



# Genomic Analysis of *Mycobacterium abscessus* Complex Isolates Collected in Ireland between 2006 and 2017

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**ABSTRACT** Members of the *Mycobacterium abscessus* complex (MABC) are multidrug-resistant nontuberculous mycobacteria and cause opportunistic pulmonary infections in individuals with cystic fibrosis (CF). In this study, genomic analysis of MABC isolates was performed to gain greater insights into the epidemiology of circulating strains in Ireland. Whole-genome sequencing (WGS) was performed on 70 MABC isolates that had been referred to the Irish Mycobacteria Reference Laboratory between 2006 and 2017 across nine Irish health care centers. The MABC isolates studied comprised 52 isolates from 27 CF patients and 18 isolates from 10 non-CF patients. WGS identified 57 (81.4%) as *M. abscessus* subsp. *abscessus*, 10 (14.3%) as *M. abscessus* subsp. *massiliense*, and 3 (4.3%) as *M. abscessus* subsp. *bolletii*. Forty-nine (94%) isolates from 25 CF patients were identified as *M. abscessus* subsp. *abscessus*, whereas 3 (6%) isolates from 2 CF patients were identified as *M. abscessus* subsp. *massiliense*. Among the isolates from non-CF patients, 44% (8/18) were identified as *M. abscessus* subsp. *abscessus*, 39% (7/18) were identified as *M. abscessus* subsp. *massiliense*, and 17% (3/18) were identified as *M. abscessus* subsp. *bolletii*. WGS detected two clusters of closely related *M. abscessus* subsp. *abscessus* isolates that included isolates from different CF centers. There was a greater genomic diversity of MABC isolates among the isolates from non-CF patients than among the isolates from CF patients. Although WGS failed to show direct evidence of patient-to-patient transmission among CF patients, there was a predominance of two different strains of *M. abscessus* subsp. *abscessus*. Furthermore, some MABC isolates were closely related to global strains, suggesting their international spread. Future prospective real-time epidemiological and clinical data along with contemporary MABC sequence analysis may elucidate the sources and routes of transmission among patients infected with MABC.

**KEYWORDS** *Mycobacterium abscessus* complex, clusters, cystic fibrosis, epidemiological analysis, transmission, whole-genome sequencing

Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms that commonly cause chronic pulmonary infections, particularly in patients with pre-existing inflammatory lung diseases, such as cystic fibrosis (CF), but they can also cause infections outside of the respiratory tract in immunologically susceptible individuals (1). An increasing number of CF patients infected with NTM is being reported (2). The majority of NTM infecting CF individuals globally are members of the *Mycobacterium*

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**TABLE 1** MABC isolates recovered from both CF and non-CF patients investigated in this study<sup>a</sup>

Patient group	No. of MABC isolates			No. of isolates from the following specimen types:		
	Paired	Single	Total	Pulmonary	Extrapulmonary	Unknown
CF patients ( <i>n</i> = 27)	54	0	54	52	0	2
Non-CF patients ( <i>n</i> = 10)	16	2	18	16	2	0
Total	70	2	72	68	2	2

<sup>a</sup>Data are for 72 MABC isolates.

*avium* complex and *M. abscessus* complex (MABC), with the latter appearing more commonly in Europe within the CF population (3). Three subspecies of MABC have been described: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*.

MABC is a group of rapidly growing mycobacteria with intrinsic multiple-antibiotic resistance, necessitating prolonged antimicrobial treatment with potential toxic side effects (4). MABC infections are associated with an accelerated decline in lung function and increased morbidity and mortality and remain a contraindication to lung transplantation in many centers (2, 5). Until recently, NTM infections were thought to be acquired by individuals through exposure to environmental sources, such as soil or water (6). However, recent studies have shown that MABC can be transmitted from patient to patient or can be acquired from a single environmental source and spread to patients within health care facilities (5, 7).

Ireland has the highest incidence rate of CF in Europe, and therefore, a significant number of people may be at risk of MABC infection (8). A previous study on the molecular epidemiology of MABC in Ireland was performed using *rpoB* fragment analysis typing and multilocus sequence typing (9). That study showed a predominance of *M. abscessus* subsp. *abscessus* isolates among the isolates investigated, with *M. abscessus* subsp. *massiliense* isolates forming a minority of isolates (9). The aim of this study was to perform whole-genome sequencing (WGS) on MABC isolates recovered from both CF and non-CF patients to characterize at a genomic level the circulating strains of MABC in Ireland.

## MATERIALS AND METHODS

**MABC isolates.** A total of 72 MABC isolates, recovered from Irish patients, were referred to the Irish Mycobacteria Reference Laboratory (IMRL) and included in this study. The MABC isolates had been recovered from both pulmonary (*n* = 68) and extrapulmonary (*n* = 2) specimens, while two isolates were from unknown sites. The MABC isolates comprised 54 isolates from 27 CF patients and 18 isolates from 10 non-CF patients (Table 1).

The American Thoracic Society guidelines recommend screening CF patients at least yearly for NTM infection (10). Therefore, in the interest of this study, the first and most recent MABC isolates from each patient were selected. There were 70 paired MABC isolates from 35 of 37 patients and single isolates from two non-CF patients (70 + 2 = 72). Each pair of isolates had been collected a minimum of 6 months apart, while a single isolate from each of two patients was also included (see Table S1 in the supplemental material). The MABC isolates had been referred to the IMRL for identification from nine different health care centers (centers A to I) between 2006 and 2017. All MABC isolates had been previously identified using the GenoType Mycobacterium CM assay (Bruker-Hain Diagnostics, Germany).

**Whole-genome sequencing.** The MABC isolates were cultured in BD Bactec MGIT 960 liquid medium (Becton, Dickinson, NJ, USA) and heat inactivated at 95°C for 30 min. Genomic DNA was extracted using a QuickGene-Mini80 device (Kurabo Industries Ltd., Osaka, Japan) according to the manufacturer's instructions. DNA libraries were prepared using a Nextera XT DNA library preparation kit (Illumina, Cambridge, UK) according to the manufacturer's instructions. Paired-end sequencing was performed on an Illumina MiniSeq platform using a High Output 300 cycle kit (Illumina, Cambridge, UK).

WGS was performed on 70 MABC isolates (paired isolates from 33 patients and single isolates from each of 4 patients). Two isolates were lost due to technical issues; therefore, only single isolates from two CF patients (patients 25 and 26) were investigated.

**Whole-genome SNP analysis.** MABC FASTQ files and the sequences of 11 published MABC strains (shown in Table S2) were mapped to the sequence of *M. abscessus* type strain ATCC 19977 using the Burrows-Wheeler alignment tool (v0.7.17-r1188) (11). Variant calling was performed using the FreeBayes program (v1.1), a minimum coverage of 10× was used, and a maximum likelihood tree was constructed using the PhyML program in Seaview software (v4.7). Additionally, MABC FASTQ files were analyzed using BioNumerics software (v7.6; Applied Maths, Sint-Martens-Latem, Belgium). The SPAdes assembly of

the earliest recovered isolate (2006) was used as the reference genome to construct the minimum spanning tree (MST). Single nucleotide polymorphisms (SNPs) were called exclusively in positions shared by all samples. Only SNPs with at least 5× coverage (including 1× coverage in each direction) were considered. Potential indel-related SNPs occurring within 12 bp of each other were removed. Positions with ambiguous base calls were excluded. A distance matrix was generated, and an MST was constructed using permutation resampling (1,000 replicates).

**Ethics.** All isolates used in this study had been previously referred for identification to the IMRL and stored as an archival collection. WGS analysis was performed on pseudonymized data, and the study was completed in March 2018. No personal patient data are reported in this study, and patient consent was considered to not be required.

**Data availability.** Raw sequence reads of all 70 sequenced MABC genomes in this study were submitted to the European Nucleotide Archive database under project accession number [PRJEB37730](https://www.ebi.ac.uk/ena/record/PRJEB37730).

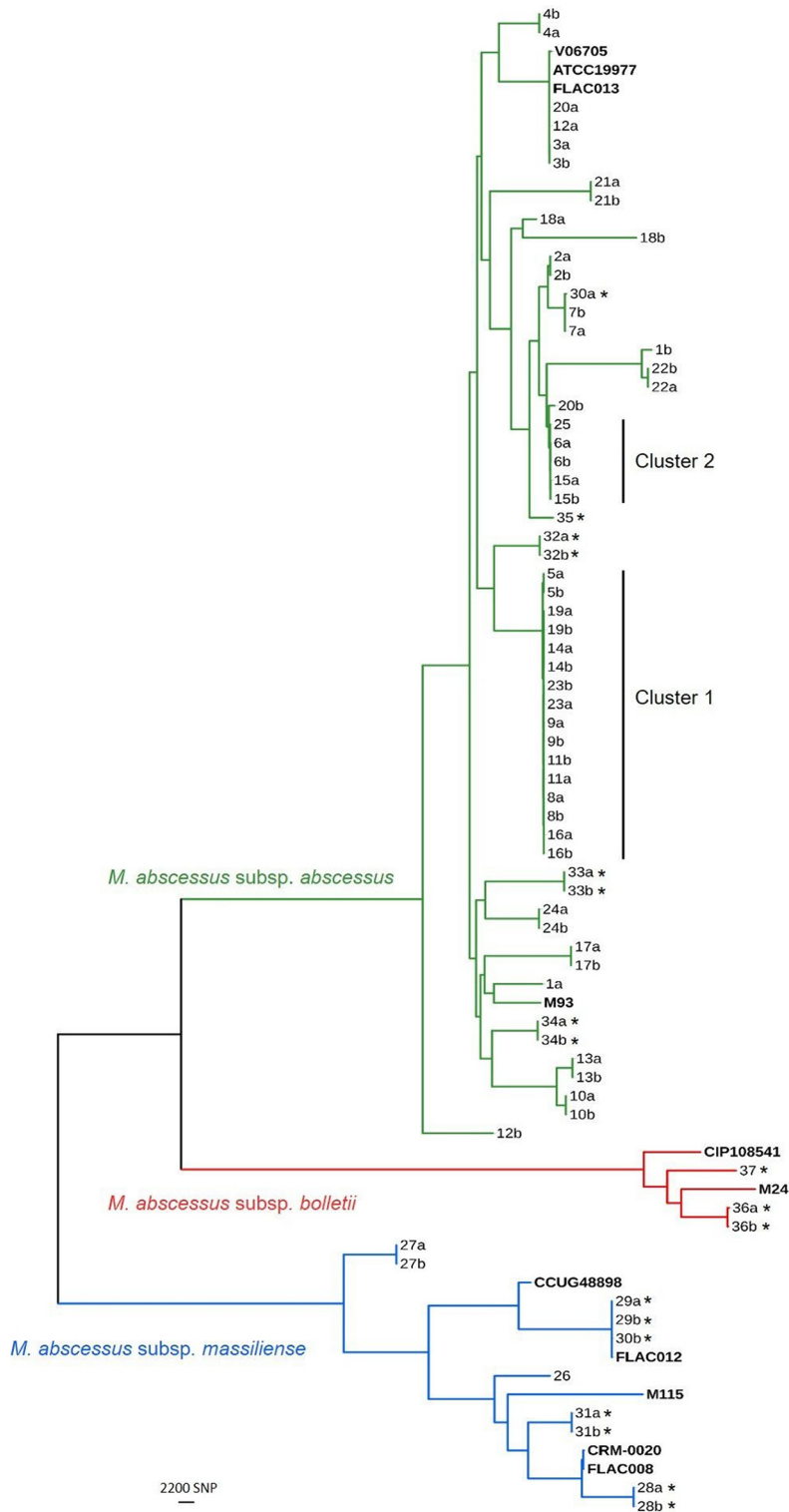
## RESULTS

**Relatedness of MABC subspecies to published strains.** The paired and single MABC isolates recovered from CF and non-CF patients from different health care centers and investigated by WGS are listed in Table S1 in the supplemental material. WGS analysis distinguished the three subspecies of MABC, and the sequences of the isolates aligned with those of previously published MABC strains, as shown in Fig. 1. The analysis identified 57/70 (81.4%) isolates as *M. abscessus* subsp. *abscessus*, 10/70 (14.3%) as *M. abscessus* subsp. *massiliense*, and 3/70 (4.3%) as *M. abscessus* subsp. *bolletii*. Four *M. abscessus* subsp. *abscessus* isolates from three patients had genomic sequences that were related (with an average of 79 SNP differences) to those of published strains that originated in France (*M. abscessus* subsp. *abscessus* strain ATCC 19977 and *M. abscessus* subsp. *abscessus* strain V06705) and the United States (*M. abscessus* subsp. *abscessus* strain FLAC013) (11–15). In contrast, *M. abscessus* subsp. *abscessus* strain M93 was distinct from these *M. abscessus* subsp. *abscessus* isolates, with approximately 11,317 SNP variations detected (16). Among the *M. abscessus* subsp. *massiliense* strains, three isolates from two non-CF patients (isolates 29a, 29b, and 30b) had genomic sequences that differed from the sequence of *M. abscessus* subsp. *massiliense* strain FLAC012, originating in the United States, by 59 SNP variations (14). These were distinct from strains originating from France (*M. abscessus* subsp. *massiliense* strain CCUG48898), Malaysia (*M. abscessus* subsp. *massiliense* strain M115), Brazil (*M. abscessus* subsp. *massiliense* strain CRM-0020), and the United States (*M. abscessus* subsp. *massiliense* strain FLAC008) (14, 17–19). Our three *M. abscessus* subsp. *bolletii* isolates were distinct from the *M. abscessus* subsp. *bolletii* strains that originated from France (*M. abscessus* subsp. *bolletii* strain CIP108541) and Malaysia (*M. abscessus* subsp. *bolletii* strain M24) (20, 21).

**Distribution of MABC isolates among CF and non-CF patients. (i) CF patients.** Forty-nine isolates (94%) collected from 25/27 CF patients were identified as *M. abscessus* subsp. *abscessus*, whereas only 3 (6%) isolates from 2/27 CF patients were identified as *M. abscessus* subsp. *massiliense* (Fig. 1 and Table S1). No *M. abscessus* subsp. *bolletii* isolate was identified among the isolates collected from CF patients, and no mixed MABC infections were detected in this cohort.

**(ii) Non-CF patients.** There was a greater diversity among the isolates ( $n = 18$ ) collected from the 10 non-CF patients than among the isolates collected from the CF patients; eight *M. abscessus* subsp. *abscessus* isolates (44%) were recovered from 5 patients; seven *M. abscessus* subsp. *massiliense* isolates (39%) were recovered from 4 patients, and three *M. abscessus* subsp. *bolletii* (17%) were recovered from 2 patients. One non-CF patient (patient 30) was infected with both subspecies *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*, which were recovered within the same year from two different pulmonary specimens (Table S1).

**Relatedness among MABC isolates from CF and non-CF patients.** Overall, 85% (28/33) of the patients' paired isolates were highly related, indicating homogeneous populations (Table S1). Identical genomic sequences were found in 39% (13/33) of the paired isolates collected at different time points. Among the CF patients, 84% (21/25) of the isolates from the same patient differed by  $\leq 11$  SNPs, while among the non-CF patients, 87.5% (7/8) of the isolates from the same patient differed by less than 10 SNPs.



**FIG 1** Maximum likelihood tree of *Mycobacterium abscessus* complex. A maximum likelihood tree was built from 222,210 core SNP sites of 70 *M. abscessus* complex (MABC) isolates from 37 patients and 11 published strains (see Tables S1 and S2 in the supplemental material). All sequenced genomes were mapped to the *M. abscessus* ATCC 19977 genome as a reference genome, and the mean coverage was 82×. MABC isolates classified as *M. abscessus* subsp. *abscessus* are represented in green. Likewise, *M. abscessus* subsp. *massiliense* isolates are represented in blue and *M. abscessus* subsp. *bolletii* isolates are represented in red on the tree. The first and last isolate from each patient is indicated by a and b, respectively. Single isolates were available only from patients 25, 26, 35, and 37. \*, isolates recovered from non-CF patients in this study. Published MABC strains are highlighted in bold.

Large SNP differences (ranging from 6,770 to 29,350) between paired isolates were found in five patients (patients 1, 12, 18, 20, and 30), suggesting infection with a different MABC strain in each case. These included four CF patients from center A and one non-CF patient (patient 30) from center I. *M. abscessus* subsp. *massiliense* was identified from a pulmonary specimen taken 6 months after *M. abscessus* subsp. *abscessus* had been recovered from a pulmonary specimen from the same patient.

While *M. abscessus* subsp. *abscessus* was the predominant (81.4%) subspecies of MABC identified, isolates collected from different patients were clearly segregated from one another when investigated by WGS (Fig. 2). Two distinct clusters of *M. abscessus* subsp. *abscessus* strains, named clusters 1 and 2, were identified. In total, 21 (37%) *M. abscessus* subsp. *abscessus* isolates were grouped in these two clusters, and all these isolates were from CF patients. Cluster 1 comprised eight pairs of *M. abscessus* subsp. *abscessus* isolates each from eight CF patients and had been collected between 2006 and 2017. The *M. abscessus* subsp. *abscessus* isolates within cluster 1 were from centers A ( $n = 7$ ), E ( $n = 6$ ), C ( $n = 2$ ), and D ( $n = 1$ ) (Fig. 2B). There was evidence from genomic analysis of both probable and possible recent transmission events (whether direct or indirect) between patients in centers A and E when a previously defined threshold for indicating probable (<20 SNPs) and possible (20 to 38 SNPs) transmission events between patients was applied (3).

In cluster 2, there were five *M. abscessus* subsp. *abscessus* isolates from three CF patients, dating between 2013 and 2015 and consisting of two pairs of *M. abscessus* subsp. *abscessus* isolates recovered from each of two CF patients and one *M. abscessus* subsp. *abscessus* isolate from one CF patient (Fig. 2C). Similar to cluster 1, the majority of isolates in cluster 2 were recovered from specimens collected in center A ( $n = 4$ ); one isolate was collected in center C. Within this cluster, there was also evidence of both probable and possible recent transmission events between patients (3).

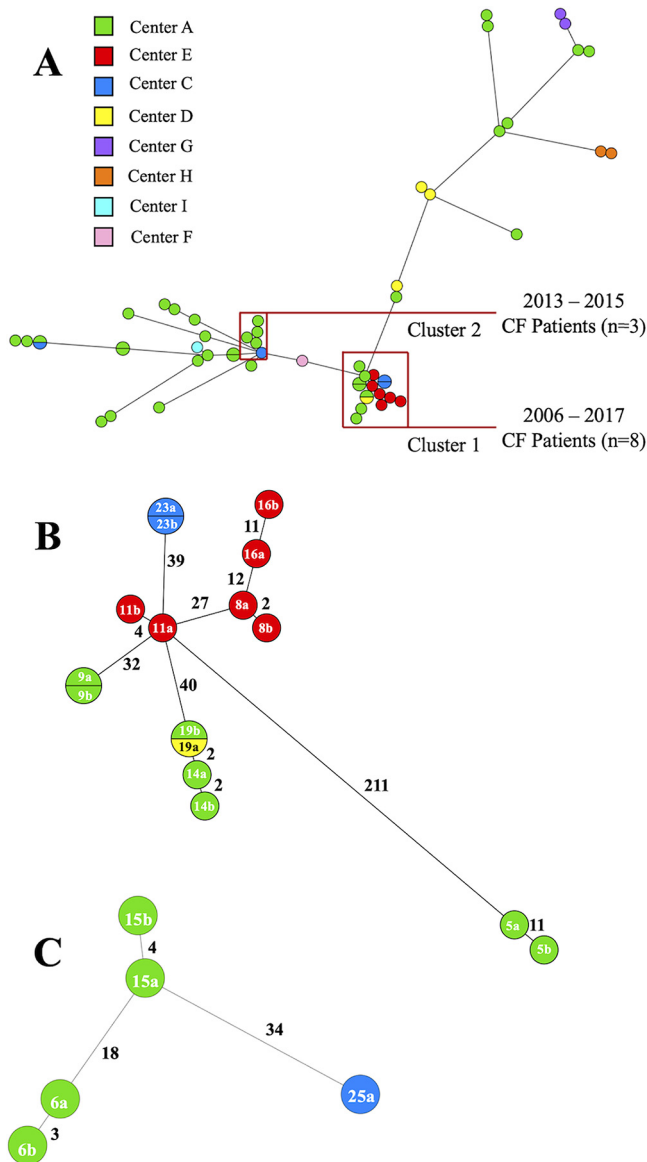
Three *M. abscessus* subsp. *abscessus* isolates recovered from three CF patients (patients 3, 12, and 20) collected between 2008 and 2015 differed by 39 to 62 SNPs (Fig. 1). *M. abscessus* subsp. *abscessus* isolates from one patient (patient 3) had been collected at different centers (centers C and A) 144 days apart, and there were no SNP variations between the isolates, as measured by pairwise SNP comparison. Among the *M. abscessus* subsp. *massiliense* strains, three isolates from two non-CF patients (patients 29 and 30) collected between 2009 and 2017 in centers A and I, respectively, were closely related and distinguished by only 111 SNPs (Fig. 1).

There was no clustering between the three *M. abscessus* subsp. *bolletii* isolates collected from 2 non-CF patients (patients 36 and 37) in 2013, 2014, and 2016 from center A ( $n = 2$ ) and center G ( $n = 1$ ).

## DISCUSSION

This is the first comprehensive investigation of the molecular epidemiology of MABC in Ireland based on WGS analysis. There was a predominance of *M. abscessus* subsp. *abscessus* (81.4%), followed by *M. abscessus* subsp. *massiliense* (14.3%) and *M. abscessus* subsp. *bolletii* (4.3%), which is consistent with reports from other countries (1, 12, 13).

In some cases, our MABC isolates aligned with previously published strains from the United States and France, consistent with the global spread and human-to-human transmission of MABC organisms (5). The low genetic variation and the presence of clusters among *M. abscessus* subsp. *abscessus* isolates collected from more than one center over prolonged time periods suggest that there are dominant circulating strains in Ireland. The genomic similarities observed within an individual patient's isolates and between the patients' isolates suggest the possibility of *M. abscessus* subsp. *abscessus* transmission between patients rather than point source or independent acquisition from environmental sources. This finding is consistent with earlier reports (5, 7). In a previous global study, it was observed that the majority of patients are infected with clustered rather than unclustered MABC isolates and that less than 20 SNPs between patient isolates could indicate a probable recent transmission event (5). MABC isolates recovered from 14 Irish patients in that study were represented in dense clusters (5).



**FIG 2** Minimum spanning tree (MST) of *M. abscessus* subsp. *abscessus*. (A) MST representing 57 *M. abscessus* subsp. *abscessus* whole-genome SNP differences among CF and non-CF patient isolates. SPAdes assembly of the earliest isolate resulted in 68 contigs with a mean coverage of 104× across the assembly. The MST was constructed from 87,491 core SNP sites. Isolates were recovered from multiple centers, as indicated by the colored nodes. While disperse, two distinct groups of isolates were noted as clustering together and are represented as cluster 1 and cluster 2. Cluster 1 comprised isolates recovered from multiple centers, while cluster 2 comprised isolates recovered from 2 centers. (B) MST of cluster 1, representing the SNP difference among the isolates. While these isolates clustered closely together, the average SNP difference was 88, with a minimum and maximum SNP difference of 2 and 211, respectively. (C) MST of cluster 2, representing the SNP difference among the isolates. The average SNP difference was 2, with a minimum and maximum SNP difference of 3 and 34, respectively.

Prospectively collected epidemiological and clinical data for MABC-infected patients along with contemporaneous WGS of environmental MABC isolates may help to elucidate reservoirs and routes of acquisition and transmission among patients infected with MABC.

Our study has shown that CF patients were mainly infected with *M. abscessus* subsp. *abscessus* (93%) rather than *M. abscessus* subsp. *massiliense* (7%) or *M. abscessus* subsp. *bolletii* (0%). Infections caused by *M. abscessus* subsp. *abscessus* are associated with worse clinical outcomes following lung transplantation, partly due to the high



frequency of macrolide resistance, and other factors, such as immunological status, can also impact clinical outcomes (22). Further work is needed to elucidate the presence of virulence factors or pathogenic mechanisms linked to MABC infection in CF patients.

There was greater diversity among MABC isolates recovered from non-CF than among those recovered from CF patients. However, the clinical significance of this finding is uncertain, in view of the relatively small number of isolates from non-CF patients.

We have shown the high discriminatory power of WGS for analyzing MABC strains circulating in Ireland, where MABC infection is a nonnotifiable disease. This results in knowledge gaps regarding antimicrobial resistance, clinical characteristics, and epidemiology. However, the high prevalence of CF in Ireland and the genomic data presented here may influence a change in the MABC public health policy to monitor MABC infections and detect the transmission of dominant clones of MABC occurring across different geographical settings. The implementation of both a national and an international MABC surveillance system based on conventional and WGS surveillance would allow greater insights into the prevalence of MABC, would guide infection prevention and control policies within health care facilities, and may help avoid the onward transmission of MABC between patients.

#### SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, XLSX file, 0.01 MB.

**SUPPLEMENTAL FILE 2**, XLSX file, 0.01 MB.

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We declare that we have no conflict of interest.

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