Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study

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Summary

Background Increasing numbers of individuals with cystic fibrosis are becoming infected with the multidrug-resistant non-tuberculous mycobacterium (NTM) Mycobacterium abscessus, which causes progressive lung damage and is extremely challenging to treat. How this organism is acquired is not currently known, but there is growing concern that person-to-person transmission could occur. We aimed to define the mechanisms of acquisition of M abscessus in individuals with cystic fibrosis.

Method Whole genome sequencing and antimicrobial susceptibility testing were done on 168 consecutive isolates of M abscessus from 31 patients attending an adult cystic fibrosis centre in the UK between 2007 and 2011. In parallel, we undertook detailed environmental testing for NTM and defined potential opportunities for transmission between patients both in and out of hospital using epidemiological data and social network analysis.

Findings Phylogenetic analysis revealed two clustered outbreaks of near-identical isolates of the M abscessus subspecies massiliense (from 11 patients), differing by less than ten base pairs. This variation represents less diversity than that seen within isolates from a single individual, strongly indicating between-patient transmission. All patients within these clusters had numerous opportunities for within-hospital transmission from other individuals, while comprehensive environmental sampling, initiated during the outbreak, failed to detect any potential point source of NTM infection. The clusters of M abscessus subspecies massiliense showed evidence of transmission of mutations acquired during infection of an individual to other patients. Thus, isolates with constitutive resistance to amikacin and clarithromycin were isolated from several individuals never previously exposed to long-term macrolides or aminoglycosides, further indicating cross-infection.

Interpretation Whole genome sequencing has revealed frequent transmission of multidrug resistant NTM between patients with cystic fibrosis despite conventional cross-infection measures. Although the exact transmission route is yet to be established, our epidemiological analysis suggests that it could be indirect.

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Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental organisms that cause chronic pulmonary infection in patients with inflammatory lung diseases—such as cystic fibrosis, non-cystic fibrosis bronchiectasis, and chronic obstructive pulmonary disease—as well as in certain individuals with no definable risk factors.1-5 Of the rapidly growing NTM species, the multidrug resistant Mycobacterium abscessus has emerged as a major respiratory pathogen particularly in individuals with cystic fibrosis, where it leads to accelerated decline in lung function and can prevent safe lung transplantation.6 Treatment of M abscessus is challenging, requiring extended therapy with poorly tolerated combination antibiotics, and frequently fails.6-8 Recent evidence9,10 has suggested that M abscessus can be divided into three subspecies (subsp): Mycobacterium abscessus subsp abscessus, M abscessus subsp massiliense, and M abscessus subsp bolletii. These subspecies are not currently distinguished by hospital laboratories, but they could have different clinical behaviours.9,10 Worryingly, pulmonary infections with M abscessus have become more common. Studies from Taiwan,11 USA,12 and Australia13 have all reported significant increases in the prevalence of M abscessus pulmonary infection over the past decade. Between 3% and 10% of individuals with cystic fibrosis in the USA and Europe are now infected with M abscessus,14,15 with increasing prevalence not attributable to changes in screening intensity or laboratory culture methods.16 The reasons for increased M abscessus infections in patients with cystic fibrosis are unclear but could include: greater exposure to NTM from biofilms in showerheads,17 the creation of permisive lung niches through increased inhaled antibiotic usage,18 and impairment of host antimycobacterial immunity through autoagglutination by chronic azithromycin therapy.19 Although person-to-person transmission of NTM has never been definitively proven,
the concern that *M abscessus* could be spread between patients with cystic fibrosis has recently increased.

Previous studies have suggested independent acquisition of NTM from the environment by patients with cystic fibrosis. In the USA, Olivier and colleagues\(^ {19} \) analysed 140 NTM isolates (16% of which were *M abscessus*) obtained from cystic fibrosis centres and found near-unique *hsp65* sequences. In France, Sermet-Gaudelus and colleagues\(^ {20} \) examined *M abscessus* isolates from 14 patients, and found them to be unique by pulsed field gel electrophoresis (PFGE). However, both studies had few patients with NTM from the same cystic fibrosis centre and analysed patient cohorts from more than a decade ago. More recently a potential outbreak of *M abscessus* subsp *massiliense* was reported after the transfer of a smear-positive patient to a cystic fibrosis centre in Seattle.\(^ {21} \) Four other patients were identified as infected with a strain that was indistinguishable by PFGE and PCR analysis from that of the index case. Other studies have also suggested that *M abscessus* isolates from patients attending the same hospital could be genetically very closely related.\(^ {22,23} \)

However, definitive proof of person-to-person transmission has been lacking. Conventional typing methods such as PCR or PFGE do not have the molecular resolution to allow accurate analysis of the population structure and modes of acquisition of mycobacteria, owing to their slow mutation rate.

To understand how individuals with cystic fibrosis acquire *M abscessus* infection and to establish whether patient-to-patient transmission does occur, we undertook whole genome sequencing on 168 consecutive isolates from 31 infected patients attending the Cambridge Centre for Lung Infection at Papworth Hospital, UK, a large adult cystic fibrosis centre.

**Methods**

**Sample collection**

From October, 2007 to April, 2011, we collected and stored every isolate of NTM as frozen aliquots from mycobacterial growth indicator tube (MGIT) samples. We analysed all recoverable isolates from every patient with pulmonary *M abscessus* infection. After initial culture on solid media, we collected sweeps of *M abscessus* colonies to subculture (to remove contamination while maintaining genetic diversity). One isolate was excluded because of likely culture contamination. We thereby obtained pure cultures of 168 separate isolates of *M abscessus* from 31 patients. DNA was extracted from these isolates for sequencing and, in parallel, antibiotic susceptibility testing was done (appendix). The date and location of hospital attendances (inpatient and outpatient) and bronchoscopies were obtained for patients from the first date they were seen in Papworth until the end of the study period. Sampling was done on hospital water supplies, showerheads, and bronchoscopes, as well as local ponds and rivers.

**Whole genome sequencing and phylogenetic analysis**

Multiplexed paired end sequencing was done on the Illumina HiSeq platform. Variation in the form of single nucleotide polymorphisms (SNPs) was detected using a mapping approach applying stringent filters. A maximum likelihood tree was built using the SNPs detected. For more accurate phylogenetic analysis within individual subspecies, additional representative references were assembled. To estimate mutation rate and the age of clusters, Bayesian inference was implemented in BEAST\(^ {24} \) version 1.6.1, a program used for Bayesian Markov chain Monte Carlo analysis of genetic sequences (appendix). Raw reads were deposited on the European Nucleotide Archive under study accession number ERP001039.

**Statistical analysis**

Date and location data were analysed to identify possible cross-infection events between patients. Social network analysis was used to compare the occurrence of cross-infection between genetically clustered and unclustered cases (appendix). Data were extracted from the following electronic systems: Patient Administration System (PAS), the Clinical Research Information System (CRIS), the Cardiovascular Information System (CVIS) and the Respiratory Physiology Database. Data for patient location was also collected from nursing handover notes and discrepancies were verified from the original notes. All clinical data were initially stored in Excel and subsequently managed using a SQL database. Analysis was done with Excel, Cytoscape, and Stata 12.0.

**Role of the funding source**

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

Clinical details, demographic information for infected patients, and dates of isolates analysed are given in the appendix. Using whole genome sequencing, we constructed a phylogenetic tree from *M abscessus* isolates obtained from patients within our adult cystic fibrosis and bronchiectasis centre (figure 1; appendix). We identified deep genetic divisions corresponding to the three *M abscessus* subspecies,\(^ {26} \) which nevertheless showed high overall genetic similarity with an average nucleotide identity of 99.1%. Although combining *M abscessus* subsp *massiliense* and *M abscessus* subsp *bolletii* has been suggested,\(^ {27} \) these are clearly as distinct from each other as each is from *M abscessus* subsp *abscessus*. Of the 31 patients, 13 were infected with *M abscessus* subsp *abscessus*, 15 with *M abscessus* subsp *massiliense*, and two with *M abscessus* subsp *bolletii*. One patient was coinfected with both *M abscessus* subsp...
*abscessus* and *M abscessus subsp massiliense* and was excluded from further analysis.

We noted tight clustering of isolates from each individual, indicating near-identical genomic sequences, and many examples of large genetic differences between isolates from different individuals, consistent with independent acquisition by patients of genetically diverse organisms from the environment. However, among both *M abscessus subsp abscessus* and *M abscessus subsp massiliense* samples, there were also clear examples of grouping of isolates from different samples, there were also clear examples of grouping of isolates from different individuals (figure 1). Within the *M abscessus subsp abscessus* cluster, isolates from six patients showed high levels of relatedness but were clearly segregated from one another.

By contrast, within both *M abscessus subsp massiliense* clusters (figure 1), we found that isolates from different individuals had near-identical genomic sequences that, on phylogenetic analysis, were often more closely related to each other than to other samples from the same individual. Furthermore, in cluster 2 the genetic diversity of isolates from patient 28 was entirely encompassed within that of samples from patient 2, indicating immediate relatedness by direct descent. These findings strongly indicate multiple episodes of transmission of *M abscessus subsp massiliense* between patients.

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**Figure 1:** Phylogenetic tree of *M abscessus* isolates indicates different modes of likely transmission
(A) Maximum likelihood tree of all isolates sequenced generated by mapping reads against the *M abscessus* (from Malaysian patients without cystic fibrosis [M115, M154, M139]; from a French patient without cystic fibrosis [CCUG48898]; from a patient in Birmingham UK with cystic fibrosis [47J26]; and from a surgical outbreak in Brazil [GO-06]) and *M abscessus subsp bolletii* (from a sputum isolate from France [CIP108541]).
The phylogeny was also supplemented with publicly available isolates from the UK, Brazil, and Malaysia. None of the additional isolates fell within the clusters identified, but were distributed broadly across the tree, indicating that our sample is representative of the species diversity. One isolate from a patient in Birmingham was found to be 76 base pairs different from one of the *M abscessus* subsp *massiliense* clusters, suggesting that this clade (*M abscessus* subsp *massiliense* cluster 1 and 2) is not specific to Papworth.

To further analyse the possibility of transmission of *M abscessus* subsp *massiliense*, we examined the distribution of genetic similarity between individual isolates, expressed as pairwise distances, and identified three distinct modes of similarity (figure 2). The first mode, with greater than 10000 base pair differences, constitutes comparisons of isolates from different *M abscessus* subspecies and from non-clustered *M abscessus* strains. The second mode, encompassing pairwise differences of 50–200 base pairs, is made up of comparisons of isolates from within the *M abscessus* subsp *abscessus* cluster and between different *M abscessus* subsp *massiliense* clusters. The final mode, with less than 25 base pair differences, represents the diversity of samples from single individuals and the similarity of different patients’ isolates within each *M abscessus* subsp *massiliense* cluster.

These results suggest that for *M abscessus* subsp *abscessus*, patients have independently acquired either genetically diverse strains (non-clustered isolates) or a dominant circulating clone (loosely-clustered isolates). In the case of *M abscessus* subsp *massiliense* however, although some patients acquired strains which were unclustered, most have been infected with one of two clones. The genetic difference between these patients' isolates is often less than that among isolates from a single individual, strongly indicating transmission between patients.

We next examined the pattern of antibiotic resistance in *M abscessus* subsp *abscessus* and *M abscessus* subsp *massiliense* isolates obtained from patients before or shortly after starting treatment (table). We focused on macrolide and aminoglycoside resistance, since the underlying molecular mechanisms are well established and *in vitro* testing predicts clinical response to treatment.28 As expected, most *M abscessus* subsp *abscessus* isolates showed inducible macrolide resistance,29 known to be mediated through upregulation of the ribosomal methyltransferase *erm*(41), while all non-clustered *M abscessus* subsp *massiliense* isolates remained fully susceptible to
clarithromycin after prolonged in vitro macrolide exposure, resulting from an inactivating erm(41) deletion found universally in this subspecies. However, M abscessus subsp massiliense isolates from cluster 1 and 2 were found to have high-level, constitutive macrolide resistance due to single point mutations in the 23S ribosomal rRNA (A2058C for cluster 1 and A2058G for cluster 2). Although constitutive macrolide resistance in NTM is thought to result from previous antibiotic exposure, we noted that three of the patients from cluster 1 had not previously taken long-term macrolide antibiotics, once again suggesting transmission between patients, rather than independent acquisition from the environment.

M abscessus subsp massiliense cluster 1 isolates showed high-level amikacin resistance (minimum inhibitory concentration [MIC] >64 mg/mL) owing to the same point mutation (A1408G) in their 16S ribosomal rRNA. Isolates from the presumed index case, patient 8, became resistant to aminoglycosides during NTM treatment with an amikacin-based regimen. Of the other patients in cluster 1, four had not previously taken nebulised aminoglycosides (table), again suggesting transmission of a resistant clone from patient 8.

Although not supported by our genetic data, we next examined whether an ongoing point source of infection might be responsible for the outbreak of each clustered M abscessus subsp massiliense clone. Given the period during which new patients were infected, any potential point source would have to be genetically stable and persistent in the environment for more than 3 years.

We first established whether patients infected with the transmitted M abscessus subsp massiliense clones might live close together or share the same potable water supply, which has been implicated as a possible source for acquisition of M abscessus. As shown in the appendix, patients infected with the clustered M abscessus subsp massiliense strains or the dominant M abscessus subsp abscessus clone are not geographically grouped and do not share the same home water supply.

We also undertook extensive environmental sampling within the hospital cystic fibrosis centre, which began during the outbreak in June, 2010. The hospital water

| Macrolide response | Aminoglycoside response |
|--------------------|-------------------------|
| Prior chronic macrolide exposure | Clarithromycin sensitivity | Minimum inhibitory concentration (μg/mL), day 5/14 | Erm(41) 23S rRNA A2058G residue | Prior chronic aminoglycoside exposure | Amikacin sensitivity | Minimum inhibitory concentration (μg/mL) 16S rRNA residue 1408/1409 |

### M abscessus subsp abscessus cluster

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 4 | No | Inducible | 2/>16 | T28 | A | No | Sensitive | 16 | A/C |
| 5 | Azithromycin | Inducible | 8/>16 | T28 | A | Nebulised tobramycin | Resistant | >64 | G/C |
| 11 | No | Inducible | 0 6/16 | T28 | A | No | Sensitive | 4 | A/C |
| 15 | No data | Inducible | 0 5/>16 | T28 | A | No data | Resistant | >64 | A/T |
| 21 | No data | Inducible | 0 5/>16 | T28 | A | No data | Sensitive | 8 | A/C |
| 23 | No data | Inducible | 0 5/>16 | T28 | A | No data | Sensitive | 8 | A/C |

### M abscessus subsp abscessus non-clustered

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 13 | No | Sensitive | 0 6/0 06 | Deletion | A | Nebulised tobramycin | Sensitive | 2 | A/C |
| 18 | No | Sensitive | 0 6/0 06 | Deletion | A | No | Sensitive | 2 | A/C |
| 25 | No data | Sensitive | 0 12/0 5 | Deletion | A | No data | Sensitive | 8 | A/C |
| 27 | No data | Sensitive | 0 25/0 5 | Deletion | A | No data | Sensitive | 16 | A/C |

### M abscessus subsp massiliense cluster 1

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 8 | Azithromycin | Resistant | >16/>16 | Deletion | C | No | Resistant* | >64 | G/C |
| 12 | Azithromycin | Resistant | >16/>16 | Deletion | C | No | Resistant* | >64 | G/C |
| 14 | No | Resistant* | >16/>16 | Deletion | C | Nebulised tobramycin | Resistant | >64 | G/C |
| 17 | Azithromycin | Resistant | >16/>16 | Deletion | C | No | Resistant* | >64 | G/C |
| 19 | No | Resistant* | >16/>16 | Deletion | C | No | Resistant* | >64 | G/C |
| 20 | Azithromycin | Resistant | >16/>16 | Deletion | C | Nebulised tobramycin | Resistant | >64 | G/C |
| 22 | Azithromycin | Resistant | >16/>16 | Deletion | C | Nebulised tobramycin | Resistant | >64 | G/C |
| 29 | Azithromycin | Resistant | >16/>16 | Deletion | C | Nebulised amikacin | Resistant | >64 | G/C |
| 30 | No | Resistant* | >16/>16 | Deletion | C | No | Resistant* | >64 | G/C |

### M abscessus subsp massiliense cluster 2

|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 2 | No data | Resistant | >16/>16 | Deletion | G | No data | Sensitive | 16 | A/C |
| 28 | Azithromycin | Resistant | >16/>16 | Deletion | G | No data | Sensitive | 16 | A/C |

Antibiotic resistance determined by broth microdilution was compared with molecular analysis of the ribosomal methyltransferase erm(41), and 23S and 16S ribosomal RNA sequences. Isolates from patient 8 became resistant to aminoglycosides during NTM treatment with an amikacin-based regimen. Resistance defined as minimum inhibitory concentration >16 μg/mL for clarithromycin and >64 μg/mL for amikacin. *Patients with isolates resistant to clarithromycin or amikacin through ribosomal mutations but with no prior chronic exposure to macrolides or aminoglycosides.

Table: Antibiotic resistance patterns for M abscessus isolates, by patient number
supply, which is chlorinated on site, was extensively sampled and repeatedly found to be culture-negative and PCR-negative for mycobacteria. Showerheads, dishwashers, and bronchosocopes (to which only four of the patients within cluster 1 and 2 were exposed before their first positive sample) were also shown to be free from NTM. Furthermore, samples from the River Cam and the Papworth Hospital pond were also negative for *M abscessus*. These findings, together with our genetic data, indicate that independent environmental acquisition of *M abscessus subsp. massiliense* from the same point source is unlikely.

We next investigated whether there were opportunities for transmission of *M abscessus subsp. massiliense* between patients with cystic fibrosis. Although we could not find occasions outside the hospital where direct patient-to-patient transmission might have occurred, we did identify clear opportunities for cross-infection within the cystic fibrosis centre for all patients from the two *M abscessus subsp. massiliense* clusters (figure 3). Except for the presumed index cases (patient 8 in cluster 1 and patient 2 in cluster 2), all previously uninfected patients were present at the centre at the same time as an infected individual on multiple occasions.

By contrast, patients infected with the grouped *M abscessus subsp. abscessus* isolates had no clear opportunities for cross-infection within or outside the cystic fibrosis centre (figure 3), further supporting our view that these isolates represent a dominant circulating clone rather than recent transmission within the hospital environment.

The possibility of within-hospital transmission of NTM was further supported by more detailed epidemiological analysis. Individuals within *M abscessus subsp. massiliense*...
Whole genome sequencing has provided the first convincing evidence for transmission of M abscessus by cystic fibrosis patients. We recorded numerous examples of independent acquisition of genetically diverse strains of all three subspecies, most likely representing independent acquisition from the environment. We also found clusters of patients with genetically related clones of M abscessus which, although identical by hsp65 PCR techniques, have roughly 50–200 base pair differences from each other and are estimated to have diverged from a common ancestor decades ago. These loose clusters are likely to represent independent acquisition of a dominant clone circulating in the wider community; a pattern frequently seen with Pseudomonas aeruginosa. Additionally, we identified multiple examples of genetically identical or very similar isolates from different patients. The recent increase in frequency of M abscessus infections in cystic fibrosis patients is likely related to the emergence of a specific clone which has been transmitted through the hospital environment.

**Discussion**

Whole genome sequencing has provided the first convincing evidence for transmission of M abscessus (panel). Although limited to analysis of one centre, our study indicates that acquisition of M abscessus subsp massiliense by cystic fibrosis patients is frequently through cross-infection.
near-identical strains of \textit{M abscessus} subsp \textit{massiliense} found in different individuals, indicating direct or indirect person-to-person transmission. Since previous studies,\textsuperscript{19,20} done at a time of low infection rates with \textit{M abscessus}, showed genetically unique isolates from individual patients with cystic fibrosis (on the basis of PFGE or \textit{hsp65} sequencing), we could speculate that transmissible clones have emerged over the past decade and spread through this patient group in a similar way to epidemic strains of \textit{P aeruginosa}.

Recent transmission of \textit{M abscessus} subsp \textit{massiliense} is also supported by several lines of evidence. The genetic variation of isolates from particular individuals is entirely encompassed by that seen within another patient’s samples, indicating direct sequential evolution of strains—a pattern incompatible with infection from a point source. Diversity analysis shows that the genetic variation of isolates seen within one person (representing \textit{de novo} accumulation of mutations by an infecting clone) is often greater than the genetic diversity seen between isolates from different individuals, once again indicating transmission events between patients. By examining patterns of antibiotic resistance, we find that the clustered \textit{M abscessus} subsp \textit{massiliense} strains have high level, constitutive clari-thromycin and amikacin resistance, which is usually only seen in isolates from patients receiving long-term macrolide and aminoglycoside therapy. However, several individuals infected with these resistant isolates had no history of relevant antibiotic exposure, again further indicating acquisition of another patient’s strain. This genetic evidence of transmission was supported by the fact that we were clearly able to define multiple opportunities for transmission of these specific clones between patients. Furthermore, Bayesian estimates place the age of the most recent common ancestral clone for each of the \textit{M abscessus} subsp \textit{massiliense} clusters within the periods when these transmission opportunities existed.

Despite our evidence for transmission of \textit{M abscessus} subsp \textit{massiliense} between patients, the exact mechanism of cross-infection remains to be established. There are several possibilities. We believe that direct person-to-person spread is unlikely. The Cystic Fibrosis Centre at Papworth Hospital adopted strict infection control policies in 2004 enforcing individual patient segregation in accordance with UK Cystic Fibrosis Trust guidelines.\textsuperscript{13} Thus, patients are advised not to meet socially and are cared for in individual rooms while receiving inpatient treatment and during outpatient clinic review. We therefore assume that transmission probably occurs indirectly. The ability of \textit{M abscessus} to withstand desiccation and other physical stresses and its resistance to many disinfectants\textsuperscript{19} might promote transmission via fomite contamination. Alternatively, aerosol generation during physiotherapy and lung function testing could lead to cross-infection

**Panel: Research in context**

**Systematic review**

Increasing numbers of individuals with cystic fibrosis are becoming infected with the multidrug-resistant non-tuberculous mycobacteria (NTM) \textit{Mycobacterium abscessus}.\textsuperscript{16–14}

How this organism is acquired is not currently known but there is growing concern that person-to-person transmission might occur. We searched Medline and Pubmed for articles published in any language before January, 2013, with the keywords “nontuberculous” or “\textit{Mycobacterium abscessus}” for evidence of studies addressing the mechanism of acquisition of \textit{M abscessus}. Several studies have identified shared genotypes between patients with cystic fibrosis attending the same clinic, indicating possible transmission,\textsuperscript{19,20} whereas others observed unique strains suggesting no such relationship.\textsuperscript{19,20} However, all of these studies were limited both by the number of patients and the resolution of typing techniques used. Our study describes the first use of whole genome sequencing to analyse the mechanisms of acquisition of NTM.

**Interpretation**

The combination of whole genome sequencing and detailed epidemiological analysis has provided the first convincing evidence for transmission of \textit{M abscessus}. Although limited to analysis of one centre, our study indicates that acquisition of this NTM by cystic fibrosis patients is frequently through cross-infection and occurs despite conventional infection control measures. As such these findings will have profound implications for how patients with cystic fibrosis are cared for in hospital. They also raise the possibility for cross-infection in other patient groups and with other NTM species.
through inhalation of airborne water droplets, from which NTM have been cultured in the environment.\(^{11}\) We have also shown that transmission can occur from patients with persistently smear-negative, culture-positive sputum, suggesting that the inoculum needed for successful infection could be low.

Although we cannot exclude the possibility that M. abscessus subspecies abscessus and M. abscessus subspecies bolletii could also be transmissible, our data only identified cross-infection events for M. abscessus subspecies massiliense clones, raising the possibility that this subspecies might be more infectious. The extent of M. abscessus cross-infection between individuals in other cystic fibrosis centres or indeed among patients without cystic fibrosis is unclear, although the sequence of a patient isolate from Birmingham is closely related to the two M. abscessus subspecies massiliense clusters we have identified, suggesting that this clade is not unique to Papworth and could be particularly adapted to cystic fibrosis infection. We are currently sequencing isolates from across the UK to define the genetic population structure and determine the frequency of transmission events in other cystic fibrosis centres. In response to our findings we have implemented new infection control measures including: continuous sputum screening for NTM of our whole patient cohort; outpatient segregation of infected patients within a dedicated outpatient clinic with single-use rooms, and use of negative pressure rooms for inpatient care. However, we cannot yet say whether we have prevented further transmission of M. abscessus subspecies massiliense in our patient cohort.

The finding of frequent M. abscessus subspecies massiliense transmission in patients with cystic fibrosis raises several important questions about current infection control measures used in treatment centres, the potential for cross-infection in other patient groups and with other NTM species, and whether mandatory notification of infections with M. abscessus complex and routine whole genome sequencing might be required to identify and control the spread of these organisms.

**Contributors**

JMB and DMG contributed equally to the work. DMG, DG, JF, MC, and SJF contributed to sample collection, culture, antibiotic sensitivity testing, and DNA extraction; JMB and SRH to sequence analysis; DMG, IR, TI, and MR to epidemiological analysis; CSH and SJF to study design and analysis of clinical data. Manuscript was written by JMB, DMG, JP, and RAF, with input from coauthors. Project was conceived, planned, and supervised by JP and RAF.

**Conflicts of Interest**

JP has received funding for conference travel and accommodation from Illumina Inc. The other authors declare that they have no conflicts of interest.

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