Mycobacterium abscessus in patients with cystic fibrosis: low impact of inter-human transmission in Italy

To the Editor:

Mycobacterium abscessus is a rapidly growing nontuberculous mycobacterial species increasingly isolated worldwide [1]. It may cause chronic pulmonary infections, mainly in elderly patients with underlying bronchiectasis or chronic obstructive pulmonary disease, and it may also cause soft tissue, bone and joint infections [2]. *M. abscessus* has been isolated frequently from patients with cystic fibrosis (CF) [3], although its role in the decline of lung function remains unclear as it can be found both in patients with severe decrease in forced expiratory volume in 1 second (FEV_1) and progressive worsening on computed tomography (CT) scan [4], and in asymptomatic patients. We investigated at the whole genome level *M. abscessus* isolated from all patients attending four Italian CF centres in the past decade with the aim of assessing the role of inter-human transmission.

The four centres are located in geographically distinct regions of Italy. All of the centres routinely perform sputum screening for mycobacteria using conventional protocols [5], together with extended incubation culture on *Burkholderia cepacia* selective agar (BCSA). The selective activity of BCSA for rapidly growing mycobacteria [6] allows the risk of missing *M. abscessu*s through contamination or overgrowth of other bacterial species to be minimised. No special segregation policy was in force in any of the centres during the years covered by this study.

*M. abscessu*s consists of three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* [7]. We identified 306 isolates at the subspecies level by *rpoB* gene sequencing [8]. In our study, *M. abscessus* subsp. *abscessus* accounted for 62% of isolates, in agreement with reported data [9]. Unexpectedly, *M. abscessus* subsp. *bolletii* was the second most prevalent subspecies (22%), suggesting that this subspecies is not as rare as previously thought.

In total, 162 isolates were used for whole genome sequencing (WGS); one isolate was taken from each patient and at least two (the first and the last) if multiple isolates were found. In patients shown to be infected by more than one strain belonging either to the same or different subspecies, a representative proportion of isolates were selected for WGS, with the objective of differentiating persistent infections from occasional re-infections.

Cluster analysis of the isolates was performed by single nucleotide polymorphism (SNP) detection using an arbitrary cut-off based on the average difference of isolates collected in a short time interval from the same patient [10]. Reads were mapped to the *M. abscessus* ATCC19977 genome (NC_010397.1) using BWA (http://bio-bwa.sourceforge.net), and mappings refined with the GATK and Samtools toolkits. SNPs were called by Samtools and perl scripts with a minimum coverage of four reads in both forward and reverse orientation, with the four reads calling the allele with a phred score of at least 20 and allele frequency of 75%. SNP positions were then combined, and positions with a reliable base call in at least 95% of the isolates were concatenated to a sequence alignment, excluding SNPs within a window of 12 bp from each other.

In 42 out of 48 patients with multiple isolates of *M. abscessus* over time, the average difference in SNPs was seven (range 0–22), a low number suggesting the persistence of the same strain and not a re-infection. In four patients, we isolated more than one subspecies of *M. abscessus* at different time points. In another

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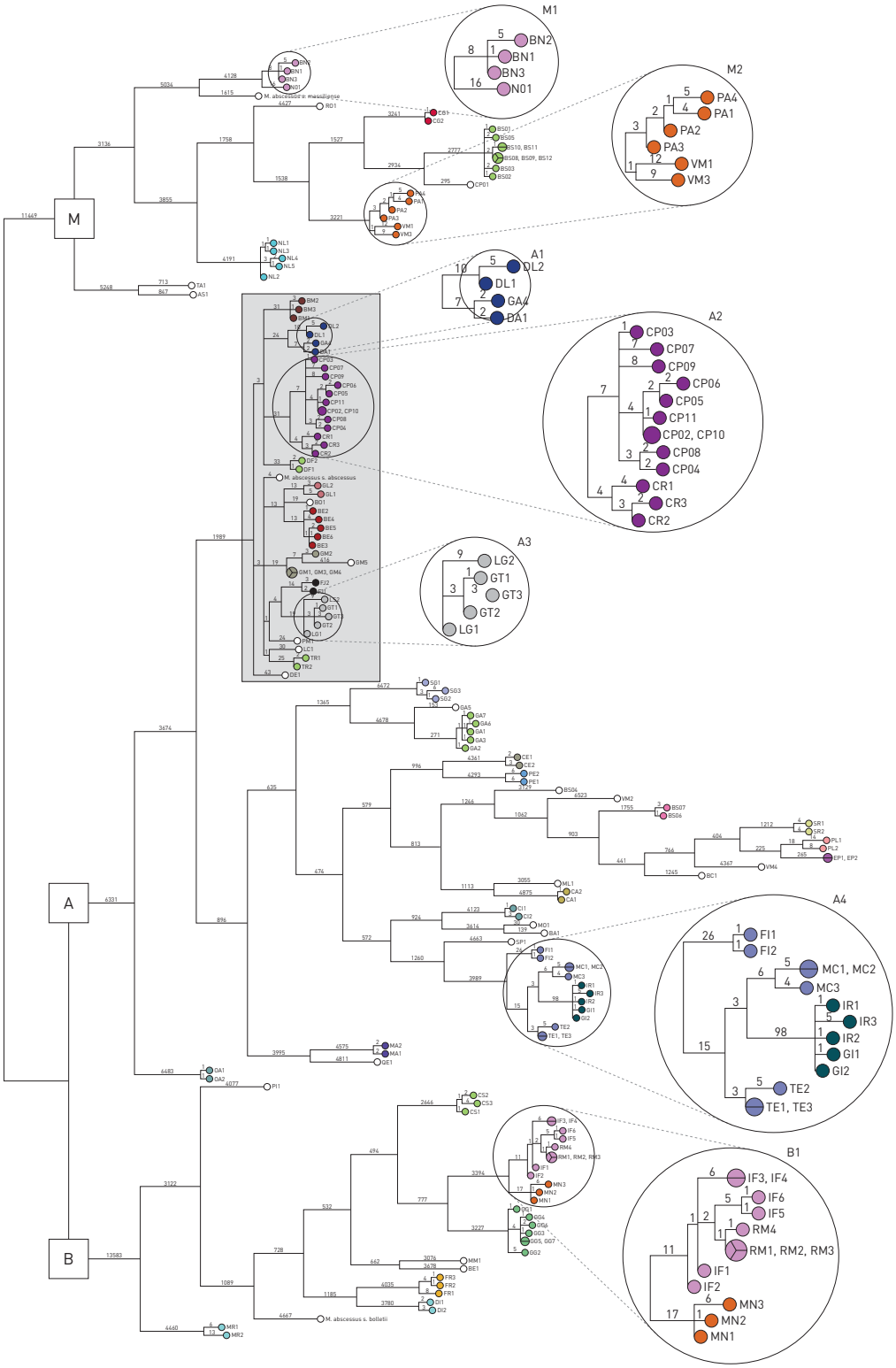

FIGURE 1 Maximum parsimony phylogenetic tree constructed on 110551 SNP positions of 162 clinical isolates and three reference strains of *Mycobacterium abscessus* on a logarithmic scale. Numbers on the branches indicate the number of distinct SNP positions between isolates. All isolates were grouped together (as indicated by the same colour) with a maximum distance of 25 SNPs to the nearest group member. M, A and B indicate the branches corresponding to the three subspecies: *M. a.* subsp. *massiliense*, *M. a.* subsp. *abscessus* and *M. a.* subsp. *bolletii* respectively. The grey shaded box highlights the dominating clone of *M. abscessus* subsp. *abscessus*. Clustered isolates are shown in circles M1, M2, A1, A2, A3, A4 and B1; each isolate is indicated by a two-letter identifier, unique to each patient, followed by a number representing the order of isolation. SNP: single nucleotide polymorphism.

two patients, two unrelated strains of the same subspecies were detected. In almost all cases presenting more than one subspecies (5/6), we observed persistence of the same strain over time.

WGS confirmed the species identification performed by *rpoB* Sanger sequencing in all cases except two strains that, despite the presence of a *rpoB* sequence specific of *M. abscessus* subsp. *abscessus*, were assigned to *M. abscessus* subsp. *massiliense* by WGS.

Figure 1 shows the minimum parsimony tree of the 162 isolates analysed by WGS. Three main clusters corresponding to the subspecies are clearly identifiable (rectangles A, B and M). Within *M. abscessus* subsp. *abscessus* (figure 1A), we observed one group of clustered isolates (grey shading) originating from 15 (25%) different patients, whereas the remaining isolates were dispersed. The median branch length of the clustered isolates was 21 SNPs (range 1–144).

A recent paper by Bryant *et al.* analysing WGS of a vast number of *M. abscessus* isolated from patients with CF worldwide [11] reported the existence of at least three dominant clones responsible for at least three outbreaks postulated to be consequent to direct or fomite-mediated transmission. The analysis of our genomes, together with 30 of those reported by Bryant *et al.* [11] (10 for each for the three major clusters: Abscessus 1 and 2 and Massiliense) revealed that Abscessus 1 overlapped the largest cluster identified in our collection (grey shading in figure 1). To avoid the risk of overlooking transmission episodes, we intentionally kept the threshold for flagging possible patient-to-patient transmission wide (⩽30 SNPs, with our same-patient difference being ⩽21 SNPs in 99% of the cases). By applying this threshold, we detected in the aforementioned cluster three possible patient-to-patient transmission episodes involving seven patients in total (3 in A1 and 2 each in A2 and A3). An additional cluster was identified within *M. abscessus* subsp. *abscessus* dispersed isolates, which involved five patients (A4). The number of SNPs confirmed possible occurrence of transmission for only two patients (IR and GI). Two clusters were detected within *M. abscessus.* subsp. *massiliense* (M1 and M2), each involving two patients, while one cluster (B1) involving three patients was present for *M. abscessus* subsp. *bolletii.*

We were able to collect information on attendance of each patient at the different centres included in this study. Of the seven "possible" transmission episodes identified by WGS analysis using a cut-off of SNPs difference ⩽30, only three (M1, M2 and B1 [patients IF and RM only]) involved patients who had attended the same CF centre at the same time. For the remaining four patients, transmission appears very unlikely based on the geographical location. In fact, these cases involved either patients attending centres located in different cities (A1), or couples of patients (one adult and one paediatric) referred to the same centre but cared for in different and distant locations of the clinic (A2, A3 and A4). One of our small clusters (A4), which included strains that were not very closely related, coincided with the Abscessus 2 of Bryant *et al.* [11], while Massiliense was far from our *M. abscessus* subsp. *massiliense* genomes with the exception of one strain (CP01), isolated only once from one patient. Importantly, our investigation could exclude the occurrence in the past 12 years of major outbreaks of *M. abscessus* strains in any of the four Italian centres participating in this study. The discrepancy with the paper by Bryant *et al.* [11] that reported large outbreaks in multiple countries may be explained by their much larger sample size; however, in an older study by Bryant *et al.* [10], which was very similar to ours in terms of number of patients recruited, two large outbreaks of *M. abscessus* subsp. *massiliense* were reported in a single CF centre. Another explanation may be the very low incidence of *M. abscessus* subsp. *massiliense* in our study.

We conclude that the cluster Abscessus 1 described by Bryant *et al.* [11] is present in Italy in the population of patients with CF. In addition, we identified very few highly clustered cases, each involving a maximum of three patients, for which the possibility of healthcare-related transmission could not be excluded. No major outbreaks were identified in our study in any of the participating centres. Our findings, far from minimising the importance of preventive infection control measures in CF centres, will hopefully reduce the concern in the CF community provoked by the emphasis given to the risk of inter-human transmission of *M. abscessus*.

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