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Original Article

Pseudomonas aeruginosa genotypes acquired by children with cystic fibrosis by age 5-years☆

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Abbreviations: ACFBAL, Australasian Cystic Fibrosis BronchoAlveolar Lavage; BAL, bronchoalveolar lavage; CF, cystic fibrosis; CFU, colony forming units; CI, confidence interval; ERIC, enterobacterial repetitive intergenic consensus; IQR, interquartile range; MLST, multilocus sequence typing; NSW, New South Wales; NZ, New Zealand; OR, odds ratio; PCR, polymerase chain reaction; SA, South Australia; SD, standard deviation; ST, sequence type.

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Abstract

Background: We describe *Pseudomonas aeruginosa* acquisitions in children with cystic fibrosis (CF) aged ≤5-years, eradication treatment efficacy, and genotypic relationships between upper and lower airway isolates and strains from non-CF sources.
Methods: Of 168 CF children aged ≤5-years in a bronchoalveolar lavage (BAL)-directed therapy trial, 155 had detailed microbiological results. Overall, 201/271 (74%) *P. aeruginosa* isolates from BAL and oropharyngeal cultures were available for genotyping, including those collected before and after eradication therapy.
Results: Eighty-two (53%) subjects acquired *P. aeruginosa*, of which most were unique strains. Initial eradication success rate was 90%, but 36 (44%) reacquired *P. aeruginosa*, with genotypic substitutions more common in BAL (12/14) than oropharyngeal (3/11) cultures. Moreover, oropharyngeal cultures did not predict BAL genotypes reliably.
Conclusions: CF children acquire environmental *P. aeruginosa* strains frequently. However, discordance between BAL and oropharyngeal strains raises questions over upper airway reservoirs and how to best determine eradication in non-expectorating children.

Keywords: Pseudomonas aeruginosa; Cystic fibrosis; Infection; Genotyping; Eradication

1. Introduction

In cystic fibrosis (CF), antibiotic-susceptible *Pseudomonas aeruginosa* isolates are acquired from an early age [1,2]. Eventually, a single strain dominates and establishes a chronic infection marked by mucoid colonies, altered phenotypes and increasing antibiotic resistance [3,4]. At this point the organisms can no longer be eradicated and patients have an accelerated pulmonary decline and increased mortality [5,6]. Fortunately, early diagnosis and treatment can prevent or delay the onset of chronic infection [7]. Nevertheless, uncertainties still exist over the origins of the initial infecting *P. aeruginosa* strains, especially in settings where some strains are shared by several patients, suggesting person-to-person transmission [8]. There are also limited and conflicting genotypic data upon whether re-isolated strains differ following eradication treatment [9–14] and if paired upper and lower airway isolates are genotypically indistinguishable [1,15–17]. A better understanding of these issues is crucial for planning *P. aeruginosa* eradication, surveillance and infection control strategies for CF clinics and the CF community.

The Australasian Cystic Fibrosis BronchoAlveolar Lavage (ACFBAL) study, which was designed to determine the role of BAL in directing therapy during pulmonary exacerbations, allowed the opportunity for genotypic evaluation of *P. aeruginosa* strains in young children up to age 5-years and to conduct a secondary analysis of data collected from this randomised controlled trial [18]. Specifically, we describe in this cohort: (i) *P. aeruginosa* acquisition, (ii) efficacy of eradication therapy, (iii) clearance of upper and lower airway strains and (iv) the genotypic relationships between paired upper and lower airway isolates. To help identify the origins of these early strains we also compared their genotypes with strains highly-prevalent in Australian CF clinics and with relatively abundant strains from non-CF clinical, veterinary and environmental sources within Australia [8,19].

2. Methods

2.1. Subjects

The ACFBAL randomised controlled trial is described in detail elsewhere [18]. Briefly, infants aged <6-months and diagnosed with CF following newborn screening in Australian states of New South Wales (NSW), Queensland, South Australia (SA) and Victoria, and in New Zealand (NZ) between June 1999 and April 2005 were recruited. With stratification by site (Australian state or NZ) and gender, they were allocated randomly to either BAL-directed therapy or standard management from before 6-months until age 5-years. Routine clinic review was every 3-months. Each centre followed the Australian CF infection control guidelines that recommend segregating CF patients with *P. aeruginosa* from all other CF patients [20]. Infants recruited from NSW and SA also received flucloxacillin as anti-staphylococcal prophylaxis until aged 12-months. Ethics committees from each centre approved the study and caregivers provided informed consent.

2.2. Interventions

Participants had oropharyngeal swabs taken for culture at enrolment, during pulmonary exacerbations, and after *P. aeruginosa* eradication treatment. Those receiving BAL-directed therapy also had a BAL at enrolment, during hospitalisation for pulmonary exacerbations, if *P. aeruginosa* was detected in oropharyngeal specimens, and after *P. aeruginosa* eradication therapy. Finally, every child had a BAL when completing the study around their 5th birthday.

When a pulmonary exacerbation developed [21], participants came to the CF clinic where staff collected oropharyngeal swab samples and prescribed oral (non anti-pseudomonal) antibiotics. Hospitalisation occurred if *P. aeruginosa* was cultured from oropharyngeal swabs, the treating doctor judged the illness to be severe, or there was no improvement after 6-weeks treatment. Eradication therapy began when *P. aeruginosa* infection was diagnosed by BAL culture ($>10^3$ colony-forming units [CFU]/mL) [22] in the BAL-directed group or by a positive oropharyngeal swab culture in the standard management group. This involved an initial 2-weeks of intravenous tobramycin combined with either ticarcillin-clavulanate or ceftazidime, followed by 1-month of oral ciprofloxacin and 2-months of tobramycin by inhalation solution [18]. Following this round of treatment, *P. aeruginosa* clearance was determined by BAL in the BAL-directed group and oropharyngeal swab cultures in