dominant clone (clone D), which was also the most abundant in a parallel study that we conducted on keratitis-associated isolates from around the UK (Stewart et al., 2011). In order to compare intraclonal diversity between two isolate sets from very different types of infection, and to determine how well AT genotyping compares with other commonly used methods, we subjected the two sets of clone D isolates to analysis using PFGE, RAPD, VNTR and MLST.

For all but two of the keratitis isolates identified as clone D, there was good concordance between the AT method, VNTR and MLST. In contrast, much more diversity was apparent when using PFGE and RAPD, especially among the keratitis isolates. It is a feature of PFGE and RAPD that they have good discriminatory power (Mahenthiralingam et al., 1996), but the utility of such discrimination has been questioned, especially in relation to long-term chronic
infections, during which populations diversify and the bacteria can undergo genome rearrangements (Fothergill et al., 2010b; Murase et al., 1999; Spencer et al., 2003). Indeed, variations among strains can be due to insertions or deletions of genomic material up to 500 kb in size (Römlinger et al., 1997; Schmidt et al., 1996). The accessory genome includes large insertions such as prophages and genomic islands, which, in some cases, can contribute to the ability of strains to cause infections (Winstanley et al., 2009).

Genotyping methods fall into two broad categories: those sampling the whole genome, which includes core and accessory regions (such as RAPD and PFGE); and those sampling variations in the core genome (such as MLST and the SNP typing using AT genotyping). Although VNTR falls into the former category and has good discriminatory powers (Onteniente et al., 2003; Turton et al., 2010), our analysis suggested that it is less susceptible to the genomic variations that can compromise RAPD and PFGE. However, this is dependent on how many loci are included in the analysis. VNTR in this study revealed identical tandem repeats for all isolates except the two keratitis isolates 39267 and 39131 at seven of the nine loci. Inclusion of the ninth locus (061) divided the strains into seven types. Thus, although the ninth locus did increase discrimination, in some circumstances, a less discriminatory approach involving the selection of fewer loci or loci with larger repeat sizes might be more appropriate (van Mansfeld et al., 2010).

MLST was the most concordant of the genotyping methods used when compared with AT genotyping. Both methods target conserved regions of the genome, and although the loci are different, the selection criteria were similar (Curran et al., 2004; Morales et al., 2004). In contrast to MLST, the AT design also allowed comparative analysis of the flexible accessory genome. Both are portable between laboratories and lend themselves to the development of large searchable strain databases, but the AT method does not rely on expensive sequencing technology (Wiehlmann et al., 2007). However, with the reducing costs of next-generation sequencing, it seems likely that, in the future, it will be feasible to move straight to whole-genome sequencing.

It has been demonstrated that MLST not only has good discriminatory power but remains effective in the context of CF chronic infections (Kidd et al., 2011). Although a recent study questioned the use of the mutL gene as an appropriate marker for MLST, highlighting the possible pitfalls of using MLST as a stand-alone method (García-Castillo et al., 2012), MLST gives information about clonal relationships and is useful for detecting genetic relatedness (Johnson et al., 2007).

All but two of the clone D isolates in this study were assigned to ST-235, which was identified as the primary founder within the clonal complex (CC) 235 in a recent study of P. aeruginosa isolates from five Mediterranean countries (Maatallah et al., 2011). This study reported that CC235 (consisting of five STs) was the major CC, comprising 27 strains (from a total of 141), 25 of which were serotype O11, which correlates with the majority of keratitis clone D isolates used in this study (Stewart et al., 2011; Winstanley et al., 2005). The exceptions were the two isolates 39267 (serotype 08) and 39131 (serotype O1), which were not identified as ST-235. These two isolates represented a discrepancy between AT genotyping and all of the other methods used in this study. Because of this, we repeated the AT analysis for these two strains at the end of the study. The repeated analysis confirmed their identification according to the AT method as clone D, highlighting the fact that reliance on single genotyping methods may be misleading.

Although our survey of CF isolates from the CF paediatric unit highlighted the possibility that clone D may represent an emerging transmissible strain, a follow-on study of both paediatric (n=38) and adult (n=42) CF patients in Liverpool, conducted in 2008–2011, failed to identify any clone D isolates (unpublished data). This suggests that clone D was transient in the CF community. It is notable that the clone D CF isolates exhibited less genotypic diversity than the clone D keratitis isolates. Although on the face of it this makes perfect sense, given that the CF isolates were from an individual CF unit whereas the keratitis isolates were from diverse UK locations, previous studies have demonstrated that P. aeruginosa populations diversify considerably during chronic lung infections (Cramer et al., 2011; Fothergill et al., 2010a; Mowat et al., 2011). This lack of divergence among our CF isolates also supports the notion that they were transient and unable to establish the kind of long-term infections associated with divergence and diversity.

Although the accessory genome data obtained for the clone D isolates cannot be used to identify specifically the reasons why isolates differed according to the various genotyping methods, variations among strains were apparent and could potentially impact on methods such as PFGE and RAPD. We also observed a greater carriage of the genomic islands represented on the AT chip among the CF isolates. However, this does not preclude the possibility that the keratitis isolates carried alternative unrelated islands that were not represented on the chip, as was shown to be the case for the genome-sequenced isolate 39016 (Stewart et al., 2011). The variation within the CF isolates does suggest that some divergence has occurred, even though there has not been much impact on the ability of the genotyping methods to identify them as clonal.

In the context of CF infections, genotype-based surveillance of P. aeruginosa strains is crucial if emerging transmissible strains are to be identified before they become widespread. However, our study demonstrated that regular surveillance would be advisable in order to identify genuine transmissible strains, and that the use of PFGE or RAPD in this context may be misleading.
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