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Complete List of Authors:	Fletcher, Louise; University of Leeds, Department of Civil Engineering Chen, Yang; University of Leeds, Department of Civil Engineering Whitaker, Paul; Leeds Teaching Hospitals NHS Trust, Regional Adult Cystic Fibrosis Unit Denton, Miles; Leeds Teaching Hospitals NHS Trust, Department of Microbiology, Peckham, Daniel; Leeds Teaching Hospitals NHS Trust, Respiratory Medicine Clifton, Ian; Leeds Teaching Hospitals NHS Trust, Respiratory Medicine
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Survival of *Mycobacterium abscessus* isolated from people with cystic fibrosis in artificially generated aerosols

LA Fletcher[1], Y Chen[1], PA Whitaker[2], M Denton[3] DG Peckham[2], IJ Clifton[2]

1. Department of Civil Engineering, University of Leeds, Leeds, UK
2. Regional Adult Cystic Fibrosis Unit, St James's University Hospital, Leeds, UK
3. Department of Microbiology, Leeds General Infirmary, Leeds, UK

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Non-tuberculous mycobacterium (NTM) are increasingly found in the sputum of people with CF, both in Europe and North America [1]. Specifically, *Mycobacterium abscessus* has emerged as a potentially important pathogen, with evidence of accelerated lung function decline [2]. Studies from two CF centres have found evidence of cross-infection between individuals with CF [3, 4], whereas studies from other centres have not replicated this finding [5-7]. *M. abscessus* has been isolated from household water and has been previously isolated from shower aerosols of people with pulmonary NTM disease [8, 9].

The aim of this study was to determine whether NTM could survive within artificially generated aerosols using a previously described laminar airflow model [10].

Four clinical isolates of *M. abscessus*, one clinical isolate of *M. chelonae* and a reference stain of *M. abscessus (*NCTC 13031 / ATCC 19977) were studied. The clinical NTM strains were isolated from individuals with CF attending a regional CF Centre. All NTM isolates were identified at a reference laboratory using a commercial kit (GenoType Mycobacterium, Hain Lifescience, GmbH, Nehren, Germany). Isolates confirmed as Mycobacterium abscessus were sub-speciated by PCR and sequencing of hsp65 and rpoB targets. Genotyping to identify strain clusters was performed using Variable Number Tandem Repeat (VNTR) based on the method of Harris et al [11]. The *M. abscessus* sub sp. *massiliense* strains studied were all isolated from unique individuals with CF. The strain of *M. abscessus* sub sp. *abscessus* (VNTR type ST26) studied had been isolated from more than one individual in our CF cohort. All clinical isolates were associated with chronic infection as defined by ATS/IDSA criteria [12].

All strains were examined in a laminar airflow model as previously described within a negatively pressurised Class II aerobiological chamber [10]. Aerosols were generated using a Collison 3-jet nebuliser (BGI, USA) containing suspensions of bacteria within 100mL ¼xRingers solution. The concentration of bacteria within the nebuliser suspension was determined both pre- and post-nebulisation using serial dilution.

The aerosols were delivered into a 110mm diameter air-tight pipe with a variable length. In order to prevent cross-contamination was the pipe was sterilised by cleaning thoroughly with Virkon solution before each experiment. The pipe was then vented with sterile air via a HEPA filter for 30 minutes before each experiment. Steady state conditions were ensured by allowing the apparatus to run for 10 minutes prior to air sampling. During each sampling event 56.6L of air was drawn through an Andersen 6-stage impactor (Andersen Inc, USA) containing nutrient agar plates. The plates were then incubated and the concentration of viable bacteria in the air sample was determined. During experimentation the length of the laminar flow apparatus was varied, and 5 air samples were taken at lengths of 2m and 4m, which equates to aerosol ages of 40.3s and 80.6s respectively. In order to determine the size distribution of the droplet nuclei generated, Stages 1-6 of the Andersen sampler were used at all lengths of the model.

Statistical analysis was undertaken using GraphPad (Version 6.07, GraphPad Inc, USA)

All strains of NTM were able to survive in particles of ≤2µm in diameter within the artificially generated aerosols (See Figure 1A). All of the strains studied were able to survive for 80.6s and travel 4m within the aerosols (See Figure 1B). There was a semi-log relationship between the concentration of NTM in the nebuliser suspension and the concentration of viable organisms in the aerosol (R^2=0.8728) (See Figure 1B).

This study demonstrates that NTMs can survive within aerosolised droplet nuclei particles within the respirable size range. The particle size distribution of the aerosols within this model were smaller

than that demonstrated to be produced during coughing by individuals from CF by Wainwright et al [13]. It would be important to demonstrate that people with CF can produce aerosols containing these pathogens. The behaviour of NTMs within respiratory secretions from people with CF may be different to that demonstrated in these artificially generated aerosols. The ability of NTMs to survive within this model appeared superior to that of *P. aeruginosa.* Different *P. aeruginosa* strains at a concentration of 10^6 CFU/mL in ¼Ringer solution produced aerosols containing less than 40,000 CFU/M3 of viable bacteria [10]. We subsequently demonstrated these strains of *P. aeruginosa* could survive for at least 40 minutes within droplet nuclei in a different aerobiological model [14].

All strains appeared to have similar characteristics in terms of airborne surivival. There does seem to be a relationship between organism load in the nebuliser and concentration in the aerosol. This raises the possibility that individuals with high mycobacterial load in the sputum may represent a higher risk of generating potentially infectious aerosols. Wainwright et al demonstrated that the higher concentrations of bacteria within sputum was associated with a greater concentration of bacteria within aerosols proceeded from people with CF during coughing [13].

Bryant et al did not demonstrate a common environmental source of their outbreak despite extensive sampling, but air samples in clinical areas were not taken [4]. They postulated that an airborne route of cross-infection may be possible and these data would support this hypothesis. This has important implications for the care of people with CF and reinforces the need for strict infection control practices. In response to the outbreak the Papworth group have introduced segregation of individuals infected with *M. abscessus* in the out-patients environment and the use of negative pressure rooms for in-patient stays.

This study is limited due to the small number of strains studied, and further work needs to be undertaken to examine the survival of other mycobacterial and bacterial pathogens important to the care of people with CF within the air in both laboratory and clinical conditions. This may then lead to the development of strategies and interventions that may reduce down the risk of cross-infection of harmful pathogens between people with CF.

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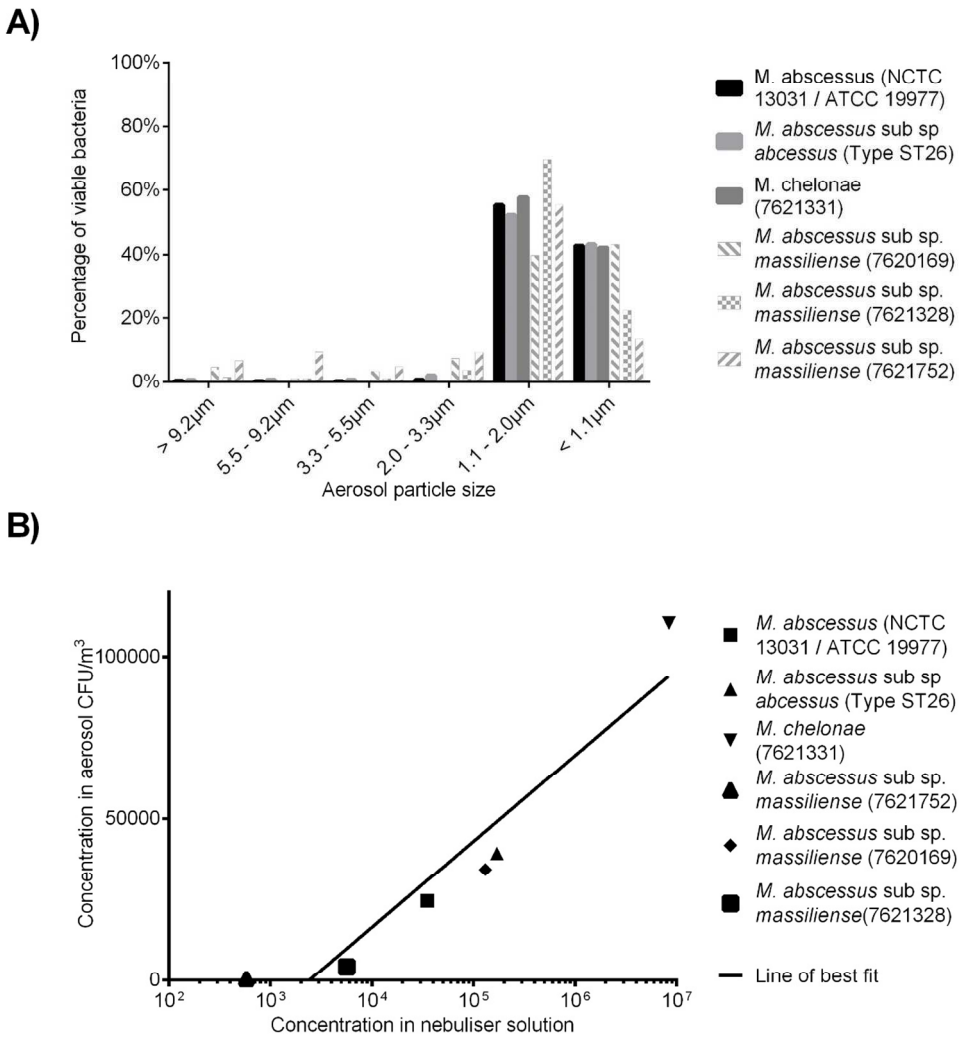

Figure 1 – 1A) Size distribution of aerosol particles containing NTM in the laminar air flow model. 1B) Relationship between concentration of viable bacteria within an aerosol age of 81 seconds and concentration in nebuliser solution. Solid line represents line of best fit.

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