An Update on Mycobacterial Taxonomy, 2016–2017

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ABSTRACT This minireview provides an update on recent taxonomic changes for the genus *Mycobacterium* with an emphasis on newly identified species isolated from humans or associated with human disease.

KEYWORDS mycobacteria, taxonomy

At the time of this writing, the List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.net>) includes 197 species and 14 subspecies (including synonyms) within the genus *Mycobacterium*. This number continues to expand annually, as both clinical and research laboratories utilize molecular sequencing methods which have greater discriminatory power than conventional phenotypic methods. Most of the novel species identified are nontuberculous mycobacteria (NTM), which are ubiquitous in the environment, especially in water, including fresh and salt water as well as piped water systems. Over the past several years, infections due to these organisms have increased globally due to a number of factors, including more discriminatory identification methods and an increased awareness to look for them in clinical specimens as the causative agent of disease, especially as opportunistic pathogens. NTM disease can range from localized superficial infections of the skin to pulmonary and disseminated infections in both immunocompetent and immunocompromised patients. The clinical significance of many of these species has changed and will continue to do so as our understanding of their role in human infections increases, and as species-specific information such as intrinsic antimicrobial resistance becomes available. As a result, clinical microbiologists should keep abreast of taxonomic changes in the genus, especially given the pace at which novel species are being identified, their widespread presence in the environment, and the potential for human exposure, transient colonization, and infection. This minireview provides an update for mycobacterial taxonomy from January 2016 through December 2017.

METHODS

Only those mycobacterial species isolated from humans or associated with human disease are reported in this minireview spanning the interval from January 2016 through December 2017. This minireview is by no means comprehensive in scope. A number of newly described mycobacterial species which were recovered from environmental sources and/or animals were excluded. Several databases and other resources were utilized to identify newly described species, including (i) the List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.net/allnamesmr.html>), (ii) the *International Journal of Systematic and Evolutionary Microbiology*, and (iii) the PubMed database (<https://www.ncbi.nlm.nih.gov/pubmed>), using “nov. sp. *Mycobacterium*” as the search term.

RESULTS

Table 1 summarizes the novel mycobacterial species identified between January 2016 and December 2017 that were isolated from humans, some of which were associated with human disease. A brief description of each species follows.

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TABLE 1 Novel mycobacterial species identified from human clinical specimens or associated with human disease between January 2016 and June 2018

Scientific name	Yr identified	Source	Clinical relevance ^a	Selected characteristics	Gene(s) used for unique identification	Reference
<i>M. aquaticum</i>	2017	Hemodialysis water, sputum	Undetermined	Rapid grower; mucoid colonies, nonpigmented; most closely related to <i>M. brisbanense</i>	16S rRNA, <i>rpoB</i> , <i>hsp65</i>	1
<i>M. eburneum</i>	2017	Sputum	Undetermined	Rapid grower; nonpigmented colonies; most closely related to <i>M. paraense</i>	16S rRNA, <i>hsp65</i> , <i>rpoB</i>	2
<i>M. grossiae</i>	2017	Sputum, blood	Isolated from patients with COPD and post-bone marrow transplant	Rapid grower; scotochromogen, dark orange/yellow colonies; most closely related to <i>M. wolinskyi</i>	16S rRNA	3
<i>M. lehmannii</i>	2017	Undefined clinical specimen	Undetermined	Rapid grower; scotochromogen, pigmented, yellow-orange colonies; most closely related to <i>M. novocastrense</i>	16S rRNA (sequence differs from other mycobacteria except <i>M. neumannii</i>), <i>rpoB</i> , <i>hsp65</i>	4
<i>M. neumannii</i>	2017	Unknown	Undetermined	Rapid grower; scotochromogen, yellow-orange colonies; most closely related to <i>M. novocastrense</i>	16S rRNA (sequence differs from other mycobacteria except <i>M. lehmannii</i>), <i>rpoB</i> , <i>hsp65</i>	4
<i>M. paraintracellulare</i>	2016	Sputum	Pulmonary infection	Slow grower; scotochromogen, yellow colonies; most closely related to <i>M. arupense</i> , a member of the <i>M. terrae</i> complex	<i>rpoB</i> , <i>hsp65</i> , <i>gnd</i> , <i>argG</i> , <i>pgm</i>	5
<i>M. persicum</i>	2017	Sputum	Pulmonary infection	Slow grower; photochromogen, yellow colonies; most closely related to <i>M. kansasii</i> ; member of the <i>M. kansasii</i> complex	16S rRNA, <i>rpoB</i> , <i>hsp65</i>	6
<i>M. talmoniae</i>	2017	Sputum	Undetermined	Slow grower; unpigmented; most closely related to <i>M. avium</i>	16S rRNA, <i>rpoB</i> , <i>hsp65</i>	7
<i>M. virginiae</i>	2017	Synovial tissue, joint fluid, bone biopsy specimen	Tenosynovitis	Slow grower; nonpigmented; most closely related to <i>M. arupense</i> , a member of the <i>M. terrae</i> complex	16S rRNA, <i>rpoB</i> , <i>hsp65</i>	8, 9

^aCOPD, chronic obstructive pulmonary disease.

***M. aquaticum*.** *M. aquaticum* was isolated from hospital water systems in Iran, specifically from hemodialysis water at two different hospitals and subsequently from the sputum of a patient with asthma. Isolation of this organism from this particular patient was not considered clinically significant, as she had no symptoms consistent with mycobacterial disease. Phenotypically, this species was classified as a rapid grower forming nonpigmented, smooth colonies on solid medium in 3 to 5 days at 25°C and 37°C but not at 42°C. Based on 16S rRNA sequencing (1,518 bp), *M. aquaticum* was found to be most closely related to *M. brisbanense* and *M. cosmeticum*, with 99.03% and 99.01% similarity. This relatively high similarity was insufficient to establish *M. aquaticum* as an independent species; thus, further investigation was performed utilizing analysis of two additional housekeeping genes, *hsp65* and *rpoB*, and determination of the average nucleotide identity (ANI) between the test strains and *M. brisbanense* and *M. cosmeticum*. Analysis revealed an ANI of <95%, considered an indication of a unique species. This finding was later confirmed when the genome-to-genome distance, equivalent to *in silico* DNA-DNA hybridization (DDH), was determined to be <70% between the test strains, *M. brisbanense*, and *M. cosmeticum*, confirming a unique mycobacterial species (1). Thus, analysis of the concatenated sequences of all three genes resulted in a phylogenetic position for *M. aquaticum* that was distant from all other *Mycobacterium* species (1). Most strains of this species recovered to date have demonstrated resistance to tobramycin, doxycycline, minocycline, cefoxitin, and imipenem, whereas susceptibility was observed for most of the other drugs used against rapidly growing mycobacteria, i.e., trimethoprim-sulfamethoxazole, linezolid, ciprofloxacin, moxifloxacin, amikacin, tigecycline, and clarithromycin (1).

***M. eburneum*.** *M. eburneum* was first isolated from the sputum of a patient in Switzerland in 1998. However, the role of this species in human infections is unknown. Nonpigmented colonies form within 7 days on solid media at 28°C to 37°C. *M. eburneum* shows the highest similarity (98%) to *M. paraense* (a 39-nucleotide [nt] difference) and *M. kumamotoense* (a 31-nt difference) based on standard 16S rRNA sequence analysis, differences which are insufficient to establish it as a unique species. However, multilocus sequence tree analysis using concatenated sequences of 16S rRNA, *hsp65*, and *rpoB*, as well as an investigation of the digital DNA-DNA relatedness between *M. eburneum* and *M. paraense*, showed a DDH of 23.8%, below the threshold of 70% used to delineate a novel prokaryotic species. This species was found to be resistant to amikacin, carbenicillin, cephalothin, chlortetracycline, spiramycin, tetracycline, and tobramycin (2).

***M. grossiae*.** Unlike some of the other newly described mycobacterial species that were recovered from a single case, *M. grossiae* has been isolated from two patients with distinctly different clinical histories. In the first case, *M. grossiae* was isolated from the sputum of a 76-year-old male from Connecticut with chronic obstructive pulmonary disease and a history of tuberculosis exposure (30 years previously) for which isoniazid prophylaxis was not completed. On presentation, the patient had a symptomatic pulmonary infection with a 1-year history of hemoptysis, weight loss, and a chest computed tomography scan showing bronchiectatic changes and the presence of micronodules indicative of an infection. Initially, one of three sputum cultures grew the *M. avium* complex, for which he was not treated. Seven months after the onset of symptoms, *M. grossiae* was recovered from a subsequent sputum specimen. Unfortunately, it was not determined what role, if any, *M. grossiae*, played in the observed disease symptoms, and the patient was lost to follow-up. The second patient was a 15-year-old male, post-bone marrow transplant, who relapsed with acute lymphoblastic leukemia. *M. grossiae* was recovered from three different blood cultures, requiring the removal of a central venous catheter. The patient was placed on an initial combination regimen consisting of meropenem, azithromycin, and amikacin, which successfully converted his blood cultures to negative, at which time he was transitioned to an oral, long-term regimen of azithromycin, trimethoprim-sulfamethoxazole, and doxycycline (3).

M. grossiae is a rapidly growing, scotochromogenic species of mycobacteria which forms dark-orange to yellow colonies on solid media in less than 7 days of incubation at 24°C to 42°C. Both isolates described above were susceptible to amikacin, ciprofloxacin, moxifloxacin, clarithromycin, doxycycline, imipenem, trimethoprim-sulfamethoxazole, and linezolid, with intermediate susceptibility to cefoxitin. Phylogenetic trees based on the complete sequence of the 16S rRNA gene demonstrated that these two strains were most closely related to *M. wolinskyi* (98.8%). Partial sequencing of *hsp65* (429 bp) and *rpoB* region V (744 bp) revealed these strains to be most closely related to *M. neoaurum* (94.8%) and *M. aurum* (92.1%). However, the *rpoB* region V sequence divergence of >3% in these two strains argues for this being a novel mycobacterial species (3).

***M. lehmannii* and *M. neumannii*.** *M. lehmannii* and *M. neumannii* are rapidly growing, scotochromogenic NTM of unknown importance with respect to colonization or infection in humans. Both were obtained from the German Collection of Microorganisms and Cell Cultures and were initially identified as *M. flavescens* in the early 1990s. Both species form yellow-pigmented colonies in an average of 5 days at temperatures ranging from 28°C to 37°C. Phenotypically, they are nearly identical with regard to both cellular fatty acid and mycolic acid compositions as well as biochemical tests often used for mycobacterial identification (e.g., heat-stable catalase, urease, niacin accumulation, and others) (4). However, *M. neumannii* is capable of growth in 8% (wt/vol) sodium chloride, whereas *M. lehmannii* is not. Genetically, they are identical to one another based on 16S rRNA sequencing and most closely related (98.5%) to *M. novocastrense* (4). However, analysis of the concatenated sequences of the *hsp65*, *rpoB*, and 16S rRNA genes demonstrated ANIs of 89.3% and 89.5% for the two test strains, respectively, and a DDH of <43% versus *M. novocastrense*; the DDH between the test strains was 61.0%. Taken together, these values are well below those used for delineation of a unique prokaryotic species (ANI < 95%; DDH < 70%) and indicate that not only is *M. lehmannii* phylogenetically distinct from *M. neumannii* but that both represent novel species in the genus *Mycobacterium* (4).

***M. paraintracellulare*.** *M. paraintracellulare* was initially isolated from the sputum of three different patients with pulmonary infections in South Korea. This species was found to be resistant to amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, moxifloxacin, and rifampin. *M. paraintracellulare* is a slow-growing mycobacterial species that requires 37°C for optimal growth and forms nonpigmented colonies after 7 days of incubation. Sequence analysis of the 16S rRNA and the internal transcribed spacer (ITS1) revealed that this species was identical to the type strain of *M. intracellulare* (ATCC 13950) (99.2% similarity) and belonged to the *M. intracellulare* genotype 1 (INT-1) group. However, genomic distance analysis based on the sequencing of five additional housekeeping genes, *hsp65*, *rpoB*, *gnd*, *argG*, and *pgm*, revealed a DDH value of 57.9% versus *M. intracellulare* (ATCC 13950), differentiating this species from *M. intracellulare* (ATCC 13950) and phylogenetically establishing it as a distinct species within the *M. avium* complex (5).

***M. persicum*.** *M. persicum* was first isolated in Iran (2009) from three sputum specimens from a 50-year-old female patient with fever, productive cough, and shortness of breath. The patient was initially placed on an antituberculosis (anti-TB) regimen (3 months) due to the presence of acid-fast bacilli in her sputum. However, this regimen was later supplemented with imipenem once the NTM was recovered in culture, and treatment was continued for an additional 3 months. Subsequently, three other strains of this species were isolated during a prevalence survey of multidrug-resistant TB (MDR-TB) in Iran. Importantly, these strains were isolated from patients in different regions of the country with no epidemiological connections who were initially diagnosed as new TB cases and placed on an empirical anti-TB regimen. All strains recovered to date have been susceptible to amikacin, clarithromycin, linezolid, moxifloxacin, and the rifamycins but resistant to ethambutol (6).

M. persicum is phenotypically nearly indistinguishable from *M. kansasii*. Like *M. kansasii*, it requires an average of 2 weeks at 37°C for recovery in culture, is a photochromogen forming yellow colonies in the presence of light, and has a similar mycolic acid profile. The only biochemical characteristic identified to date which separates the two species is the absence of urease activity in *M. persicum*, in comparison to *M. kansasii*, which is positive for urease (6). As with the phenotypic characteristics, *M. persicum* is also most closely related to *M. kansasii* genotypically (99.5% similarity; a difference of 7 bp) based on sequencing of the 16S rRNA gene (1,527 bp). However, further phylogenetic analysis based on the concatenated sequences of the 16S rRNA, *hsp65*, and *rpoB* genes revealed that although the test strains clustered close to *M. kansasii*, they were distinct from the type strain, suggesting inclusion as a member of a related complex. Further analysis of orthologous genome fragments of the test strains versus *M. kansasii* demonstrated an ANI of <95% and a genome-to-genome distance of >0.0170, both consistent with status as an independent species. As such, *M. persicum* is considered a new member of the *M. kansasii* complex. It is important to note that currently available, commercial line probe assays (Genotype; Hain Lifescience) identify this species as either the *M. kansasii* complex or *M. kansasii*/*M. gastri* (6).

***M. talmoniae*.** *M. talmoniae* was first isolated in 2000 from a sputum specimen of a patient in Oregon and subsequently in 2012 in Nebraska from the respiratory samples of a patient with chronic pulmonary disease. This slow-growing, nonchromogenic mycobacterial species is capable of growth at temperatures ranging from 25°C to 42°C, with optimal growth seen after incubation for 7 to 10 days at 37°C. Strains to date have been susceptible to rifabutin, moxifloxacin, amikacin, clarithromycin, rifampin, and ethambutol but resistant to clofazimine, linezolid, ciprofloxacin, and streptomycin. This species is most closely related to *M. avium* based on the *rpoB* gene sequence, which shows 92% similarity (7). However, a pairwise genome comparison utilizing the 16S rRNA, *hsp65*, and *rpoB* genes demonstrated an ANI between the test strains and other mycobacterial species of ≤81.5%, indicative of a unique species (<95% to 96%). As such, *M. talmoniae* is phylogenetically separate from other known slowly growing NTM (7).

***M. virginiense*.** *M. virginiense* was first isolated from a 58-year-old female from Virginia with tenosynovitis. The isolate was part of a collection of 26 strains previously identified using nonmolecular methods as either the *M. terrae* complex or *M. nonchromogenicum*, which were for many years associated with tenosynovitis or osteomyelitis. These specific isolates were recovered between 1984 and 2014 from patients with diagnosed tenosynovitis or osteomyelitis from 13 different states within the United States. Importantly, no sequencing analysis had been performed to confirm the identification previously established using phenotypic methods such as high-performance liquid chromatography (HPLC) and/or biochemicals. Complete sequencing of the 16S rRNA gene revealed that of the 26 isolates tested, none was a match to either *M. terrae* or *M. nonchromogenicum*. Twenty-one of the isolates were identified as more recently described species within the *M. terrae* complex: *M. arupense* (*n* = 10), *M. heraklionense* (*n* = 10), and *M. kumamotonense* (*n* = 1). The 5 remaining strains were found to have the same 2-nt insertion in helix 18 of the 16S rRNA gene as that seen in other members of the *M. terrae* complex but represented a previously undescribed species based on the following sequences: the complete 16S rRNA gene, a fragment of the *hsp65* gene, and regions III and V of the *rpoB* gene (8, 9). Three of the five strains shared 100% identity to each other by 16S rRNA sequencing but differed from other close matches within the *M. terrae* complex, namely, *M. arupense* (99.7%), *M. nonchromogenicum* (99.4%), and *M. heraklionense* (99.3%). Further examination of the genetic relatedness of these three strains to members of the *M. terrae* complex by use of neighbor joining and pairwise deletion analysis demonstrated that they represented a previously unknown species within the *M. terrae* complex (8, 9).

M. virginiae was susceptible to clarithromycin, ethambutol, rifabutin, and trimethoprim-sulfamethoxazole and resistant to rifampin, the fluoroquinolones, amikacin, doxycycline, and minocycline. This species is a slow-growing, nonpigmented mycobacterium which grows best at 35°C but is unable to grow at 42°C (8, 9).

Other updates. In 2010, the first description of *M. paraterrae* was effectively but not validly published by Lee and coworkers and was also described by Tortoli et al. in 2014. In 2016, this species was added to Validation List number 172, confirming valid publication of the previously effectively published new name (10, 11, 12).

DISCUSSION

Advances in molecular sequencing methods have led to an increase in the number of species and subspecies in the genus *Mycobacterium*. From a clinical perspective, this is particularly useful in furthering our understanding of the role of individual species in human and animal diseases, which was previously hindered by the lack of accuracy and reproducibility of phenotypically based identification methods, methods which were potentially confounded by the presence of multiple, previously unidentified species. However, the lengths to which laboratories must go to identify not only the species detailed in this review but also many others previously described in recent years are beyond the capabilities of most clinical laboratories. In fact, many clinical laboratories do not have such testing capacity due both to a paucity of trained personnel and the logistic and budgetary constraints required to sequence and analyze multiple targets (16S rRNA, *rpoB*, *hsp65*, and others). Thus, from a practical standpoint, operationalizing genetic identification to the species level illustrated in this review may not be possible on a larger scale and may be relegated to larger reference or specialty laboratories. Justification for advanced molecular sequencing is also hindered by the facts that the NTM are ubiquitous in the environment and that the clinical significance of many species, including those newly described, is not well known; most examples are illustrated by a single or limited number of cases. Moving forward, it is important for clinical laboratories to note that while more conventional methods of identification may not be able to provide the same level of differentiation as that of advanced molecular sequencing techniques, careful attention must be paid to standard probe-based or other identification methods such as MALDI-TOF (matrix-assisted laser desorption ionization–time of flight mass spectrometry), which produce indeterminate or erroneous results that cannot be explained by instrument or operator error, which in some instances may suggest the presence of a unique species. Clinical laboratories should also be aware of emerging information regarding newly described species associated with clinical disease, especially those considered to be part of a complex (e.g., *M. chimera*, part of the *M. avium* complex), where it may be necessary to know exactly which species is present not only to determine the appropriate treatment but also for epidemiological purposes. Likewise, for laboratories conducting advanced molecular sequencing, reporting to clinicians should include whether the isolate is a subspecies within a complex or, if a unique species, the name and the closest genetic relative. Such an approach provides a frame of reference for the clinician which, along with other phenotypic characteristics such as growth rate and pigment production, can help guide early interventions in clinical care.

Advances in molecular sequencing will continue to expand the number of unique species in the genus *Mycobacterium*. This is especially true in the era of whole-genome and next-generation sequencing. We can expect that the increased discriminatory power of current and emerging sequencing technologies will not only help to further our understanding of the role of NTM in human and animal disease but also facilitate epidemiological studies and aid in the determination of molecular markers of resistance. As the number of phylogenetically distinct species increases, clinical microbiologists should be mindful of changes which occur on a continuing basis and focus on those which are problematic with regard to more conventional identification methods and which have a significant impact on patient care.

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