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How and why to monitor *Pseudomonas aeruginosa* infections in the long term at a cystic fibrosis centre

L. Kalferstova [a], K. Vilimovska Dedeckova [a,b], M. Antuskova [a], O. Melter [a], P. Drevinek [a,b,*]

[a] *Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic*
[b] *Department of Paediatrics, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic*

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SUMMARY

Background: *Pseudomonas aeruginosa* is a major cystic fibrosis (CF) pathogen causing chronic respiratory infections and posing a risk for cross-infection between patients with CF.
Aim: To propose an algorithm for long-term surveillance of *P. aeruginosa* and assess its suitability for monitoring the epidemiological situation at a CF centre with approximately 300 patients.
Methods: Over a nine-year period, over 300 *P. aeruginosa* isolates from 131 infected patients were tested by multi-locus sequence typing (MLST) and/or random amplified polymorphic DNA (RAPD) assay.
Findings: MLST analysis led to the identification of 97 different sequence types which were distributed among 17 RAPD-generated (pseudo)clusters. This indicates that the easy-to-perform RAPD assay is only suitable for intra-individual, not interindividual, strain analyses. No epidemic strains were observed. Longitudinal analysis revealed that 110 of the 131 patients were infected with the same strain over the observation period, whereas 21 patients had a strain replacement or a new infection. Chronic infection was found in 99 of the 131 patients, and the remaining 32 patients met the criteria for intermittent infection (as defined by the Leeds criteria). Eighteen of the 32 patients (56%) with intermittent infection were infected with the same strain for up to nine years.
Conclusion: The strain type only changed in 16% of 131 patients with chronic or intermittent infection. As many as 56% of patients considered to have intermittent infection were actually chronically infected with the same strain for many years.

Introduction

Cystic fibrosis (CF) is the most common hereditary disease of the Caucasian population, caused by a mutation in the gene for chloride ion channels (cystic fibrosis transmembrane conductance regulator). A CF-mediated lung disease is characterized by airway obstruction with thick mucus and altered mucociliary

* Corresponding author. Address: V Uvalu 84, 150 06 Prague, Czech Republic. Tel.: +420 22443 5350; fax: +420 22443 5352.
E-mail address: pavel.drevinek@lfmotol.cuni.cz (P. Drevinek).

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clearance. Both processes lead to persistent inflammation and recurrent and chronic respiratory infections, caused most notably by *Pseudomonas aeruginosa*.[1,2]

Infections with *P. aeruginosa* usually start without any clinical symptoms. Based on microbiological findings, they are commonly interpreted as single positivity or intermittent infections (i.e. less than 50% of samples yielded *P. aeruginosa* over the last 12 months, as per the Leeds criteria).[3] Clinical trials have shown that early antibiotic intervention can eradicate the first episode of *P. aeruginosa* colonization/infection with a success rate of 65–90%.[4,5] Nevertheless, successive re-infections over the years lower the chances of clearing *P. aeruginosa* from the CF respiratory tract, and eventually, the infection becomes persistent and clinically significant. Chronic *P. aeruginosa* infection (i.e. more than 50% of samples grew *P. aeruginosa* over the last 12 months, with a minimum of four samples examined) is reported in 12.5–57% of European adults with CF,[6] and is characterized by repeated events of pulmonary exacerbation and progressive decline in lung function.[7]

Traditionally, acquisition of *P. aeruginosa* was linked to bacterial contamination of a CF patient's immediate environment, and each infected patient was believed to harbour their own unique strain of *P. aeruginosa*. However, in 1996, the Liverpool children's CF centre reported alarming data on cross-infection caused by a highly transmissible beta-lactam resistant strain, known today as the 'Liverpool epidemic strain' (LES).[8] In subsequent years, more *P. aeruginosa* strains were found to be shared by multiple patients, and several other epidemic strains were described.[9,10] The transmission risk of *P. aeruginosa* between patients, highlighted in these studies, has justified the need for infection control and patient cohort segregation at CF centres.[11] More recent studies have demonstrated that compliance with infection control policies reduced or completely eliminated the spread of transmissible strains within a CF community.[12,13] New cases of *P. aeruginosa* infection indicate that the environment (including hospitals) is still a significant, if not the only, source of infection today.

In 2004, the Prague CF centre performed a cross-sectional epidemiological study on *P. aeruginosa* isolates from 69 patients with CF. The analysis revealed strain diversity and good in-vitro susceptibility to antibiotics.[14] It was hypothesized that the absence of single-strain dominance in the studied CF population was due to timely implementation of infection control measures. These were introduced in the Prague CF centre in the mid-1990s during an ongoing large epidemic of *Burkholderia cenocepacia* infection.[15]

The aim of this study was to assess the epidemiological situation of *P. aeruginosa* infections at the Prague CF centre. Nine years ago, an initial study was undertaken and a surveillance programme of CF infections was put in place with optimized laboratory procedures for rapid strain identification. Based on the authors' experience, an easy-to-implement and easy-to-follow protocol for long-term monitoring of *P. aeruginosa* infection in patients with CF is proposed.

Methods

Patients

The Prague CF centre is the largest in the Czech Republic; approximately 300 patients (representing approximately three-fifths of the total Czech population with CF) attend, with a minimum frequency of two visits per year. In 2005, the CF population included 174 children and 77 adults (aged ≥19 years); in 2013, there were 237 children and 139 adult patients with CF. Over the study period (2005–2013), the authors collected and processed 9282 samples from the lower respiratory tract (mostly sputum) from 394 patients. The median sampling frequency was two samples per patient per year, with a range of one to 13 samples per year. In total, 844 *P. aeruginosa* isolates from 222 patients with CF (median 2, range 1–17 per patient) were cultured.

Microbiological investigation related to P. aeruginosa

Prior to strain assignment, every culture of putative *P. aeruginosa* was subjected to confirmatory *P. aeruginosa*-specific polymerase chain reaction that targeted the *oprL* gene.[16] Matrix-assisted lased desorption ionization-time of flight (MALDI-TOF) would also have been acceptable as a confirmatory test, but was not available at the time of the study. Strain typing was performed from extracted bacterial DNA[17] using multi-locus sequence typing (MLST) and/or random amplified polymorphic DNA (RAPD) assay.

MLST was performed according to previously published protocols.[18,19] Briefly, to obtain the best sequence data, the *acsA*, *guaA*, *nuoD*, *ppsA* and *trpE* genes were amplified and sequenced with primers proposed by Curran *et al.*,[18] while primers for the *mutL* gene were adopted from van Mansfeld *et al.*[19] The primers used are detailed in Table A (see online supplementary material). Allele numbers for all seven genes and resulting sequence types (STs) were determined from the publicly available *P. aeruginosa* MLST database (www.pubmlst.org/paeruginosa).

RAPD was performed with primer RAPD 272 (5′-AGCGGGC-CAA-3′) according to the previously described protocol.[20] RAPD-generated DNA fragments were separated on the Agilent 2100 Bioanalyzer (Agilent, Stockport, UK) micro-fluidics platform, and the fingerprint profiles were analysed with BioNumerics Version 5.00 (Applied Maths, Sint-Martens-Latem, Belgium). A cluster analysis with Pearson's correlation was applied to determine similarity values among profiles (similarity >85% was deemed to be representative of the same strain), and MEGA 4 software (UPGMA phylogeny construction algorithm) was used to draw a dendrogram.

Results

Hurdles in P. aeruginosa genotyping for the purpose of CF infection surveillance

To monitor the epidemiological situation in real-time, the authors searched for laboratory methods that would allow inexpensive, less laborious but reliable discrimination of CF *P. aeruginosa* isolates. The plan was to adopt a system in the laboratory for the surveillance of *B. cepacia* complex (Bcc) infections.[21] This Bcc strain-specific diagnostic algorithm combined MLST with RAPD. A single ST matched a single RAPD Bcc cluster; thus, the RAPD analysis was considered to be as suitable and informative as MLST at both intra- and interpatient levels. However, this was not the case for *P. aeruginosa*, where the RAPD-generated similarity trees contained pseudo-clusters