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Original Article

Molecular epidemiology of *Mycobacterium abscessus* complex isolates in Ireland

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Abstract

Background: The *Mycobacterium abscessus* complex are the rapidly growing mycobacteria (RGM) most commonly causing lung disease, especially in cystic fibrosis (CF) patients. Ireland has the world's highest CF incidence. The molecular epidemiology of *M. abscessus* complex in Ireland is unreported.
Methods: We performed *rpoB* gene sequencing and multi-locus sequence typing (MLST) on *M. abscessus* complex strains isolated from thirty-six patients in 2006–2012 (eighteen known CF patients).
Results: Twenty-eight strains (78%) were *M. abscessus* subsp. *abscessus*, eight *M. abscessus* subsp. *massiliense*, none were *M. abscessus* subsp. *bolletii*. Sequence type 1 (ST1) and ST26 (*M. abscessus* subsp. *abscessus*) were commonest. Seven *M. abscessus* subsp. *abscessus* STs (25%) were novel (two with novel alleles). Seven *M. abscessus* subsp. *massiliense* STs were previously reported (88%), including two ST23, the globally successful clone. In 2012, of 552 CF patients screened, eleven were infected with *M. abscessus* complex strains (2%).
Conclusions: The most prevalent *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* strains in Ireland belong to widely-distributed STs, but there is evidence of high *M. abscessus* subsp. *abscessus* diversity.

Keywords: *Mycobacterium abscessus*; Epidemiology; Cystic fibrosis; Ireland; MLST

1. Introduction

Clinically significant mycobacterial isolates can be divided into the *Mycobacterium tuberculosis* complex and non-tuberculous mycobacteria. The non-tuberculous mycobacteria are classifiable as slowly growing or rapidly growing [1]. Members of the *M. abscessus* complex (or *M. abscessus* sensu lato) are the most frequent rapidly growing mycobacteria (RGM) causing lung disease in humans [2,3], and are also associated with postsurgical [4] or traumatic infections outside the respiratory tract and occasionally disseminated disease in immunocompromised patients. The taxonomy of *M. abscessus* complex strains has been in flux in recent years. A division into three species *M. abscessus*, *M. massiliense* [5] and *M. bolletii* [6] was called into question by a lack of inter-species differentiation by phenotype, DNA–DNA

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hybridisation and individual sequences of *rpoB* and *hsp65* genes when larger numbers of strains were evaluated [4,7]. However, a clear phylogenetic signal supporting these three groups has now been obtained by extensive Multi Locus Sequence Typing (MLST) [8] and multiple genome sequence comparisons [9,10] of widely geographically distributed strains. It is likely that three subspecies will be re-proposed based on this evidence. For the purposes of this manuscript we use the terms *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* to describe these three taxa individually following usage in the current clinical literature on cystic fibrosis patients [9,11].

Respiratory infections with *M. abscessus* complex are especially associated with cystic fibrosis [12]. The largest prospective survey in western Europe (in France) cultured *M. abscessus* complex from 3.2% of cystic fibrosis patients [13], with a peak incidence between 11 and 15 years of age (5.8%).

Case reports of fatal disseminated infection with *M. abscessus* complex in immunosuppressed cystic fibrosis (CF) patients following lung transplant [14,15] means carriage is viewed as a contraindication to lung transplant in some centres, but not others where longitudinal surveys have revealed no excess mortality with *M. abscessus* complex carriage [16].

A variety of different strains of *M. abscessus* complex is generally revealed on molecular typing of clinical isolates, but large outbreaks of surgical site infections with a genetically uniform (on PFGE and single gene sequencing) glutaraldehyde resistant strain of *M. abscessus* subsp. *massiliense* have occurred in Brazil associated with laparoscopy and arthroscopy [17]. Genome sequencing allowing very fine inter-isolate comparison by single nucleotide polymorphisms (SNPs) has enabled two recent reports of horizontal transmission of *M. abscessus* subsp. *massiliense* between cystic fibrosis patients within hospitals in Seattle, USA [18] and Papworth, UK [9]. The outbreak strains involved at the two centres were also very closely related on genome sequencing SNP phylogeny [9,19], despite the lack of documented inter-continental contact. Genome sequencing of the Brazilian surgical infection clone [20] showed that it was also phylogenetically very similar to the two cystic fibrosis outbreak strains [9,19].

The molecular epidemiology of *M. abscessus* complex strains from Ireland has not been reported. Ireland has the highest incidence of cystic fibrosis in the European Union [21] and therefore the population contains a relatively large number of individuals susceptible to *M. abscessus* complex infection. As the country is an island, individual strain prevalence may differ from other countries. We describe *rpoB* fragment typing and MLST for isolates from 36 patients from Ireland, including eighteen cystic fibrosis patients attending two different regional centres. Six patients were from one centre serving adult and paediatric patients (Cork University Hospital, Cork, CUH) and twelve from an adult centre (St Vincent's University Hospital, Dublin, SVUH).

2. Materials and methods

Thirty six isolates of the *M. abscessus* complex, obtained between 2006 and 2012 from 36 individuals were included in this study. Six respiratory isolates were obtained from a single adult and paediatric cystic fibrosis centre (Cork University Hospital, CUH). Thirty were anonymous isolates sent to the Irish National Mycobacterial Reference Laboratory for speciation. Twelve of these were respiratory isolates from adult cystic fibrosis patients at St Vincent's University Hospital, Dublin (SVUH), three were respiratory isolates from non-cystic fibrosis patients. Clinical details were not available for the remaining fifteen isolates. Culture and initial identification: *M. abscessus* complex strains from CF patients at CUH were identified by rapid growth in BacT/ALERT 3D broth (bioMérieux) and subsequent GenoType Mycobacterium assay (Hain Lifescience) targeting the 23S rRNA gene. Twice yearly mycobacterial culture screens have been carried out at CUH on CF patients since 2008, before 2008 mycobacterial cultures were taken on clinical suspicion. At SVUH cultures were obtained by extended incubation (7–10 days) on *Burkholderia cepacea* selective agar [22], and *B. cepacea* screening was carried out 4–5 times per annum. DNA extractions were carried out on fresh cultures using Tris–EDTA, lysozyme, and proteinase as described [23]. *Aspergillus* culture positivity was defined as more than one isolation of *Aspergillus* sp. within a month before or after *M. abscessus* complex isolation

Molecular typing: a 940-bp fragment of the *rpoB* gene was amplified by PCR using AmpliTaq gold polymerase (Applied Biosystems) with primers MYCOF1 and MYCOR2 [23] and trimmed to 752 bp [24]. 4–600 bp fragments from *argH*, *cya glpK*, *gnd*, *murC*, *pta*, and *purH* were amplified in 25 μl of ReddyMix PCR master mix (Thermo Fisher Inc.) with 1 μl of each primer (10 pmol) as described [8]. Minimum spanning tree created with PhyloWeb at Institut Pasteur *M. abscessus* MLST Database using the minimum spanning tree (MST) algorithm [25] on 118 sequence types (ST) including 22 ST from this study http://www.pasteur.fr/recherche/genopole/PF8/mlst/Myco-abscessus.html.

The neighbour-joining algorithm was used as implemented in MEGA5 [26] as described [8] using concatenated DNA sequences of each MLST type aligned with MUSCLE [27]. Codon positions were in frame and there were a total of 3576 bp in the dataset. The concatenated ST sequence of the *M. massiliense* type strain (ST37) was added to the alignment, strains from Ireland with the same ST as the *M. abscessus* type strain (ST1) were already present in the dataset. Bootstrap confidence values were based on 500 replications. Where *rpoB* species assignment and MLST species assignment based on localization in the minimum spanning tree or phylogenetic trees constructed with concatenated multiple sequence alignments using MLST loci were discordant, the MLST assignment was chosen because of the known horizontal transfer of *rpoB* in some strains [8]. An estimate of prevalence of *M. abscessus* complex infection in CF patients in Ireland was determined from the number of CF patients attending CUH and SVUH clinics in 2012 and the recorded number of patients from these centres from whom *M. abscessus* complex strains were isolated in that year.

3. Results

Twenty eight strains (78%) were assigned to *M. abscessus* subsp. *abscessus* by *rpoB* sequencing and eight to *M. abscessus*