Nontuberculous Mycobacteria in Respiratory Infections

Advances in Diagnosis and Identification

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KEYWORDS

• Nontuberculous mycobacteria • NTM • Mycobacterium • Identification
• Antimicrobial susceptibility testing

KEY POINTS

- Among adults 65 years or older, from 1997 to 2007, the annual prevalence of pulmonary NTM disease significantly increased from 20 to 47 cases per 100,000 persons, or 8.2% per year. Women were 1.4 times more likely to be a pulmonary NTM case than men. Relative to white individuals, Asian/Pacific Islander individuals were twice as likely to be a case, whereas black individuals were half as likely.
- For optimal recovery of mycobacteria, clinical specimens from nonsterile body sites must be subjected to digestion, decontamination, and concentration. This procedure aims to eradicate more rapidly growing contaminants, such as normal flora (other bacteria and fungi), while not seriously affecting the viability of the mycobacteria.
- One of the most urgent questions that needs to be addressed rapidly by the mycobacteriology laboratory is whether *Mycobacterium tuberculosis* complex or NTM is involved. NAA assays are excellent tools for the purpose, and can be used directly on the clinical specimens of patients suspected of having mycobacterial disease, allowing same-day reporting of results. However, these tests are usually evaluated primarily with respiratory specimens and adequate information of their performance on nonrespiratory specimens stratified to different body compartments is often lacking.
- The Centers for Disease Control and Prevention recommends the use of both liquid and solid media for the growth detection of mycobacteria to decrease the time to detection and to increase the yield of growth detection.
- With the recent advances in chemistry and automation of instrumentation, DNA sequencing of variable genomic regions offers a rapid, accurate, and relatively inexpensive method for the identification of mycobacteria. The most routinely used and reliable method of this kind is the amplification and sequence analysis of hypervariable regions of the gene encoding 16S rRNA.

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Clin Lab Med 34 (2014) 271–295
http://dx.doi.org/10.1016/j.cll.2014.03.001 **labmed.theclinics.com**
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MICROBIOLOGY

Together with the genera *Corynebacterium* and *Nocardia*, the genus *Mycobacterium* forms a monophyletic taxon, the so-called CMN group, within the phylum Actinobacteria. The genus *Mycobacterium* is highly diverse, thanks to its ancient origin and years of evolution in multiple habitats. Historically, the species within the genus *Mycobacterium* have been classified based on their growth rate in a subculture as rapid (visible growth in <7 days) and slow growers (growth detection >7 days), and on their pigment production as scotochromogenic (pigment production in dark), photochromogenic (pigment production after exposure to light), or nonchromogenic.[1–5]

Previously, the identification of mycobacteria used a panel of cultural characteristics and biochemical tests; however, these assays are not only unacceptably time consuming, but also often inaccurate, laborious, or not capable of identifying the mycobacterium species at all. In addition, some fastidious species (eg, *Mycobacterium haemophilum* or *Mycobacterium genavense*) require special growth conditions (hemin source or unusually acidic pH), necessitating rarely used and special media, as well as an exquisite collaboration between the clinician requesting the test and the laboratory professional performing the test.[1–5]

The plethora of newly described species seen in the past decades (**Table 1**) is in part the consequence of the availability and increased reliability of new DNA-sequencing methods that are capable of differentiating even closely related species and an increased frequency of isolation of mycobacteria. The latter may be the result of newly emerging manmade reservoirs for certain species. From 41 valid species in 1980, currently this genus encompasses 169 recognized species and 13 subspecies (**Fig. 1**) (http://www.bacterio.net/mycobacterium.html).[1–5]

The *Mycobacterium* genus includes strict pathogens, potentially or opportunistic pathogens, and nonpathogenic saprophytic species. According to the presently prevailing terminology, the mycobacteria species that earlier were referred to as atypical mycobacteria or mycobacteria other than tuberculosis are now called nontuberculous mycobacteria (NTM). Gene sequence similarities within the genus sequences (>94.3% for 16S rRNA gene) and robust phylogenetic reconstructions using concatenated sequences of housekeeping genes have confirmed the natural division among slow-grower and rapid-grower mycobacteria, and also have demonstrated that all slow growers belong to a single evolutionary branch that emerged from the rapidly growing mycobacteria.[2–5] This feature is intrinsically linked to their pathogenic ability to infect humans and, therefore, all obligatory pathogens and most opportunistic pathogens belong the slow-growing evolutionary branch.[5]

EPIDEMIOLOGY

NTM are ubiquitous environmental microorganisms that can be recovered from soil and fresh water and seawater (natural and treated).[1–5] Until recently there was no evidence of human-to-human or animal-to-human transmission of NTM. However, 2 recent findings investigating outbreaks in patients with cystic fibrosis using thorough conventional epidemiologic and state-of-the-art molecular typing investigations, such as whole-genome sequencing, have challenged the dogma of person-to-person transmission indicating potential transmission of *Mycobacterium abscessus* subspecies *massiliense* and *M abscessus* between these patients.[6,7] Because NTM may be found in both natural and manmade reservoirs, human infections are suspected of being acquired from these environmental sources. However, the identification of the specific source of infection is usually not possible. NTM diseases are usually not

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