Isolation and identification of '*Mycobacterium angelicum*' from a patient with type II respiratory failure: suggested reporting guidelines to molecular clinical laboratories

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This is the first case report and subsequent discussion of a new mycobacterial species, namely '*Mycobacterium angelicum*', isolated from a patient with type II respiratory failure. This case is noteworthy as i) it represents the first report of a new mycobacterial species in a human host, ii) its association and potential clinical significance in this respiratory patient, iii) its difficult conventional identification, and iv) the difficulty of reporting a new species which has yet to be accepted as an approved bacterial name, and without any phenotypic description.

A 49-year-old lady was admitted to hospital with significantly raised inflammatory markers, following an episode of sudden-onset breathlessness in the early hours of the morning. The patient had a background history of chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, right knee replacement, humeral fracture, anaemia of chronic disease, medullary sponge kidney, mild oesophagitis/antritis and chronic renal impairment. Previously, she had been on methotrexate therapy and on admission was on salazopyrin.

Chest X-ray demonstrated left sided pulmonary infiltrates and a background of interstitial shadowing. She was in type II respiratory failure and was commenced on broad-spectrum antibiotic therapy. She was noted to be a current smoker on admission. She deteriorated, had multi-organ failure and was mechanically ventilated.

Sputum was negative on direct examination for acid/alcohol-fast bacilli (AAFB), but cultured positive for *Staphylococcus aureus*. While she was being ventilated, flexible bronchoscopy was performed, which demonstrated fungal plaque in the distal trachea and also vesicles at the carina and proximal right and left main bronchi. Bronchial washings were positive for *S. aureus*, *Candida* sp., herpes simplex virus (HSV) and respiratory syncytial virus (RSV).

Initially, the patient was commenced on aztreonam and clarithromycin, but subsequently changed to meropenem and clarithromycin. Fluclonazole and acyclovir were then added. Subsequently, her clarithromycin was switched to teicoplanin. Although clinically she had been improving overall, she suffered further temperature spikes and bronchoalveolar lavage (BAL) AAFB cultures became positive for a non-tuberculosis *Mycobacterium*. The patient continued to improve clinically and the inflammatory markers continued to decrease.

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The *Mycobacterium* isolate was difficult to identify phenotypically using routine bacteriological methods and was genotypically negative for *M. tuberculosis* when employing the Hain *Mycobacterium* CM/AS genotyping assay (Hain Lifescience, Nehren, Germany), although this assay did confirm that the isolate belonged to the *Mycobacterium* genus.

Subsequently, the isolate was subjected to molecular identification with 16S rRNA gene sequencing, as previously described.[1] Analysis (FASTA; www.ebi.ac.uk/FASTA) of the resulting sequence showed 100% similarity (with 817 bases called) with '*Mycobacterium angelicum*' (GenBank Accession number: AM884328). The resulting sequence has now been submitted to GenBank with the accession number GU907552, and the isolate has been deposited in the strain collection of the Northern Ireland *Mycobacterium* Reference Laboratory, Royal Group of Hospitals, Belfast, Northern Ireland (contact email: timothy.stanley@belfasttrust.hscni.net).

The 16S rRNA gene sequence of '*M. angelicum*' was first described in September 2007 by F. Pourahmed at the Institute of Aquaculture, University of Stirling, and since then this group has submitted an additional three DNA sequences obtained from the strain, including hsp65, ITS1 and *rpoB* gene sequences. The strain has been submitted to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (www.dsmz.de), with reference number DSM 45057.

In addition, the isolation of '*M. angelicum*' from *Crocidura olivieri* (the African giant shrew) was reported from near water in the Allada region of Benin, West Africa, in 2006.[2] Phylogenetically, this organism is most closely related to *M. aemonae* and *M. szulgai*, and a phylogenetic tree is included in Figure 1 to facilitate the positioning of '*M. angelicum*' among other species within this genus. Previously, others workers have shown this species to be closely related (96.8%) to *M. avium*[3] when employing polymerase chain reaction (PCR)-restriction enzyme analysis using BstEII and HaeIII restriction enzymes.

The role of atypical mycobacteria, including *M. szulgai*, in lung disease has recently been reviewed by Sexton and Harrison[4] and Alvarez-Uria,[5] although any reference to '*M. angelicum*' is absent from these two reports. Hence, the current authors believe that this is the first report of the isolation of '*M. angelicum*' from a human host, but, with the complicating co-pathogens isolated, it is difficult to interpret the current case in the absence of clinical reports of the virulence of this organism from previous work. In the present case, the consequence of the isolation of this atypical *Mycobacterium* in a BAL sample from this symptomatic patient is unclear. The co-presence of *S. aureus*, as well as HSV and RSV, from the BAL sample complicates the interpretation of the role of this *Mycobacterium* isolate in this case.

The genus *Mycobacterium* currently consists of 149 listed species with approved bacterial names (www.bacterio.cict.fr/); however, '*M. angelicum*' is not, as yet, included as one of these species, hence the use of its name in quotation marks in this report. The original sequence description and

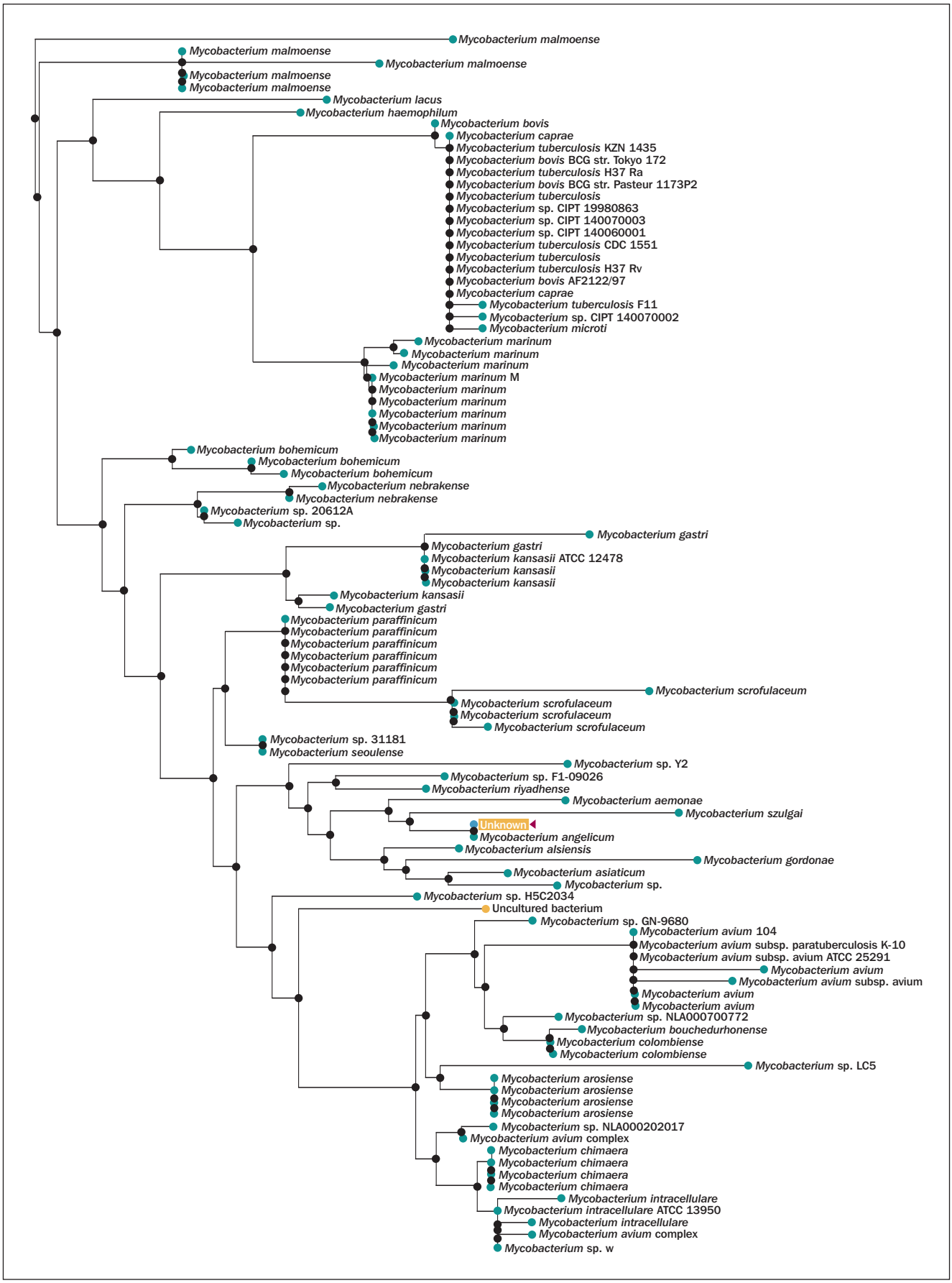

Fig. 1. A phylogenetic genetic tree based on 16S rRNA gene sequences showing the relative position of '*Mycobacterium angelicum*' with its closest phylogenetic neighbours.

deposition in the DSMZ strain repository detailed this organism to have been isolated from dermal lesions of the freshwater angelfish (*Pterophyllum* spp.). Aquatic mycobacteria have been reported in fish, which are susceptible to infection with these environmental organisms.[3,6,7] Indeed, this information prompted the authors to return to the patient from whom the organism was isolated, in order to enquire about any potential zoonotic association. A subsequent history revealed that the patient lived in a household in which a large goldfish had been kept in a bowl for approximately two years prior to hospital admission. The fish died shortly before she became unwell; however, the patient claimed never to have changed the water in the fish bowl, nor did she ever feed the fish. In addition, interestingly, for about two years prior to her hospital admission, she accompanied her daughter and grandchildren approximately every two weeks to her local pet shop in order to look at the exotic fish.

In order to achieve full and approved species status, further taxonomical work is required and the species described in a formal taxonomic report. This poses a problem with regard to reporting such results to physicians. In the present case, molecular diagnostic identification yielded an identity for the organism which as yet has not been fully and formally described in the literature. The ability to identify this organism was reliant on the Scottish group's deposition of its 16S rRNA gene sequence in GenBank. Given the vast biodiversity still to be elucidated within the bacterial kingdom, with the rapid advancement of molecular ability in clinical microbiology laboratories, and an expanding phylogenetic database (eg GenBank), it is likely that clinical microbiology's molecular diagnostics ability will overtake its taxonomic ability, as was the case here.

The authors therefore propose the guidelines detailed in Table 1 to help molecular diagnostics laboratories deal with such eventualities. From this table, there is a recommendation to forward such isolates and their 16S phylogenetic data to the isolating laboratory's national reference laboratory for further examination. As a result, reference laboratories should be properly funded to facilitate the receipt of isolates from diagnostic laboratories along with accompanying high-quality 16S phylogenetic data. As most diagnostic laboratories, including those with enhanced molecular experience, have limited taxonomic ability, such departments would look towards their reference laboratory for taxonomic positioning of new isolates.

Table 1. Suggested guidelines to molecular clinical diagnostic laboratories dealing with a new organism.

• Molecular laboratories should attempt to sequence the full-length of the 16S rRNA gene employing universal or 'broad range' 16S rRNA gene primers.
• Full-length 16S rRNA gene sequence data are corroborated with forward and reverse strand reads.
• Resulting 16S rRNA gene sequence data are carefully checked with CHROMAS software against the coloured chromatogram, and any sequencing anomalies corrected.
• BLAST analyses will reveal the uniqueness of the taxon being investigated. Where there is sufficient evidence to make a confirmation of the correct genus (i.e., >92% sequence homology with the existing genus), then the report is sent out as singularly the genus (e.g., *Mycobacterium* sp.), with no attempt to name the organism under examination, at this stage.
• Corrected 16S rRNA gene sequence data should be deposited in GenBank as simply the genus, or in more complicated cases as 'unidentified' bacterium, with as much clinical, epidemiological and other available information uploaded to help other diagnostic laboratories in similar circumstances.
• The new organism is safely archived and the isolate and its 16S rRNA gene sequence forwarded to the national reference laboratory for further taxonomical examination.

References

1 Xu J, Stanley T, Millar BC *et al*. Difficult-to-identify bacteria: how use of 16S rDNA PCR and gene sequencing can help. *Br J Biomed Sci* 2008; **65** (1): 33–6.

2 Durnez L, Suykerbuyk P, Nicolas V *et al*. Terrestrial small mammals as reservoirs of *Mycobacterium ulcerans* in Benin. *Appl Environ Microbiol* 2010; **76** (13): 4574–7.

3 Pourahmad F, Thompson KD, Adams A, Richards RH. Comparative evaluation of polymerase chain reaction-restriction enzyme analysis (PRA) and sequencing of heat shock protein 65 (hsp65) gene for identification of aquatic mycobacteria. *J Microbiol Methods* 2009; **76** (2): 128–35.

4 Sexton P, Harrison AC. Susceptibility to nontuberculous mycobacterial lung disease. *Eur Respir J* 2008; **31** (6): 1322–33.

5 Alvarez-Uria G. Lung disease caused by nontuberculous mycobacteria. *Curr Opin Pulm Med* 2010; **16** (3): 251–6.

6 Pourahmad F, Thompson KD, Taggart JB, Adams A, Richards RH. Evaluation of the INNO-LiPA mycobacteria v2 assay for identification of aquatic mycobacteria. *J Fish Dis* 2008; **31** (12): 931–40.

7 Pourahmad F, Thompson KD, Adams A, Richards RH. Detection and identification of aquatic mycobacteria in formalin-fixed, paraffin-embedded fish tissues. *J Fish Dis* 2009; **32** (5): 409–19.