An aerobiological model of aerosol survival of different strains of *Pseudomonas aeruginosa* isolated from people with cystic fibrosis


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Abstract

*Pseudomonas aeruginosa* is a common and important pathogen in people with cystic fibrosis (CF). Recently epidemic strains of *P. aeruginosa* associated with increased morbidity, have been identified. The method of transmission is not clear, but there is evidence of a potential airborne route. The aim of this study was to determine whether different strains of *P. aeruginosa* isolated from people with CF were able to survive within artificially generated aerosols in an aerobiological chamber.

Viable *P. aeruginosa* could still be detected up to 45 min after halting generation of the aerosols. All of the strains of *P. aeruginosa* expressing a non-mucoid phenotype isolated from people with CF had a reduced ability to survive within aerosols compared to an environmental strain. Expression of a mucoid phenotype by the strains of *P. aeruginosa* isolated from people with CF promoted survival in the aerosol model compared to strains expressing a non-mucoid phenotype.

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Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Aerosol

1. Introduction

*Pseudomonas aeruginosa* is the most common and clinically important pathogen affecting people with cystic fibrosis. *P. aeruginosa* is a gram-negative, non-fermentative, aerobic bacillus belonging to the family Pseudomonadaceae. The organism is ubiquitous within the environment and is particularly isolated from moist areas such as the soil and water. While acquisition of chronic *P. aeruginosa* infection in people with CF can occur at any age, many studies suggest that 70–80% of patients are infected during the teenage years [1], with a number of studies associating chronic infection with *P. aeruginosa* with increased mortality [2–4]. Acquisition of *P. aeruginosa* infection by people with CF is generally thought to be from the environment. However, evidence has emerged recently that cross-infection of certain strains of *P aeruginosa* can occur between people with CF [5–8]. Infection with some of these strains is associated with increased morbidity and treatment burden compared to infection with non-epidemic strains [9,10]. The method of transmission between patients is not clear, however there is some evidence that the airborne route may play an important contributory role [11,12]. In a previous study involving a laminar airflow model, it was found that while little difference existed between strains of *P. aeruginosa* expressing a non-mucoid phenotype, *P. aeruginosa* strains expressing a mucoid phenotype appeared to have a survival advantage in artificially generated aerosols [13]. However, the laminar flow model was limited in its ability to study the long-term survival of droplet nuclei in air, and therefore the present study was devised to characterise differences in aerosol survival for a number of strains of *P. aeruginosa* expressing mucoid and non-mucoid phenotypes using a decay model in an aerobiological chamber.
2. Materials and methods

2.1. Bacterial strains

Isolates of 3 of the strains, Unique CF, Leeds Paediatric and Liverpool, were obtained expressing both mucoid and non-mucoid phenotypes. The remaining strains were only expressed a non-mucoid phenotype (see Table 1). P. aeruginosa (NCIMB 10848) was obtained from the National Collection of Industrial and Marine Bacteria. Clinical isolates of P. aeruginosa were obtained from Department of Microbiology, Leeds General Infirmary and Centre for Infectious Diseases, University of Edinburgh.

2.2. Preparation of bacteria for nebulisation

The bacteria were grown to their maximum stationary phase cell concentration in nutrient broth then stored in stored in 40% (v.v⁻¹) glycerol solution at −20 °C until required. The frozen cultures were defrosted and placed in the nebuliser solution 1 h prior to the start of nebulisation.

2.3. Aerosol decay model

The decay experiments were undertaken in a Class II aerobiological chamber, which had a volume of 32 m³. During experimentation the air temperature, relative humidity and ventilation air change rate were maintained at 21 °C±1 °C, 50%±5% RH and 6 AC h⁻¹ respectively. The environmental conditions were chosen to approximate conditions found within a hospital environment [14]. Aerosols were delivered into the centre of the chamber by a Collison 3-jet nebuliser [15] (BGI, USA) operating at 6 L min⁻¹ and 103.4 kPa, containing 10⁶ CFU mL⁻¹ of P. aeruginosa suspended in 100 mL of 1/4× Ringers solution for 1 h. Aerosol generation was then stopped and standard air samples were taken at 0 min, 3 min, 6 min, 9 min, 12 min, 15 min, 25 min, 35 min and 45 min following stopping of aerosol generation. Air samples were taken by drawing 56.6 L of air for 1 h. Aerosol particles to settle out under the influence of gravity.

2.4. Statistical analysis

The experimental data was modeled to a first order decay model (see Eq. (1)) using GraphPad 5.01 (GraphPad Software Inc) using least squares error analysis. Comparisons between curves were made by determining if there was a significant difference between the inactivation rate k using the F-test. A P value of <0.05 was deemed significant.

\[
\log_c(N_t) = \log_c(N_0) - kt
\]

\(N_0\) = concentration of bacteria at time 0 (CFU L⁻¹)

\(N_t\) = concentration of bacteria at time \(t\) (CFU L⁻¹)

\(k\) = inactivation rate (h⁻¹)

\(t\) = time (h)

(Eq. 1—first order decay kinetics)

In such a model the inactivation rate \(k\) represents the combined effect of natural biological decay arising from loss of bacterial viability and the removal of airborne particles from the aerobiological chamber by the action of the ventilation air [19] (see Eq. (2)).

\[k = k_{\text{bio}} + k_{\text{vent}}\]

\(k_{\text{bio}}\) = inactivation rate due to biological inactivation (h⁻¹)

\(k_{\text{vent}}\) = inactivation rate due to ventilation (AC h⁻¹)

(Eq. 2—factors affecting the inactivation rate \(k\))

In the study the value of \(k\) was determined experimentally and then used to calculate the experimental and biological half-lives using Eq. (3).

\[t_{1/2} = \frac{-\log_c(0.5)}{k}\]

\(t_{1/2}\) = half life (h)

\(k\) = inactivation rate (h⁻¹)

(Eq. 3—calculation of half – life from inactivation rate)

Due to the relatively high ventilation rate (i.e. 6 AC h⁻¹) used in the chamber during the experiments, the mean particle residence time was only 10 min — much too short for most aerosol particles to settle out under the influence of gravity. Consequently, the effect of particle deposition on the chamber surfaces was assumed to be minimal and thus ignored in the model.

3. Results

The environmental strain and all three strains of P. aeruginosa expressing a mucoid phenotype could be isolated...
mucoid phenotype exhibited significantly smaller values for the environmental strain, the strains of strain Bacterial aerobiological chamber model. The concentration of all the strains of samples from the nebuliser suspension demonstrated that between the various strains tested, despite the fact that control in Table 2. From this it can be seen that there is great variability in small numbers up to 35 min after cessation of nebulisation (see Fig. 1).

The overall results of the various experiments are presented in Table 2. From this it can be seen that there is great variability between the various strains tested, despite the fact that control samples from the nebuliser suspension demonstrated that the concentration of all the strains of *P. aeruginosa* was 10^6 CFU mL^-1 both pre- and post-nebulisation. With the exception of the environmental strain, the strains of *P. aeruginosa* expressing a mucoid phenotype exhibited significantly smaller values for \( k_{\text{bio}} \) and therefore correspondingly longer biological half-lives, \( t_{1/2\text{bio}} \), compared with their non-mucoid counterparts (Unique vs Unique Liverpool vs Liverpool mucoid \( P<0.0001; \) Paediatric vs Paediatric mucoid \( P<0.0001; \) Liverpool vs Liverpool mucoid \( P<0.0001 \)).

There was no significant difference between the value for \( k \) of the 3 CF strains of *P. aeruginosa* expressing a mucoid phenotype and the environmental strain of *P. aeruginosa* (F-test \( P=0.9373 \)). The CF strains of *P. aeruginosa* expressing a non-mucoid phenotype formed two groups; one contained the Manchester, Seacroft, Paediatric and Unique strains (F-test \( P=0.9059 \)) and the Liverpool and Liverpool/Seacroft strains formed the remaining group (F-test \( P=0.1932 \)). The environmental strain of *P. aeruginosa* had a significantly smaller value for \( k \) and hence a much longer biological half-life than all of the CF strains expressing a non-mucoid phenotype (Environmental vs Non-mucoid CF strains F-test \( P<0.0001 \)).

### 4. Discussion

Two studies undertaken at CF centres in the UK raised the possibility of airborne dissemination of transmissible *P. aeruginosa* from people as a potential route of cross-infection [11,12]. Using a Cassela slit sampler and a *Pseudomonas*-selective agar Panagea et al. isolated the Liverpool epidemic strain of *P. aeruginosa* from the air in cubicles and the corridors of the CF unit up to 3 h after the individuals with CF had left the area [12]. In this study the only strain of *P. aeruginosa* isolated from the air was the Liverpool transmissible strain. Jones et al. sampled the air following people with CF performing spirometry measurement, airway clearance and nebulisation using a SAS air sampler and *Pseudomonas*-selective agar and isolated both non-epidemic strains and the Manchester epidemic strain of *P. aeruginosa* [11].

A more recent study undertaken in a French CF unit used an Air Test Omega impactor air sampler combined with a *Pseudomonas*-selective media to sample the air in the CF hospital ward, spirometry measurement area and the hospital leisure centre. In this study the levels of airborne contamination within the bedroom were significantly higher after the person with CF had woken up or performed physiotherapy, than after the bedroom had been cleaned. Over half of the 22 people in this study infected with *P. aeruginosa* had air samples taken from their bedroom which were positive for *P. aeruginosa*. In 6 cases where *P. aeruginosa* was isolated from the air, the strain was genetically identical to the strain isolated from the patient’s sputum. The mean concentration of *P. aeruginosa* in the air samples after waking up and following physiotherapy was 154.3 CFU m^-3 and 40.7 CFU m^-3 respectively [20]. An Australian study using a cough aerosol sampling system demonstrated 25 of 26 people with CF chronically infected with *P. aeruginosa* were able to produce aerosols containing viable bacteria. The majority of these bacteria were contained within aerosol particles <3.3 µm in diameter [21]. Collectively the above findings suggest that aerosol particles containing *P. aeruginosa* may be frequently liberated into the air from individuals with CF and may represent a potential reservoir for cross-infection.

Gram-negative bacteria are generally not thought to survive well in the aerosolised state and this may, in part, explain why some have dismissed the possibility of *P. aeruginosa* cross-infection occurring via the airborne route. However, the results

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**Table 2** Biological inactivation rates \( (k_{\text{bio}}) \) and half life \( (t_{1/2}) \) for each strain of *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>( k ) (h^-1)</th>
<th>( k_{\text{bio}} ) (h^-1)</th>
<th>( k_{\text{vent}} ) (AC h^-1)</th>
<th>( t_{1/2\text{exp}} ) (min)</th>
<th>( t_{1/2\text{bio}} ) (min)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>15.3</td>
<td>9.3</td>
<td>6.0</td>
<td>2.727</td>
<td>4.495</td>
<td>0.6485</td>
</tr>
<tr>
<td>Liverpool</td>
<td>53.2</td>
<td>47.2</td>
<td>6.0</td>
<td>0.782</td>
<td>0.882</td>
<td>0.8051</td>
</tr>
<tr>
<td>Liverpool mucoid</td>
<td>16.4</td>
<td>10.4</td>
<td>6.0</td>
<td>2.532</td>
<td>3.989</td>
<td>0.8883</td>
</tr>
<tr>
<td>Manchester</td>
<td>43.2</td>
<td>37.2</td>
<td>6.0</td>
<td>0.964</td>
<td>1.119</td>
<td>0.9655</td>
</tr>
<tr>
<td>Paediatric</td>
<td>38.0</td>
<td>32.0</td>
<td>6.0</td>
<td>1.095</td>
<td>1.300</td>
<td>0.9265</td>
</tr>
<tr>
<td>Paediatric mucoid</td>
<td>16.6</td>
<td>10.6</td>
<td>6.0</td>
<td>2.508</td>
<td>3.989</td>
<td>0.8883</td>
</tr>
<tr>
<td>Seacroft</td>
<td>38.6</td>
<td>32.6</td>
<td>6.0</td>
<td>1.078</td>
<td>1.277</td>
<td>0.8718</td>
</tr>
<tr>
<td>Seacroft/ Liverpool</td>
<td>59.9</td>
<td>53.9</td>
<td>6.0</td>
<td>0.695</td>
<td>0.772</td>
<td>0.9681</td>
</tr>
<tr>
<td>Unique CF</td>
<td>43.6</td>
<td>37.6</td>
<td>6.0</td>
<td>0.954</td>
<td>1.106</td>
<td>0.9221</td>
</tr>
<tr>
<td>Unique CF mucoid</td>
<td>16.2</td>
<td>10.2</td>
<td>6.0</td>
<td>2.569</td>
<td>4.082</td>
<td>0.9588</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Decay curves for different strains of *P. aeruginosa* generated in aerobiological chamber model.
of the decay experiments demonstrate that mucoid strains of 
P. aeruginosa expressing a mucoid phenotype survive well in the 
aerolysed state for many minutes, suggesting that cross-
infection could occur via the airborne route. These findings 
support the data presented previously in a laminar flow study, 
which found the expression of a mucoid phenotype to be 
beneficial to the survival of the bacteria in the aerolysed state 
[13]. The strains of P. aeruginosa isolated from people with CF 
expressing a non-mucoid phenotype CF strains appear to have a 
reduced ability to survive when aerolysed when compared to the 
environmental strain of P. aeruginosa which is also non-
mucoid. This may be a reflection that the environmental strain 
is more equipped for dealing with environmental insults such as 
desiccation, whereas isolates of P. aeruginosa isolated from 
people with CF will have evolved to survive within the CF lung 
and may have lost the ability to resist environmental insults. The 
epidemic strains of P. aeruginosa did not appear to have a 
survival advantage within the aerosols when compared to the 
non-epidemic strains. This would suggest that improved 
environmental survival may not account for the spread of 
epidemic strains of P. aeruginosa which is also non-
mucoid. This may be a reflection that the environmental strain 
is more equipped for dealing with environmental insults such as 
desiccation, whereas isolates of P. aeruginosa isolated from 
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and may have lost the ability to resist environmental insults. The 
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and may have lost the ability to resist environmental insults. The 
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and may have lost the ability to resist environmental insults. The 
people with CF will have evolved to survive within the CF lung 
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people with CF will have evolved to survive within the CF lung 
and may have lost the ability to resist environmental insults. The 
people with CF will have evolved to survive within the CF lung 
desiccation, whereas isolates of P. aeruginosa isolated from 
people with CF will have evolved to survive within the CF lung 
and may have lost the ability to resist environmental insults. The 
people with CF will have evolved to survive within the CF lung 
desiccation, whereas isolates of P. aeruginosa isolated from 
people with CF will have evolved to survive within the CF lung. 

Table 3

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Ventilation rate in area (AC h(^{-1}))</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental</td>
<td>14.9</td>
<td>11.3</td>
<td>9.1</td>
<td>7.6</td>
<td>6.5</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Liverpool</td>
<td>2.9</td>
<td>2.8</td>
<td>2.6</td>
<td>2.5</td>
<td>2.3</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Liverpool mucoid</td>
<td>13.3</td>
<td>10.3</td>
<td>8.4</td>
<td>7.1</td>
<td>6.2</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Manchester</td>
<td>3.7</td>
<td>3.4</td>
<td>3.2</td>
<td>3.0</td>
<td>2.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Paediatric</td>
<td>4.3</td>
<td>3.9</td>
<td>3.6</td>
<td>3.4</td>
<td>3.1</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Paediatric mucoid</td>
<td>13.1</td>
<td>10.2</td>
<td>8.3</td>
<td>7.1</td>
<td>6.1</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Seacroft</td>
<td>4.2</td>
<td>3.9</td>
<td>3.6</td>
<td>3.3</td>
<td>3.1</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Seacroft/Liverpool</td>
<td>2.6</td>
<td>2.4</td>
<td>2.3</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Unique CF</td>
<td>3.7</td>
<td>3.4</td>
<td>3.2</td>
<td>3.0</td>
<td>2.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Unique CF mucoid</td>
<td>13.6</td>
<td>10.5</td>
<td>8.5</td>
<td>7.2</td>
<td>6.2</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

Using Eq. (4) it is possible to estimate how the concentration of bioaerosol particles will vary with time for any clinical space. The results in Table 3 are calculated using Eq. (4). These show the time required to remove 90% of bacteria from a room space under various ventilation conditions. These data suggest that, providing that the ventilation rate is greater than 6 AC h\(^{-1}\), within 10 min of an individual with CF leaving a clinical area, over 90% of the P. aeruginosa that may have disseminated into the air, will have been removed through a combination of ventilation and biological inactivation. Although, the UK Department of Health guidelines for hospital ventilation [14] do not specifically specify ventilation rates (i.e. outdoor air changes) rooms where treatment of people with CF is undertaken, they suggest a ventilation rate of 12 AC h\(^{-1}\) for areas such as infectious diseases isolation rooms, critical care areas and wards used for the care of neutropaenic patients. If this ventilation rate was adopted in the context of the care of people with CF, then allowing 7 min between one person with CF leaving an area and another person with CF entering the area should ensure the removal of >90% of the bacteria from the room air, thus greatly reducing the risk of cross-infection.

There are limitations to this study due to the small numbers of P. aeruginosa strains studied and we would suggest further studies with a greater number of strains to try and determine both genetic and phenotypic factors that may influence the survival of P. aeruginosa within aerosols.

An important consideration when trying to determine the risk of contagion is the infectious dose required to establish lung colonization/infection in people with CF following inhalation of aerosol particles containing P. aeruginosa. Unfortunately, no data exists regarding this issue and it would not be ethical to undertake studies to try and determine the infectious dose in people with CF. However, data from research related to biological warfare agents suggests that bacteria can produce disease with the inhalation of as few as 1–100 organisms [22].

Given the defects in the innate immunity of the CF lung, it is therefore entirely plausible that P. aeruginosa cross-infection could be occurring via the airborne route in the clinical setting. However, further work needs to be undertaken to quantify the infectious dose required. A methodology does exist for evaluating the infectious dose associated with airborne diseases such as tuberculosis [23,24]. However, this methodology has never been applied to the care of people with CF, mainly

\[e^{(e^{-(k_{bio} + k_{vent}) \times t})},\]

\[\text{where } N(t) = \text{concentration of bacteria at time } t (\text{CFU L}^{-1}),\]

\[\text{and } N(0) = \text{concentration of bacteria at time } 0 (\text{CFU L}^{-1}).\]

\[k_{bio} = \text{inactivation rate due to biological inactivation (h}^{-1}\)],\]

\[k_{vent} = \text{inactivation rate due to ventilation (AC h}^{-1}\)],\]

\[t = \text{time (h)}\].

(Eq. 4—first order decay taking into account biological decay and ventilation)
because of the multiple confounding factors. Consequently, little information exists with which to assess the performance of potential interventions such as improved room ventilation, ultra-violet light, ionization of the air, or even drying of the air.

Conflict of interest

The authors declare no competing financial interests.

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