Whole-Genome Sequencing and Epidemiological Analysis Do Not Provide Evidence for Cross-transmission of Mycobacterium abscessus in a Cohort of Pediatric Cystic Fibrosis Patients

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Background. Mycobacterium abscessus has emerged as a major pathogen in cystic fibrosis (CF) patients and has been associated with poor clinical outcomes, particularly following lung transplant. We investigated the acquisition of this bacterium in a cohort of pediatric CF patients.

Methods. Demographic and patient location data were used to uncover epidemiological links between patients with genetically related strains of M. abscessus that had been previously typed by variable-number tandem repeat profiling. Whole-genome sequencing was applied to 27 M. abscessus isolates from the 20 patients in this cohort to provide definitive data on the genetic relatedness of strains.

Results. Whole-genome sequencing data demonstrated that M. abscessus isolates from 16 patients were unrelated, differing by at least 34 single-nucleotide polymorphisms (SNPs) from any other isolate, suggesting that independent acquisition events have occurred. Only 2 clusters of very closely related (<25 SNPs) isolates from different patients were seen. The first cluster contained 8 isolates, differing by a maximum of 17 SNPs, from a sibling pair who had intense exposure to each other both inside and outside the hospital. The second cluster contained 3 isolates, differing by a maximum of 24 SNPs, from 2 individuals with no apparent epidemiological links.

Conclusions. We have not demonstrated cross-transmission of M. abscessus within our hospital, except between 1 sibling pair. Alternative routes of acquisition of M. abscessus infection, in particular the environment, require further investigation.

Keywords. Mycobacterium abscessus; cystic fibrosis; cross-transmission; whole-genome sequencing; VNTR.

Mycobacterium abscessus has emerged as a major pathogen in cystic fibrosis (CF) patients and has been associated with poor clinical outcomes, particularly following lung transplant [1–3]. Mycobacterium abscessus is resistant to most classes of antibiotics [4, 5]. Macrolide resistance is either due to mutations in the rrl gene or the presence of an inducible ribosomal RNA methylase gene, erm(41) [6–8].

Mycobacterium abscessus is a single species that encompasses 3 subspecies (M. abscessus subsp abscessus, M. abscessus subsp massiliense, and M. abscessus subsp bolletii) [8–11]. These 3 subspecies have been

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associated with varying clinical outcomes [8, 12–14]. Accurate identification can generally be achieved by sequencing multiple gene targets [15]. Variable-number tandem repeat (VNTR) profiling can further differentiate isolates [9, 16] and, when applied to our cohort, showed that the majority of chronically infected patients were infected with 1 of 2 dominant strains. [9].

Whole-genome sequencing can provide more definitive data on the relatedness of isolates. Several publications have reported whole-genome sequences from a single M. abscessus complex isolate [17–20] or several isolates from a single patient [21]. Whole-genome sequences of M. abscessus subsp. massiliense isolates from a cohort of adults with CF provided the first evidence that patient-to-patient spread can occur [10].

We describe the dynamics of acquisition of M. abscessus in a cohort of pediatric CF patients, using epidemiological and clinical data to uncover evidence of cross-transmission events and whole-genome sequencing to establish the resolution of the previously published VNTR typing scheme. Ultimately, this will lead to a better understanding of the impact of particular strains on clinical outcomes, especially following lung transplant.

MATERIALS AND METHODS

Patients and Microbiological Data Collection
Great Ormond Street Hospital is a large regional referral center for pediatric CF patients, including those patients for whom the hospital is their main CF clinic and patients from other centers undergoing assessment and then being listed for lung transplant (Table 1). All patients seen as outpatients or admitted to wards undergo regular respiratory microbiological diagnostic investigations, including specific stain and culture for mycobacteria on both sputum and bronchoalveolar lavage samples (which are performed at least on an annual basis).

Demographic and patient location data were extracted from the patient administration system and microbiological data from the laboratory information management system using SQL (structured query language) databases and Excel spreadsheets. Electronic patient records were used to capture all inpatient and outpatient episodes including location (general CF clinics, lung function testing, inpatient ward, and bed-days admitted). Additional sources of information included CF and transplant databases. Clinical case-note review was used to verify location, admission data, and clinical/radiological evidence of nontuberculous mycobacteria infection using American Thoracic Society consensus guidelines [5]. All investigations were performed in accordance with the hospital’s research governance policies and procedures. We sought and obtained specific informed consent in 2 instances where patients had moved from our center to an adult CF center.

For patients who had isolated M. abscessus for the first time after initial contact with the hospital, all outpatient and inpatient admission episodes and cumulative bed-days were captured up to the date of their initial culture with M. abscessus. Exposure of these M. abscessus–naive patients was defined as being at the same time and same location as another patient known to be infected with M. abscessus. For comparison purposes, basic demographic data were collected on all patients who had isolated Pseudomonas aeruginosa for the first time after initial contact with our hospital. Surveillance data on P. aeruginosa have been prospectively gathered and stored on a database since 1994. The project was registered as a service evaluation.

Data Analysis
Graphs and (where appropriate) statistical analysis were generated using GraphPad Prism software version 6.03. Advice on statistical analysis of epidemiological exposure data was provided by the Department of Paediatric Epidemiology and Biostatistics at the Institute of Child Health. Where statistical analysis was performed, nonparametric tests were utilized (Mann–Whitney U test for unpaired data and Wilcoxon signed-rank tests for paired data).

Whole-Genome Sequencing
Twenty-seven M. abscessus isolates from 20 pediatric CF patients attending either CF or transplant clinics at our hospital between January 2004 and December 2011 were analyzed by whole-genome sequencing. All isolates had previously been identified to subspecies level by sequencing of hsp65 and rpoB gene targets [15] and typed by VNTR profiling [9]. DNA was extracted from isolates as previously described [9], and the concentration was determined using a Qubit high-sensitivity (HS) assay kit (Life Technologies). Two hundred fifty nanograms of DNA was sheared on the Covaris S2 (duty cycle, 5%; intensity, 4; cycles per burst, 200; time, 90 seconds) before undergoing library preparation with the NEBNext DNA Library Prep Master Mix Set for Illumina (New England Biolabs) combined with 12 cycles of enrichment polymerase chain reaction with multiplex adapters. During library preparation, libraries were size-selected to 500 bp using Ampure XP beads (Beckman). Final libraries were quantified using the Qubit HS DNA assay, and absence of adapter-dimer was confirmed using the Bioanalsyer HS DNA Chip (Agilent). Libraries were equimolar pooled, and 12 pM was loaded onto an Illumina MiSeq to undergo 250 bp paired-end sequencing with v2 chemistry. The short reads from these studies are deposited in the short-read archive of the European Nucleotide Archive in the project PRJEB6776.

Sequence Data Analysis
Whole-genome phylogenetic analysis was performed using the sequence data from this study and from M. abscessus isolates from adult patients described in the study by Bryant et al [10], as deposited in the European Nucleotide Archive under study accession number ERP001039.
Table 1. Twenty Patients With Cystic Fibrosis From Whom *Mycobacterium abscessus* Was Isolated

| Patient | Subspecies | VNTR Cluster | Sex | CF Genotype | Clinical Diagnosis of NTM Infection | Proportion of Sputum/BAL AAFB Positive | No. of Positive *M. abscessus* Cultures | Specific *M. abscessus* Antimicrobial Treatment Given | Age at First *M. abscessus* Isolate, y | Years Between First *M. abscessus* Isolate and Contact With Study Center | Infection Status at First Contact to GOSH | Main CF Attending Center/Main CF Center | UK Residence HPU |
|---------|------------|--------------|-----|-------------|------------------------------------|----------------------------------------|----------------------------------------|------------------------------------------|---------------------------------|-----------------------------------------------|---------------------------------|------------------|----------------|
| 2       | ABS        | VNTR I       | F   | p.Phe508del/p.Phe508del | Likely                           | 6/32                                   | 6                                      | Y                                       | 13.99                           | 0                              | Already Infected  | South of England | Surrey and Sussex |
| 8       | ABS        | VNTR I       | M   | p.Phe508del/p.Phe508del | Likely                           | 7/20                                   | 10                                     | Y                                       | 10.29                           | 10.04                          | Naive              | Study center     | South Midlands and Hertfordshire |
| 9       | ABS        | VNTR I       | F   | p.Phe508del/p.Trp1089X  | Likely                           | 22/35                                  | 20                                     | Y                                       | 10.93                           | 0                              | Already Infected  | London (other)/study center   | Northwest London |
| 10      | ABS        | VNTR I       | M   | p.Phe508del/p.Phe508del | Likely                           | 5/17                                   | 6                                      | Y                                       | 13.36                           | 4.97                           | Naive              | Study center     | South Midlands and Hertfordshire |
| 21      | ABS        | VNTR I       | M   | p.Phe508del/p.Phe508del | Likely                           | 4/5                                    | 5                                      | Y                                       | 15.15                           | 11.97                          | Naive              | Study center     | South Midlands and Hertfordshire |
| 3       | ABS        | VNTR II      | F   | p.Phe508del/p.Phe508del | Likely                           | 6/25                                   | 11                                     | Y                                       | 11.33                           | 0                              | Already Infected  | North of England | Cumbria and Lancashire |
| 18      | ABS        | VNTR II      | M   | p.Phe508del/p.Gly551Asp | Likely                           | 52/82                                  | 40                                     | Y                                       | 5.90                            | 3.79                           | Naive              | Study center     | Northwest London |
| 19      | ABS        | VNTR II      | M   | p.Phe508del/p.Phe508del | Likely                           | 0/4                                    | 1                                      | Y                                       | 17.25                           | 16.18                          | Naive              | Study center     | Northwest London |
| 22      | ABS        | VNTR II      | F   | Delta F508/I507/Delta F508/I507 | Likely                           | 5/26                                   | 10                                     | Y                                       | 15.05                           | 14.24                          | Naive              | Study center     | Northwest London |
| 11      | ABS        | Unique       | F   | c.2988+1G>A/p.Tyr913X  | Likely                           | 0/40                                   | 3                                      | Y                                       | 13.76                           | 13.04                          | Naive              | Study center     | Northeast and North-central London |
| 15      | ABS        | Unique       | F   | p.Phe508del/c.1885-1G>A | Unlikely                          | 4/6                                    | 5                                      | N                                       | 10.99                           | 10.87                          | Naive              | Study center     | Anglia            |
| 17      | ABS        | Unique       | M   | p.Phe508del/p.Phe508del | Unlikely                          | 1/13                                   | 1                                      | No                                      | 14.07                           | 13.81                          | Naive              | Study center     | Southeast London |
| 24      | ABS        | Unique       | F   | UK                         | Likely                           | 1/15                                   | 1                                      | Y                                       | 13.27                           | 0                              | Already Infected  | London (other)   | South Midlands and Hertfordshire |
| 27      | ABS        | Unique       | F   | p.Phe508del/p.Phe508del   | Likely                           | 2/21                                   | 2                                      | Y                                       | 14.86                           | 5.37                           | Naive              | Study center     | South Midlands and Hertfordshire |
| 30      | ABS        | Unique       | F   | p.Phe508del/p.Phe508del   | Likely                           | 3/10                                   | 4                                      | Y                                       | 14.99                           | 14.64                          | Naive              | Study center     | Essex             |
| 7       | MAS        | VNTR III     | F   | p.Phe508del/p.Phe508del   | Likely                           | 3/3                                    | 3                                      | Y                                       | 14.49                           | 0                              | Already Infected  | Midlands          | West Midlands West |
| 23      | MAS        | VNTR III     | F   | p.Phe508del/p.Phe508del   | Likely                           | 25/40                                  | 30                                     | Y                                       | 6.97                            | 5.64                           | Naive              | Study center     | South Midlands and Hertfordshire |
| 14      | MAS        | Unique       | F   | p.Gly551Asp/c.2052delA   | Likely                           | 0/8                                    | 1                                      | Y                                       | 14.29                           | 0                              | Already Infected  | Wales              | South Wales       |
| 28      | MAS        | Unique       | F   | p.Phe508del/p.Phe508del   | Likely                           | 1/1                                    | 1                                      | Y                                       | 14.09                           | 0                              | Already Infected  | Midlands          | West Midlands East |
| 1       | BOL        | Unique       | F   | p.Phe508del/p.Phe508del   | Likely                           | 1/14                                   | 1                                      | Y                                       | 10.08                           | 0                              | Already Infected  | London (other)   | Northeast and North-central London |

Clinical and radiological diagnosis of likely NTM infection with clinician decision to treat with long-term antibiotics considered active against *M. abscessus* was recorded in patient records. Abbreviations: AAFB, acid-alcohol fast bacilli; ABS, *Mycobacterium abscessus* subsp *abscessus*; BAL, bronchoalveolar lavage; BOL, *Mycobacterium abscessus* subsp *bolletii*; CF, cystic fibrosis; GOSH, Great Ormond Street Hospital; HPU, Health Protection Unit; MAS, *Mycobacterium abscessus* subsp *massiliense*; NTM, nontuberculous mycobacteria; VNTR, variable-number tandem repeat.
The short reads were mapped to the reference ATCC 19977 (accession number CU458896) using BWA-MEM 0.7.5.a [22] and the default parameters. The sequence alignment map output from BWA was sorted and indexed to produce a binary alignment map (BAM) using Samtools 0.1.18 [23]. The Genome Analysis Toolkit 2 [24] was used to create a variant call format (VCF) file for each sequenced isolate using the BAM files as input and specifying diploid mode when calling variants. Variants within the VCF files were parsed to retain high-quality single-nucleotide polymorphisms (SNPs) based on the following conditions: DP (depth) ≥5, AD ratio (ratio between variant base and alternative bases) ≥0.8, MQ (mapping quality score) ≥30, ratio of number of reads with MQ0 (mapping quality of 0) to total number of reads ≤0.05, and distance to nearest SNP >10. All positions that fulfilled these criteria in >0.9 of the samples were joined to produce a multiple fasta format file where the sequence for each strain consists of the concatenated variants. This file was used as an input to generate a maximum likelihood tree using RAxML [25] with the following parameters: -m (substitutionModel) GTRCAT, -b (bootstrap-RandomNumberOfSeeds) 12345, -c (NumberOfCategories) 25.

Multilocus sequence types (MLSTs) were identified by mapping the reads against all 162 M. abscessus allele variants held in the Institut Pasteur MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) using a modification of the short-read sequence typing [26].

Antibiotic resistance analysis was performed by searching for SNPs previously discovered to be responsible for resistance mutations within the coding sequence of rrl and both the coding sequence and 126-bp upstream regulatory region of erm(41) [6]. Trimmed reads were mapped to the rrl and erm(41) gene sequences from the genome sequence of M. abscessus strain ATCC 19977 using bowtie2 version 2.1 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml). The resulting BAM file was converted to pileup format using samtools and the output parsed to determine the nucleotide base at the known drug-resistance locations. To generate a phylogenetic tree based on the rrl and erm(41) genes, the pileup format was converted to form a consensus sequence for each sample, aligned using muscle [27], and a maximum likelihood tree was created using FastTree [28].

RESULTS

Clinical and Microbiological Characteristics of M. abscessus–Infected CF Patients

In patients from whom M. abscessus was isolated for the first time, a number of observations were apparent. When compared with a larger cohort of M. abscessus–negative CF patients (n = 122) who acquired P. aeruginosa for the first time after contact with the hospital, children who acquired M. abscessus were significantly older (P = .0001, Mann–Whitney U test; Figure 1). The median age for acquiring P. aeruginosa was 4.35 years compared with a median age of first acquisition of M. abscessus of 14.07 years (P = .005, Wilcoxon signed-rank test; Table 1 and Figure 1). The median age difference in years between first P. aeruginosa isolation and first M. abscessus isolation was 7.56 years.
years (interquartile range, 5.18–11.00 years). Taken together, in this single but large regional referral center, infection with *P. aeruginosa* always precedes *M. abscessus* infection, which usually occurs in the early to middle teenage years.

*Mycobacterium abscessus* strains clustered by VNTR profile (VNTR I, VNTR II, or VNTR III) or had unique VNTR profiles. Among the 12 patients who acquired *M. abscessus* for the first time during contact with our hospital, 11 acquired *M. abscessus* subsp *abscessus* strains (3 VNTR I, 3 VNTR II, and 5 with unique VNTR profiles) [9], and 1 individual acquired *M. abscessus* subsp *massiliense* (Table 1). After consulting with expert statistical colleagues, we concluded that there were insufficient numbers in each VNTR-defined outcome group to make statistically robust comparisons based on multiple comparisons of exposure to ward environments or among patients. Despite the relatively low numbers of defined acquisition events, we were able to make a number of observations.

### Patient-to-Patient Contact of *M. abscessus*–Infected CF Patients

We analyzed the strength of exposure of patients who acquired *M. abscessus* after exposure to other patients with *M. abscessus* of any subspecies and VNTR profile. First, taking the patients (n = 3) who acquired VNTR I strains, there was minimal exposure to patients already infected with a VNTR I strain, either as an outpatient or as an inpatient. In contrast, patients who acquired VNTR I strains were exposed on a number of occasions both as inpatients and outpatients to other patients already infected with VNTR II strains and strains with unique VNTR profiles (Figure 2A and 2D). Where patients acquired VNTR II strains (n = 3), there was more intense exposure to patients with VNTR II strains (in particular, between patients 18 and 19) in both outpatient and inpatient settings than to patients with VNTR I strains; however, exposure to patients with strains that had a unique VNTR profile was similarly intense (Figure 2B and 2E). Patients who acquired strains with a unique VNTR

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**Figure 2.** Exposure of patients who first acquired *Mycobacterium abscessus* after contact with our center to other *M. abscessus*-infected patients. Exposure of patients who acquired *M. abscessus* subsp *abscessus* (MA) variable-number tandem repeat (VNTR) I (n = 3; A and D), MA VNTR II (n = 3; C and E), and MA VNTR unique strains (n = 5; C and F) to other patients already infected with MA VNTR I, VNTR II, VNTR unique, and *M. abscessus* subsp *massiliense* strains. A–C, Total number of bed-days on the same ward at the same time that each patient was with other *M. abscessus*-infected patients. C–E, Total number of outpatient episodes where patients were with other *M. abscessus*-infected patients. Abbreviation: OPD, outpatients department.
profile (n = 5) were also exposed on a number of occasions, in both outpatient and inpatient environments, to patients infected with strains with different VNTR profiles, including VNTR I and VNTR II strains (Figure 2C and 2F).

**Whole-Genome Sequencing of M. abscessus Isolates**

Whole-genome sequences were obtained from 27 M. abscessus isolates in this study. These and sequences from the study by Bryant et al [10] were processed to produce a multiple fasta file, which was used to generate a tree. The fasta file recorded a total of 133 683 variant positions. The maximum likelihood tree showed 3 distinct clades corresponding to the 3 subspecies. Twenty isolates from this study were M. abscessus subsp abscessus, 6 were M. abscessus subsp massiliense, and 1 was M. abscessus subsp bolletii. Sixteen isolates were assigned to 1 of 4 clades. A further 9 isolates that were not closely grouped with >1 isolate were not assigned to a clade (Figure 3). Two isolates (27–313 and 28–319) did not produce sufficient read-depth to be included in the maximum likelihood tree.

Within clade 1, there were 152 SNPs; the minimum distance between any isolate from this study and any other was 38 SNPs. Within clade 2, there were 157 SNPs. Isolates 18–114, 18–531, 18–362, 7_a, 7_b, 7_c, 7_d, 19–825, and 18–169 differed by 17 SNPs, and the smaller subset of 18–169, 19–825, 7_a, 7_b, 7_c, and 7_d differed by just 5 SNPs. Within clade 3, there were 1328 SNPs; the minimum distance between an isolate from this study and any other was 43 SNPs. Within clade 4, there were 308 SNPs. Isolates 8–768 and 8–170 were 2 SNPs different. Isolates 10–994, 21–468, and 15_a differed by 24 SNPs. Apart from these, the minimum distance between any isolate from this study and any other was 34 SNPs (Figure 4).

**MLST and Resistance Mutations**

MLSTs were deduced from the whole-genome sequences of all isolates in this study. All VNTR I strains were sequence type ST-26 and fell into sequence clade 4. All VNTR II strains were ST-1 and fell into sequence clade 2. The VNTR III strains had 2 different MLST types (ST-48 and ST-23) and fell into sequence clade 1 or no clade. All other strains had a unique VNTR profile and MLST type and did not fall into any sequence clade (Table 2).

Mutations in the rrl and erm(41) genes, known to cause antimicrobial resistance, were also examined. None of the VNTR I...
or VNTR II strains had \textit{erm}(41) mutations and therefore had a predicted phenotype of inducible macrolide resistance. All \textit{M. abscessus} subsp \textit{massiliense} strains had a truncated \textit{erm}(41) sequence, no resistance-associated \textit{rrl} mutations, and were predicted to be macrolide susceptible. Four \textit{M. abscessus} strains with unique VNTR profiles had no resistance-associated \textit{rrl} mutations and had the \textit{erm}(41) mutation (T28C) that predicts macrolide susceptibility (Table 2). A phylogenetic tree generated from the \textit{rrl} and \textit{erm}(41) sequences clustered isolates identically to the trees generated from whole-genome sequences. Two isolates that did not produce sufficient read-depth to be included in the whole-genome analysis were included in this tree (Supplementary Figure).

**DISCUSSION**

The purpose of this retrospective cohort study of \textit{M. abscessus} infection in pediatric CF patients was to explore the evidence for cross-infection between patients using a combination of epidemiology, VNTR profiling, and whole-genome sequencing.

Epidemiological data were used to investigate the intensity of exposure between patients with strains that were identical by VNTR profiling and strains with different VNTR profiles [9]. We hypothesized that if cross-transmission had occurred, we were likely to see more intense exposure prior to first detection of a VNTR I or VNTR II strain with a strain from the same cluster compared with exposure to strains from another cluster or
unique strains. There was little evidence of transmission of \textit{M. abscessus} as a result of contact with the hospital. Only 2 patients (18 and 19), a sibling pair who both acquired VNTR II strains, had frequent exposure to each other in outpatient environments.

Whole-genome sequencing data have provided a more accurate basis for assessing the degree of genetic similarity between isolates and have confirmed that VNTR profiling is an accurate method for identifying genetically related strains. Isolates with identical VNTR profiles were also of the same MLST type and belonged to the same whole-genome sequence clade, with the exception of 3 \textit{M. abscessus} subsp \textit{massiliense} isolates (Figure 3 and Table 2). All isolates with either the VNTR I or VNTR II profile (ST-26 and ST-1) were predicted to have a phenotype of inducible macrolide resistance. These are the dominant VNTR types in our patient cohort, and we have previously suggested a link with chronic infection [9]. Moreover, ST-1 and ST-26 are global lineages that appear to be successful clones [29]. Indeed, infections with strains that have inducible resistance to macrolides would be significantly harder to treat and therefore more likely to establish chronic infections [30]. However, it is possible that other parameters are important, such as expression of virulence factors, biofilm formation, adaptation to the CF microenvironment, and host–pathogen interactions [31], all of which warrant further study.

The finding that all the patients who acquired \textit{M. abscessus} were already infected with \textit{P. aeruginosa} is noteworthy, although the reasons for this are unclear. It is possible that more aggressive antimicrobial therapy associated with chronic \textit{P. aeruginosa} infection could play a role; however, the precise mechanism is as yet unknown and warrants further study.

### Table 2. Genotypic Data for All 27 \textit{Mycobacterium abscessus} Isolates in This Study

| Isolate | Date Isolated | Subspecies | VNTR Profile | MLST Type | Sequence Clade | \textit{erm}(41) | \textit{rrl} | Predicted Macrolide Resistance Phenotype |
|---------|---------------|------------|--------------|-----------|----------------|-------------|----------|----------------------------------------|
| 15–313  | Jun 2006      | ABS        | Unique       | ST-122    | 3              | T28C        | None     | Susceptible                           |
| 24–878  | Mar 2009      | ABS        | Unique       | Novel allele | None           | none        | None     | Inducible resistance                  |
| 17–257  | Apr 2009      | ABS        | Unique       | Novel ST  | 3              | T28C        | None     | Susceptible                           |
| 27–313  | Dec 2012      | ABS        | Unique       | ST-33     | Not available<sup>a</sup> | none        | None     | Inducible resistance                  |
| 30–578  | Jun 2012      | ABS        | Unique       | Novel ST  | None           | none        | None     | Inducible resistance                  |
| 11–067  | Oct 2008      | MAS        | Unique       | ST-117    | None           | Trunc158–430 | None     | Susceptible                           |
| 11–618  | May 2012      | ABS        | Unique       | Novel allele | None           | None     | None     | Inducible resistance                  |
| 2–520   | Jan 2009      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 8–046   | Jun 2007      | ABS        | Unique       | Novel ST  | None           | T28C        | None     | Susceptible                           |
| 8–170   | Oct 2009      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 8–768   | May 2012      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 9–847   | Nov 2009      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 21–468  | Oct 2009      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 10–994  | Feb 2011      | ABS        | VNTR I       | ST-26     | 4              | None        | None     | Inducible resistance                  |
| 18–169  | Jun 2007      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 18–114  | Mar 2008      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 18–362  | Mar 2010      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 18–531  | Jan 2012      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 3–390   | Jul 2009      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 22–071  | Feb 2009      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 19–825  | Jun 2007      | ABS        | VNTR II      | ST-1      | 2              | None        | None     | Inducible resistance                  |
| 14–907  | Apr 2005      | MAS        | Unique       | ST-69     | None           | Trunc158–430 | None     | Susceptible                           |
| 7–983   | Apr 2009      | MAS        | VNTR III     | ST-23     | 1              | Trunc158–430 | None     | Susceptible                           |
| 23–476  | Feb 2009      | MAS        | VNTR III     | ST-48     | None           | Trunc158–430 | None     | Susceptible                           |
| 23–816  | Nov 2011      | MAS        | VNTR III     | ST-48     | None           | Trunc158–430 | None     | Susceptible                           |
| 28–319  | Jun 2011      | MAS        | Unique       | ST-37     | Not available<sup>a</sup> | Trunc158–430 | None     | Susceptible                           |
| 1–873   | Dec 2004      | BOL        | Unique       | Novel ST  | None           | None        | None     | Inducible resistance                  |

<sup>a</sup> Insufficient sequence reads to determine sequence clade.

Sequence clade, MLST types, \textit{erm}(41) mutations, and \textit{rrl} mutations were all deduced from the genome sequencing data.

Abbreviations: ABS, \textit{Mycobacterium abscessus} subsp \textit{abscessus}; BOL, \textit{Mycobacterium abscessus} subsp \textit{bolletii}; MAS, \textit{Mycobacterium abscessus} subsp \textit{massiliense}; MLST, multilocus sequence type; ST, sequence type; VNTR, variable-number tandem repeat.
Whole-genome sequence data provided greater resolution than either VNTR profiling or MLST, differentiating isolates within each of the 4 clades. Bryant et al [10] described different modes of similarity where those isolates belonging to a “related” cluster are <25 SNPs different. Using this cutoff, there are only 2 clusters with isolates from >1 patient in our data. The first cluster contains 4 isolates from patient 18 and 1 isolate from patient 19 (sibling). There were also 4 sequences (7 a–d) from Bryant et al [10], and we have confirmed that these were also isolates from patient 19, who was transferred to the adult service. These isolates were identical or virtually identical, differing by a maximum of 17 SNPs in patient 18 over a 5-year period. Patients 18 and 19 had a great deal of exposure to each other, particularly at home. Cross-transmission of M. abscessus is likely to have occurred, although, interestingly, the older sibling acquired M. abscessus 11 years after the younger sibling. The second cluster contains isolates from patients 10 and 21 (this study) and 15 (Bryant et al [10]), and we have confirmed that patient 10 and 15 are the same individual. Patients 10 and 21 acquired M. abscessus at a similar time, after their first contact with our center. However, the epidemiological data in this study did not show any contact between these 2 individuals within the hospital environment. Furthermore, they did not attend the same local hospital, outreach clinics, or school and, to the best of our knowledge, did not know each other socially. This suggested that cross-transmission between these 2 patients was unlikely and that these individuals had acquired highly genetically related strains by another route.

Isolates from the remaining 16 patients in this study differed by at least 34 SNPs from each other, which further suggested that cross-transmission was uncommon in this pediatric CF cohort. This conclusion has been reached previously by genotyping of M. abscessus isolates from CF patients at a single center [32]. However, this is in contrast to a recent study that demonstrated person-to-person spread of M. abscessus subsp massiliense between adult CF patients [10]. It is not yet clear why this difference is seen. It is possible that M. abscessus subsp massiliense is more transmissible, or that adults have more prolonged or intense exposures or shed a higher load of bacteria into the environment, making transmission more likely. Differences in infection control practices between centers could also explain why we have seen little evidence of person-to-person spread. Another potential route of acquisition of M. abscessus is a common environmental source. A recent publication demonstrated that strains isolated from potable water were genetically similar to patient isolates [33, 34]. We suggest that a better understanding of the role of the environment in the acquisition of M. abscessus infection is critical and has not been adequately investigated.

In conclusion, we could not demonstrate cross-transmission of M. abscessus within our hospital, except between 1 sibling pair that had intense exposure both in the hospital and, perhaps more importantly, the home environment. Two patients were infected with highly genetically similar strains but appeared to be epidemiologically unrelated. The role of the environment in the acquisition of M. abscessus infection requires further investigation.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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