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MINIREVIEW

Current Updates on Mycobacterial Taxonomy, 2018 to 2019

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ABSTRACT This minireview provides an updated overview of taxonomic changes for the genus *Mycobacterium*, with a focus on new species identified from humans or those associated with human disease for the period of 2018 to 2019.

KEYWORDS mycobacteria, taxonomy

At the time of this writing, the List of Prokaryotic names with Standing in Nomenclature (LPSN) (https://lpsn.dsmz.de/genus/mycobacterium) includes over 200 species (192 validly published with a correct name and 13 synonyms) within the genus *Mycobacterium*. As molecular technologies evolve, clinical and research laboratories continue to discover new species and increase their existing knowledge of current species, especially among the nontuberculous mycobacteria (NTM) (1, 2). Over the past few years, there has been a marked increase in the identification of these species from clinical specimens and, in some cases, as causative agents of human disease, in part due to more discriminatory power in molecular diagnostics as well as an increased clinical awareness. With the emergence of new species and subspecies and the resulting updated classifications, it is imperative for clinical microbiologists to be aware of these changes, especially those which could play a role in human disease given the ubiquitous nature of these organisms in the environment. At the same time, it is equally important to recognize the context in which taxonomic changes must be viewed relevant to clinical care and therapeutic interventions for patients with mycobacterial disease. This minireview gives an update on mycobacterial taxonomy from January 2018 through December 2019.

METHODS

Novel mycobacterial species isolated from humans and/or found to be clinically relevant and validly published between January 2018 and December 2019 are included in this minireview. Newly discovered mycobacteria recovered from animals or the environment were not included. In compiling this minireview, the following sources were utilized: (i) the List of Prokaryotic Names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/mycobacterium), (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 provides a summary of the novel mycobacterial species isolated from humans identified between January 2018 and December 2019, some of which were associated with clinical disease. The following provides a short overview of each, separated as follows: novel species, new species within existing complexes, and reclassification within a complex.

Novel species and subspecies. (i) *Mycobacterium basiliense*. *M. basiliense* was first cultured from a bronchoalveolar lavage (BAL) sample in 2013 and subsequently isolated from respiratory specimens from four additional patients between 2016 and 2017, all in Basel, Switzerland (3). While four of the five patients were symptomatic with cough, dyspnea, and hemoptysis, only one met the criteria established by the

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TABLE 1 Novel mycobacterial species isolated from humans or identified as causes of human disease between January 2018 and December 2019

Scientific name	Yr identified	Source(s)	Clinical relevance	Selected characteristics	Type strain no.	Gene(s) used for unique identification	Reference(s)
M. attenuatum	2019	Bronchial secretion	Nonpathogenic	Slow-grower, photochromogen; phenotypically similar to other members of *M. kansasii* complex	MK41	16S, *hsp65*, *rpoB*, *tuf*	9
M. basiliense	2019	Bronchoalveolar lavage and secretions	Pulmonary infection	Slow-growing nonphotochromogen, closely related to *M. marinum* and *M. ulcerans*	901379	16S, *rpoB*	1
M. decipiens	2018	Cutaneous lesion, lymph node tissue	Synovitis, lymphadenitis	Slow-growing nonpigmented with rough colonies; most closely related to *M. tuberculosis* complex; positive by AccuProbe for MTBC	TBL 1200985	16S, *rpoB*, *hsp65*	2, 3
M. innocens	2019	Sputum	Undetermined	Slow-grower, photochromogen; shares similarities to *M. persicum*	MK13	16S, *hsp65*, *rpoB*, *tuf*	9
M. pseudokansasii	2019	Blood	Isolated from patient with disseminated infection	Slow-grower, rough colonies, and photochromogenic; phenotypically similar to other members of *M. kansasii* complex	MK142	16S, *hsp65*, *rpoB*, *tuf*	9
M. shigaense	2018	Skin, sputum, bronchial fluid, lymph nodes	Pulmonary and cutaneous lesions, disseminated infection	Slow-growing scotochromogen, rough or smooth pigmented yellow colonies; member of the *M. simiae* complex; most closely related to *M. simiae*	UN-152	16S, ITS, *rpoB*, *hsp65*	4
M. intracellulare subsp. *yongonense*	2018 (reclassified)	Sputum	Pulmonary infection	Slow-growing nonchromogenic, new subspecies of *M. intracellulare*	05-1390	WGS	10, 11
M. chelonae subsp. *gwanakae*	2018 (reclassified)	Sputum	Pulmonary infection	Rapid growing nonchromogenic, white smooth colonies; new subspecies of *M. chelonae*	MOTT36W	16S, *rpoB*, *hsp65*	12

American Thoracic Society for nontuberculous mycobacterial pulmonary disease (4). In most of these cases, treatment was not provided, as symptoms resolved on their own. Molecular identification using a commercial line probe assay (Hain Lifescience, Nehren, Germany) proved inconclusive. However, on review of 16S rRNA, the initial strain (European Nucleotide Archive strain 901379) was found to be most closely related to *Mycobacterium marinum* (99.7%) and *Mycobacterium ulcerans* (99.5%) (3). High-performance liquid chromatography (HPLC) showed distinct chromatographic differences in mycolic acid profiles between *M. marinum* and *M. basiliense*, with a shift in retention time of the major peaks ranging from 5.913 to 7.626 min to 6.535 to 8.163 min, respectively. Initial genomic analysis revealed that all of the patient isolates had identical 16S rRNA sequences when using hypervariable regions A and B and shared 99.5% identity with *M. marinum* (GenBank accession number AB716939) and *M. ulcerans* (GenBank accession number AB548729). Further analysis was performed by comparison of closely related whole-genome assemblies by determining the average nucleotide identity (ANI) and the digital DNA-DNA hybridization (dDDH). For strain 901379, when compared to *M. marinum* and *M. ulcerans*, the ANI and dDDH were $<$95% and $<$70%, respectively, indicating a novel species of mycobacteria (3).

Phenotypically, *M. basiliense* is a slow-growing nontuberculous mycobacteria, with nonphotochromogenic white colonies appearing after approximately 10 days of growth on agar plates at 30°C. Biochemically, this species is similar to *M. marinum* and *M. ulcerans*, with positive tests for pyrazinamidase, urease, and tellurite reduction and negative tests for Tween 80 hydrolysis, nitrate reductase, and growth on MacConkey agar. However, unlike *M. marinum* and *M. ulcerans*, *M. basiliense* grows very well at 37°C. Antimicrobial susceptibility testing revealed that *M. basiliense* is sensitive to all major antibiotics, including clarithromycin, rifampin, rifabutin, ethambutol, amikacin, moxifloxacin, clofazimine, and linezolid (3).

Although an environmental niche has not been identified for *M. basiliense*, it should be noted that this mycobacterial species is capable of growth in human macrophages *in vitro* while not able to survive within amoebae. In addition, nearly 10% of the coding sequences within the genome were found to contain a large number of virulence factors, suggesting that this particular mycobacterial species may be more of a pathogen than an environmental saprophyte. As it currently stands, the *M. basiliense* isolates recovered from the patients described in this minireview demonstrated differences of up to two single nucleotide polymorphisms (SNPs), suggesting a common source of infection (3).

(ii) *Mycobacterium decipiens*. *M. decipiens* was isolated from two different patients in 2012 and 2016, both of whom reported prior travel to the tropics (5, 6). The first patient, a 58-year-old from the United States developed synovitis in the right thumb and wrist after traveling on vacation to the U.S. Virgin Islands where a small wound to the hand was noted. Cultures obtained from resected specimens were positive for a mycobacteria species closely related to the *Mycobacterium tuberculosis* complex (MTBC) (isolate TBL 1200985). The second isolate (FI-16190) was recovered from a 5-year-old Italian girl with a history of a small wound on the heel, who developed abdominal pain and fever. Cultures from an intraabdominal lymph node were positive for mycobacteria and showed granulomatous lymphadenitis (5, 6).

In both cases, cultures grew rough, nonpigmented colonies after approximately 20 days on agar plates at both 25°C and 37°C. Growth characteristics were compatible with the *Mycobacterium tuberculosis* complex (MTBC). However, unlike *M. tuberculosis*, biochemical tests were negative for niacin accumulation, nitrate reductase, and Tween 80 hydrolysis and positive for 68°C catalase. A commercially available, probe-based assay (AccuProbe; Hologic, Marlborough, MA) misidentified the isolates as MTBC. Further molecular analysis showed 99.4% similarity to the MTBC using 16S rRNA as the target sequence and 95.3% similarity to *Mycobacterium intracellulare* using *hsp65* (5, 6). However, sequencing of the *rpoB* gene demonstrated $<$90% identity with the MTBC in either strain. When compared to all other members of the MTBC, the average

nucleotide identity (ANI) for the two patient isolates was less than the cutoff required to establish a separate species (<95% to 96%), whereas all members within the MTBC had ANI values of >95% to 96%. Taken together, these data indicate a new species outside of the MTBC. Both isolates showed susceptibility to amikacin, clarithromycin, linezolid, moxifloxacin, rifabutin, and trimethoprim-sulfamethoxazole and resistance to rifampin (5, 6).

(iii) *Mycobacterium shigaense*. Initially recovered in 2009 from infectious skin lesions of a patient in Shiga Prefecture Japan, *M. shigaense* has since been isolated from cutaneous lesions and sputum in five additional patients in Japan and China, leading to speculation that this particular mycobacterial species has limited regional distribution (4). Based on partial 16S rRNA sequencing results and the 16S to 23S internal transcribed spacer region (ITS region), alignment for the original isolate (UN-152) as well as the strains from the other patients were shown to cluster together within the *Mycobacterium simiae* complex. Sequencing comparisons of the ITS region, *rpoB1* and *rpoB2*, and hsp65 showed that the isolates were most closely related in order to the following members of the *M. simiae* complex: *Mycobacterium sherrisii*, *Mycobacterium triplex*, *Mycobacterium saskatchewanense*, and *Mycobacterium florentinum*. Next-generation sequencing (NGS) phylogenetic analysis of UN-152 revealed a clear divergency from *M. simiae*; however, the ANI values were less than the threshold of 95% to 96% required to establish a unique species, placing this organism within the *M. simiae* complex (7).

M. shigaense is a slow-growing, scotochromogen, with both rough and smooth yellow colony growth on solid medium at 30°C and 37°C and no growth at 42°C. Biochemically, *M. shigaense* does not accumulate niacin, which differentiates it from *M. simiae*. Antibiotic susceptibility profiles indicate resistance to ethambutol with an MIC of 8 μg/ml but susceptibility to clarithromycin (MIC $\leq$ 0.25 to 0.5 μg/ml), rifampin (MIC = 0.25 μg/ml), and rifabutin (MIC $\leq$ 0.125 μg/ml) (7).

Species-level identification within the *Mycobacterium kansasii* complex. Historically, *M. kansasii* has been divided into 7 closely related subtypes, which are virtually indistinguishable to any reliable extent based on phenotypic characteristics and conventional biochemical testing (8). These subtypes were based on restriction fragment length polymorphism (RFLP) analysis of the *hsp65* gene. Subtypes 1 and 2 have most often been associated with clinical disease and postulated to be the most pathogenic of the subtypes, whereas subtype 3 is thought to be mostly a colonizer. Subtypes 4, 5, and 6 are infrequently recovered from patients and appear to be relatively nonpathogenic. As for subtype 7, the pathogenicity and role in clinical disease has not been well defined, as only a single isolate has been described to date (8–11). In addition to these 7 subtypes, two closely related species have been described, *Mycobacterium gastri* and *Mycobacterium persicum*, which taken together form the *M. kansasii* complex (8–11). Recently, the whole-genome sequences (WGS) of strains from subtypes 1, 2, 3, 5, and 6 were subjected to phylogenomic analysis. Strains of the same subtype had ANI (>95 to 96%) and dDDH (>70%) values that indicated they were the same species. In contrast, the same analysis for strains from different subtypes revealed ANI (<95% to 96%) and DDH (<70%) values below this threshold, indicating species-level lineages. It was also found that *M. persicum* shared ANI and DDH values with subtype 2, suggesting that this particular subtype should be changed to *M. persicum* (8–11). The following are the species-level identifications suggested to replace subtypes 6, 5, and 3, respectively.

(i) *Mycobacterium attenuatum*. This organism was first isolated from a bronchial secretion of a patient in Switzerland who had Still's disease, although clinically it is thought to be nonpathogenic and not related to human disease (12). Phenotypically, *M. attenuatum* is a slow-growing photochromogen with pale colony growth occurring on agar plates after 14 days at 37°C. Initially, *M. attenuatum* was considered part of the *M. kansasii* complex, subtype 6, based on RFLP of the *hsp65* gene. A comparison of 16S rRNA sequences between subtypes demonstrated 99.54% nucleotide identity with subtype 1, highlighting the challenges of using methods with low discriminatory power to

identify strains to the species-level within the *M. kansasii* complex. Greater genomic discrimination within the complex was achieved using both a whole-genome approach as well as targeted sequencing of the 16S rRNA, *rpoB* and *tuf* genes. Based on these data, a maximum-likelihood phylogenetic tree was constructed with the amino acid alignment of concatenated single-copy orthologous genes, showing that *M. kansasii* is not monophyletic. Subtype 6 was found to be the most distant of the subtypes and was given the name *M. attenuatum*. It is important to note that previous studies have shown variability among phenotypic testing results for subtypes of *M. kansasii* as well as other members of the complex (*M. persicum* and *M. gastri*) (12); therefore, molecular identification is suggested.

(ii) *Mycobacterium innocens*. This species (formerly *M. kansasii* subtype 5) was first isolated from sputum and is generally considered to have rare pathogenicity (12). Phenotypic characteristics show a slow-growing photochromogen on Lowenstein-Jensen (LJ) agar at 37°C and beige colony growth on Middlebrook 7H10 agar after 2 weeks in culture at 37°C. Biochemical testing shows variability for nitrate reductase, Tween 80 hydrolysis, and urease. As part of the *M. kansasii* complex, *M. innocens* shares a comparable mycolic acid profile by HPLC with other species. Molecular identification of this species requires PCR-RFLP of the *hsp65* or *tuf* gene or alternatively by sequencing analysis of the *hsp65*, *rpoB*, and 16S rRNA genes (12).

(iii) *Mycobacterium pseudokansasii*. *M. pseudokansasii* is a new subspecies of the *M. kansasii* complex, originally referred to as subtype 3. While the original type strain was isolated from a blood culture, it is thought to be more of a colonizer than a cause of human disease (12). *M. pseudokansasii* is a photochromogen, forming rough colonies on LJ medium and beige colonies on Middlebrook 7H10 agar after 2 weeks of growth at 37°C. *M. pseudokansasii* is positive for nitrate reductase, has a positive Tween 80 reaction, but does not produce niacin. It shares variable phenotypic features with other *M. kansasii* subtypes and has a similar HPLC profile with other members of the complex (6 major peaks found between 6.5 and 8.5 min). Thus, phenotypic characteristics alone are insufficient to separate this species from the other subtypes. Proper identification is possible using PCR and sequencing of *hsp65*, 16S rRNA, *tuf*, and *rpoB* genes (12).

Reclassification of previous species. (i) *M. intracellulare* subsp. *yongonense* subsp. nov. This nontuberculous mycobacterium was first cultivated from a patient with pulmonary disease in the Republic of Korea (strain 05-1390) and identified initially as a separate species from *M. intracellulare* on the basis of genetic sequencing using several housekeeping genes from one specific strain (13). However, with the advent of whole-genome sequencing and determination of the ANI, further identification was performed by Castejon and colleagues (14). Using a phylogenetic tree based on 35 concatenated genes revealed ANI and dDDH values of $>$97% and $>$70%, respectively, suggesting that *M. yongonense* was not a separate species on the genomic level but should be placed within the *M. intracellulare* complex, specifically as a subspecies (13, 14). Subsequent genome-based taxonomic investigations by Nouioui et al. (15) and Tortoli et al. (16) support the finding that *M. intracellulare* subsp. *yongonense* is a later heterotypic synonym of *M. intracellulare* subsp. *chimaera* and suggest opting for the use of *M. intracellulare* subsp. *chimaera* as the preferred correct name (15–17). Further review and inclusion in an International Journal of Systematic and Evolutionary Microbiology (IJSEM)-approved list will validly recognize such a change.

(ii) *Mycobacterium chelonae* subsp. *gwanakae*. This rapidly growing nontuberculous mycobacteria was first identified in the Republic of Korea in sputum samples from 3 individual patients who were coinfected with *Mycobacterium yongonense* (18). Smooth white colony growth occurred at both 25°C and 30°C within 7 days on Middlebrook 7H10 agar, but growth was poor at 37°C and inhibited at 45°C. Drug susceptibility patterns showed divergence from the type strain of *M. chelonae* (ATCC 35752) and were more susceptible to doxycycline with lower MICs (0.5 to 1.0 μg/ml) but resistant to amikacin, cefoxitin, ciprofloxacin, imipenem, and trimethoprim-sulfamethoxazole. However, clarithromycin resistance was similar to other species ($\leq$0.5 μg/ml). HPLC profiles showed

late cluster peaks indicative of the *M. abscessus- M. chelonae* group, and 16S rRNA gene sequencing analysis showed similar findings with 99.9% similarity to *M. chelonae* subsp. *bovis* (KCTC 39630). Further phylogenetic analysis of 10 housekeeping genes, including *hsp65* and *rpoB* revealed clustering with *M. chelonae* subsp. *chelonae* (ATCC 35752) and *M. chelonae* subsp. *bovis* (KCTC 39630). Greater phylogenetic differentiation was revealed using whole-genome sequence analysis, which yielded an ANI of 95.89%, a value consistent with current criterion used for delineating a novel species (>95%) (18).

DISCUSSION

Mycobacterial taxonomy continues to progress forward as molecular techniques evolve, providing increased discriminatory power between closely related species. Clarification of the taxonomic relationships between mycobacteria has been complicated in the past, where various approaches have been utilized for such purposes, including phenotypic and genotypic methods. Phenotypic characterization of closely related mycobacterial species is known to be problematic, and as a result, biochemical testing has been discontinued by many laboratories. Genotypic methods have ranged from sequencing of a single or limited number of targeted genes to the more recent advanced comparisons of whole genomes. For clinical laboratories, there are pros and cons to these advances. On the pro side, increased genomic discriminatory power improves our understanding of which species are involved in human disease and enables subsequent investigations into pathogenicity and epidemiology. Thus, as new species are discovered and existing species are subdivided or reclassified, our understanding of the role of each in human disease improves as does the ability to perform particular phenotypic assays with a greater degree of accuracy, such as antibiotic susceptibility testing, which in the past may have been confounded by misidentification of the mycobacterial species present. Continued genomic advances and increased dissemination of these methods will also elucidate the geographical niche occupied by specific species and strains and reveal in a more comprehensive manner whether these organisms are ubiquitous in the environment or confined to a particular region.

On the con side, it is also essential to note that while genomic testing continues to evolve and be more readily accessible, progress is hampered by operational and feasibility challenges in many clinical mycobacteriology laboratories. Indeed, for many clinical laboratories, the ability to sequence various individual targets (*hsp65*, *rpoB*, 16S rRNA, and others) is not always possible, and whole-genome sequencing and the requisite analysis are out of reach. For this reason, many laboratories employ a combination of both conventional and genomic methods whenever possible to improve mycobacterial identification. This can be especially helpful when a particular species has been implicated by epidemiologic links to human disease as occurred with *M. chimaera* or in species for which known antibiotic resistance has been documented. As the incidence and death rate from nontuberculous mycobacterial infections continues to increase (19), laboratorians should seek to stay abreast of new and reclassified species to the extent that the information and knowledge gained helps guide clinical interventions and therapeutic options for patients when indicated.

A practical word about taxonomic changes. It has been proposed that the genus *Mycobacterium* be divided into five different genera based on phylogenomic data, which clearly separated the various species into five major clades (20). As proposed, the genus *Mycobacterium* would contain members of the MTBC, whereas most of the nontuberculous species would be classified in the following four genera: *Mycobacteroides*, *Mycolicibacter*, *Mycolicibacterium*, and *Mycolicibacillus*. Although division of the single genus, *Mycobacterium*, into five distinct genera rests on a solid scientific foundation based on criteria for establishing novel species using genomic comparisons, from a clinical and practical perspective, changing the taxonomic designation of so many mycobacterial species of clinical importance is problematic for a number of reasons as noted by others in the field (21). Reassignment of so many

NTM, many of which have been well characterized in terms of their relevance in human disease, not only creates a significant amount of confusion among laboratorians and clinicians alike but also provides little to no benefit with regard to patient care. Conversely, use of the single genus, existing nomenclature, and previous taxonomic divisions maintains a standardized way for laboratorians and clinicians to approach assessment of patients with mycobacterial exposure, colonization, and/or disease. Continued use of the single genus is less likely to result in errors and would conserve valuable resources, which include both educational and financial components. For instance, a move away from the single genus would require complete revision of laboratory standard operating procedures and testing algorithms, reconfiguration of electronic laboratory information systems, and training of laboratorians and clinicians.

While it is recognized that whole-genome and next-generation sequencing will continue to expand the phylogeny of the genus *Mycobacterium* with identification of new species and reclassification of existing ones, use of this knowledge must be tempered with what is practical from a clinical standpoint. The single genus, *Mycobacterium*, currently contains a large number of medically important species with increasing relevance in clinical care, and as such, should continue to be utilized in laboratory and clinical medicine to avoid errors and other deleterious impacts on patient care.

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