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NTM-TB INSIGHTS

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Identifying novel *Mycobacterium* species in clinical samples: Have we met before?

As of 2014, there were "over 150 officially recognized nontuberculous mycobacteria (NTM) species, only two or three dozen familiar to clinicians, and even to most microbiologists" [1]. A total of 59 new *Mycobacterium* species were described between 2006-2014, illustrating the breadth of undiscovered biology in the genus. Among the new species, two-thirds are in the slowly growing mycobacterial group and one-third in the rapidly growing mycobacterial group. Approximately 70 were isolated from human samples and the remainder from animals or the environment. The fact that so many novel strains were originally isolated from human samples suggests that we should pay careful attention to these species and monitor their frequencies and infection potential.

For identification testing in the clinical laboratory, mycobacterial isolates are first identified as acid fast positive by Ziehl-Neelsen staining methodology. Then, they are classified to the species level using molecular methods such as single- or multi-gene sequencing [2-4] or protein spectrum analysis using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Both methods rely on generating molecular or protein "signatures" and comparing them to databases with signatures of known mycobacterial species. In some cases, a reliable match will not be obtained, and the strain will be categorized into an ambiguous group called "unknown isolate". The proportion of unknown isolates in the clinical lab is relatively low, but the frequency of any one novel species is largely unknown until it is officially named and described with its unique features catalogued for future reference. Most likely, a novel species has been seen multiple times in a given lab, perhaps even spanning several years.

As an example, a novel mycobacterial species called *M. talmoniae sp. nov* (type strain: NE-TNMC-100812T = ATCC BAA-2683T = DSM 46873T) was identified and described by researchers at National Jewish Health, the University of Nebraska Medical Center and Colorado State University [5]. In 2000, an unidentified acid-fast bacillus was isolated from a sputum sample of a patient referred to National Jewish Health from her home in Oregon. The internal transcribed spacer (ITS) region was sequenced and the isolate was presumptively identified as the genus *Mycobacterium*. The isolate was given the unofficial name of "*Mycobacterium coloregonium*" and was deposited to the American Type Culture Collection (ATCC) in 2004 under the name ATCC BAA-1052. This species, however, was not published validly at the time.

Then in 2012, a presumed novel *Mycobacterium* was repeatedly isolated from respiratory samples of a patient with chronic pulmonary disease at the Nebraska Medical Center and given the strain designation, NE-TNMC-100812^T. The ITS region of NE-TNMC-100812^T was sequenced and showed the highest sequence similarity to "*M. coloregonium*" ATCC BAA-1052 (99% identity and 72% coverage) in the NCBI database. NE-TNMC-100812^T was initially assumed to be novel due to low sequence coverage compared to ATCC BAA-1052, thus the authors subjected both isolates, ATCC BAA-1052 and NE-TNMC-100812^T, to phenotypic testing, MALDI-TOF MS analysis and whole genome sequencing to test the hypotheses that 1) the two isolates are the same species and 2) the isolates belong to a novel species in the genus *Mycobacterium*. Additional analyses, including fatty acid and mycolic acid profiling and antimicrobial susceptibility testing (AST), were performed to further characterize the novel taxon.

Growth of NE-TNMC-100812^T and ATCC BAA-1052 was observed in 7-10 days at 37°C, and phylogenetic analyses of the 16S rRNA genes supported placement in the slowly growing group. MALDI TOF MS results showed no reliable matches against a database of over 130 known mycobacterial species, and the full-length *rpoB* gene showed the highest sequence similarity to *M. avium* (92%), which is well below the cutoff of 97% for a species-level identification. The mycolic acid profile of NE-TNMC-100812^T was more similar to *M. avium* than *M. simiae* and *M. abscessus* subsp. *abscessus*. But, the MIC results using the CLSI microdilution method [6] differed from *M. avium* by more than one dilution factor for three of the 10 antibiotics tested. This species is most easily identified by its unique *rpoB* sequence, which has now been deposited in NCBI Genbank sequence database under the accession number KX008971.

All phenotypic and genetic experiments suggested that the Colorado isolate from 2000 and the Nebraska isolate from 2012 were indeed the same species and an undescribed novel taxon. The name *Mycobacterium talmoniae sp. nov* was proposed in honor of Kathy Talmon, a microbiologist with over 30 years of service to the public health laboratory in Nebraska. It was Ms. Talmon who noticed the strain's unique growth characteristics in the laboratory, and its repeated isolation from the same patient. Clinically, the Nebraska strain was determined to be a colonizer and the patient was not put on treatment. It is unknown whether the National Jewish Health patient in 2000 received treatment at the time. Since then, gene sequences of two other strains of this species have been deposited to NCBI from researchers in Seattle, WA, and Newcastle, United Kingdom. This illustrates how a novel species could be potentially widespread and was likely there all along, simply known as an "unknown isolate". We will have to monitor the ongoing story of *M. talmoniae* in the clinical population to determine whether it can cause human disease.

The most effective ways to identify mycobacterial species are through molecular and MALDI-TOF MS methods that continue to be evaluated and improved for the clinical laboratory [7]. A useful method to characterize novel species, which is available primarily in research laboratories, is whole genome sequencing, as was used for *M. talmoniae* [5] and others [8,9]. This allows for in-depth whole genome comparisons against the ever-expanding mycobacterial genome database at NCBI and also has the added benefit of detailed information on drug resistance mutations and other molecular markers of interest. As whole genome sequencing slowly makes its way into the clinical laboratory routine [10], we may identify many more clinically relevant *Mycobacterium* species than we ever imagined.

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Rebecca M. Davidson, PhD, Assistant Professor, Center for Genes, Environment and Health, Department of Biomedical Research, National Jewish Health, Denver, CO

Recent Staff Publications

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