

REVIEW ARTICLE

# Laboratorial diagnosis and clinical importance of Non-Tuberculosis *Mycobacterium* (NTM)

## Diagnóstico laboratorial e importância clínica do *Mycobacterium* não tuberculoso (NTM)

## Diagnóstico de laboratorio e importancia clínica de *Mycobacterium* no tuberculoso

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### ABSTRACT

Non-tuberculosis *Mycobacterium* (NTM) is a bacterial group with pathogenic, phenotypic, and genotypic variability. These microorganisms have been grouped according to the growing and biochemical characteristics, and their ability to cause disease. Immunodeficient patients are the most frequently achieved, and laboratory identification is so difficult. The disease caused by NTM has a slow progress, and it is considered emerging and sometimes neglected. This literature review reports about major clinically relevant species of non-tuberculosis *Mycobacterium*, and the methods available for laboratory identification.

**Keywords:** Nontuberculosis *Mycobacterium*, molecular bacterial identification, pathogenicity.

### RESUMO

O *Mycobacterium* não tuberculoso (NTM) é um grupo de bactérias com variabilidade patogênica, fenotípica e genotípica. Esses microrganismos foram agrupados de acordo com as características de crescimento e bioquímicas e sua capacidade de causar doenças. Pacientes imunodeficientes são os mais fre-

qüentemente atingidos, e a identificação laboratorial é muito difícil. A doença causada pela MNT tem evolução lenta, sendo considerada emergente e, às vezes, negligenciada. Esta revisão da literatura relata sobre as principais espécies clinicamente relevantes de *Mycobacterium* não tuberculosas e os métodos disponíveis para identificação laboratorial.

**Palavras-chave:** Nontuberculosis *Mycobacterium*, identificação molecular bacteriana, patogenicidade.

### RESUMEN

*Mycobacterium* no tuberculoso (NTM) es un grupo bacteriano con variabilidad patógena, fenotípica y genotípica. Estos microorganismos se han agrupado de acuerdo con las características bioquímicas y de crecimiento y su capacidad para causar enfermedades. Los pacientes inmunodeficientes son los que se alcanzan con mayor frecuencia y la identificación de laboratorio es muy difícil. La enfermedad causada por NTM tiene un progreso lento, y se considera emergente y en ocasiones desatendida. Esta revisión de la literatura informa sobre las principales especies de *Mycobacterium* no tubercu-

losas clínicamente relevantes y los métodos disponibles para la identificación de laboratorio.

**Palabras clave:** *Mycobacterium no tuberculoso*, identificación bacteriana molecular, patogenicidad.

## INTRODUCTION

The Mycobacteriaceae family is composed by microorganisms with variable pathogenic potential, treatment choice, and growth characteristics.<sup>1</sup> Non-Tuberculosis Mycobacteria (NTM) are found widely distributed worldwide in a variety of environments such as water, soil, and animals. Acquisition of these microorganisms by the patients occurs from the environment, but the sources hardly are identified.<sup>2</sup> Transmission has been reported due to inadequately sterile medical or aesthetic equipment, caused by fast growing mycobacteria. Due to their ability to form biofilm in both organic and inorganic materials, these bacteria may contaminate medical devices such as endoscopes and surgical solutions, water systems, tattoo materials, water heaters, swimming pools and showers.

Lung infections affect patients with cystic fibrosis, emphysema, bronchiectasis, alpha-1 antitrypsin deficiency, Williams-Campbell's syndrome, Sjögren's syndrome, and the most varied primary immunodeficiencies are the main risk factors for acquire NTM infections. For the disseminated form, there is usually a relationship with severely immunodeficient patients, such as those who received tumor necrosis factor (TNF alpha) antagonists, interferon gamma, transplanted from any organ (primarily bone marrow and kidney transplantation) and untreated HIV positive patients.<sup>3</sup> In 1980, the disseminated form of the *M. avium* Complex (MAC) was identified as an important pathogen in patients with AIDS and clinical symptoms, in addition to the exclusion of other microorganisms as cause. The pathogen exclusion criteria are adopted in sample from non-sterile site, mainly pulmonary origin. The definitive diagnosis of NTM infection should be based on the second sample or a sterile site sample to determine the clinical significance. It is recommended to collect the new lung sample after one week of the first, in order to exclude transitional colonization.<sup>4</sup> In addition, the laboratory detection of NTM is not the only decisive factor in the diagnosis

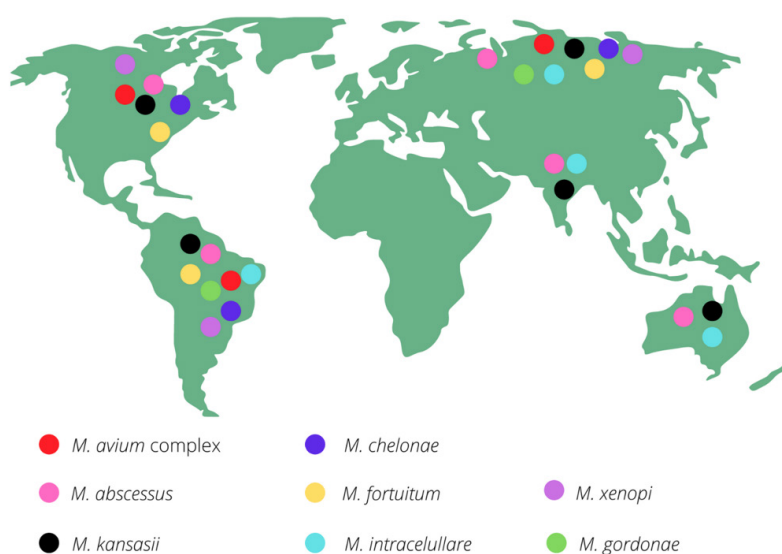
of an infection by these microorganisms, by considering their action as contaminant or colonizer. The clinical criteria such as radiological finding is used is association.<sup>5</sup>

Although there have been increasing reports of NTM infections across the world, epidemiological data are scarce. NTM infections are not always reported, neither patient conditions nor risk factors, which help the infection identification. Being a slow course disease, brings up the delay in suspecting that it is an NTM. These infections are rare and are estimated to reach 2 per 100,000 people. The geographic distribution of NTM species is represented in figure 1.<sup>6</sup>

## Pathogenicity and antibiotic resistance of NTM species

*Mycobacterium* spp. may be classified according to their ability to cause human infections. The pathogenic species include *M. leprae* and *M. tuberculosis* complex. The *M. avium* complex, *M. abscessus*, *M. chelonae*, *M. fortuitum*, *M. haemophilum*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. szulgai*, *M. ulcerans*, *M. malmoeense*, *M. scrofulaceum*, *M. szulgai*, *M. xenopi*, *M. simiae* are classified as potentially pathogenic species. Finally, *M. agri*, *M. alvei*, *M. brumae*, *M. gastri*, *M. mucogenicum*, *M. obuense*, *M. pulveris*, *M. terrae*, *M. gordonae* are classified as rarely pathogenic species. The last group is most frequently associated with environment.<sup>1</sup> This classification complements the clinical and radiological findings to conduct the patient treatment. The main clinically important NTM are described in the table 1.

In addition, NTM species present diversity of antimicrobial resistance profile, and this is determining for therapy choice. Overexpression of efflux pump, reduced affinity for the drug, or production of drug-inactivating enzymes are some mechanisms expressed by this species. So far, most of the mechanisms described in these species are encoded by chromosomal genes.<sup>7</sup> It's important to highlight a mechanism described in *M. abscessus* Group causing resistance to macrolides. When exposed to the drug, there is induction of *erm*(41) gene expression (erythromycin ribosomal methylase), which encodes a methyltransferase, thus preventing the binding of antibiotic in the 23s region of RNA, resulting in resistance.<sup>8,9</sup> Detection of the *erm*(41) gene is extremely important to good



**Figure 1.** Geographic distribution of NTM species according to the frequency of isolation.

clinical conduct, since some species have a 274 bp deletion in the *erm* gene, taking over the inactive gene, so allowing the use of clarithromycin, the first choice of drug in *M. abscessus* infections.<sup>9</sup> *M. abscessus* is resistant to first-line tuberculosis drugs (rifampicin, isoniazid, ethambutol and pyrazinamide) and has reduced susceptibility to imipenem (Figure 2).<sup>10</sup>

Diverse recommendations for NTM lung disease treatment are described, according to the species. Van Ingen<sup>11</sup>, affirmed that the use of rifampicin, clofazimine, ethambutol, macrolide, amikacin or streptomycin are recommended for *M. avium* complex and *M. kansasii*. The treatment with rifampicin, clofazimine, ethambutol, macrolide is alternative; however, for *M. abscessus* subsp. *abscessus* or subsp. *bolletii* (induced resistance), there is no alternative antibiotics. The association between three or four drugs, as amikacin, cefoxitin, imipenem, tigecycline, and linezolid (Intensive Phase) is recommended. For *M. abscessus* subsp. *massiliense* (no induced resistance) other therapeutic options are available, like macrolide plus amikacin, cefoxitin, imipenem or linezolid (Intensive Phase). Alternative options like amikacin, cefoxitin (Intensive Phase), macrolide, and ciprofloxacin can be also used. For all NTM species its recommended more than 12 months or a negative culture.<sup>11</sup>

Due to variability of pathogenicity and antibiotic resistance among NTM species, the correct bacterial species identification should always precede the treatment to avoid empirical or mistaken therapy, leading to therapeutic failure and/or development of antimicrobial resistance. However, clinicians end up with little information regarding the identification of these species. Susceptibility tests are performed for a small range of antibiotics, making it difficult to choose an effective therapeutic method.<sup>7</sup>

## Phenotypic identification of NTM

For a long time, the Runyon classification has been used as a method for NTM species differentiation based on grown time (fast - up to 7 days; slow - more than 7 days), grown temperature, pigment production, and grown in selective media.<sup>1</sup> Based on these phenotypic characteristics, we may classify the NTMs as: (I) photochromogens, that species characterized by slow growth of colonies. Cultures develop yellow pigment only when exposed to light (*M. kansasii* and *M. marinum*); (II) scotochromogen, the species characterized by slow colony growth and develop pigment in both light and dark (*M. gordonae* and *M. scrofulaceum*); (III) acromogens, the species characterized by slow colony growth and no pigment production (*M. avium*, *M. ulcerans*, *M. haemophilum*, *M. xenopi*, and *M. malmoense*); and the last group (IV) the species characterized by rapid growth, with or without pigmentation (*M. fortuitum*, *M. chelonae*, *M. abscessus*) (Table 1). Besides the hard work and the time consuming, the biochemic and phenotypic classification is not efficient in discriminating all species, leading to a late and inaccurate diagnosis. The need for identification of the species has grown in recent decades, due to the increased frequency of isolates, mainly due to use of liquid culture media in automated systems.<sup>12</sup>

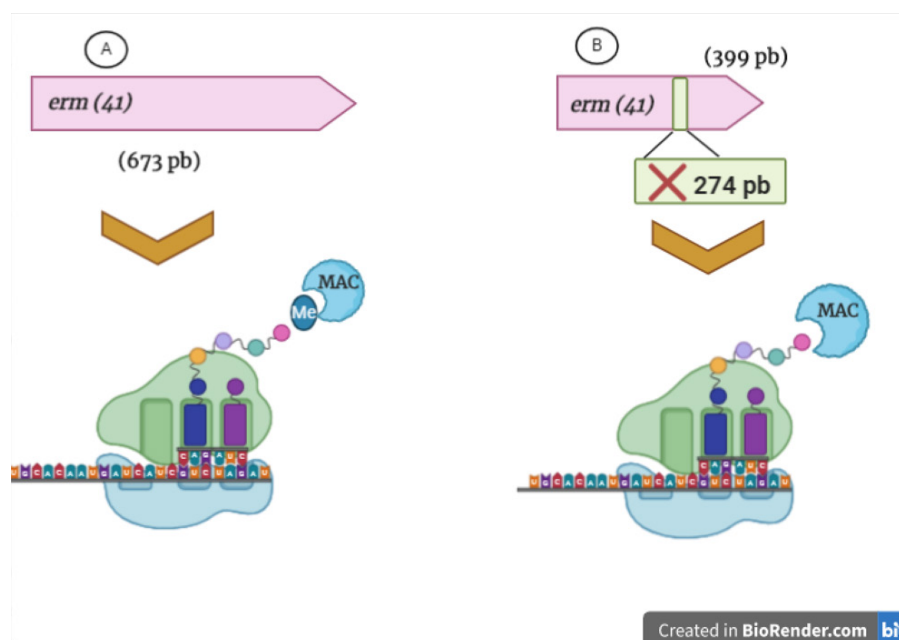
## Molecular Identification of NTM

### DNA-based identification

Molecular methods have greater reliability due to higher sensitivity and specificity rates than conventional biochemical methods, and has allowed to identify and characterize NTM group, which have up to 70% similarity between species.<sup>5,12</sup> One common molecular method used by clinical laboratories is the PRA-hsp65 (Polymerase Chain Reaction Restriction Analysis of the hsp65), which identifies by amplifying a 439 bp fragment

**Table 1.** Clinical and phenotypic characteristics of MNT species.

Specie	Phenotypic characteristics	Infection Site	Risk factors
<i>M. avium</i> Complex (MAC)	Slow grown, acromogenic	Lung infections	Cystic fibrosis, smokers, alcoholics, postmenopausal women, and HIV.
<i>M. fortuitum</i> complex	Fast grown, pseudo chain factor forming	Skin and soft tissue	Postoperative or catheter-related infections
<i>M. kansasii</i>	Fast grown, pseudo chain factor forming, rough colonies	Lung infection, with infiltrates in the cavities	Pneumoconiosis, chronic obstructive pulmonary disease, tumors, alcoholism, and HIV
<i>M. abscessus</i> Group	Fast grown	Skin and soft tissues, and disseminated infection	Gastroesophageal disorders, and cystic fibrosis
<i>M. chelonae</i>	Fast grown, pseudo chain factor forming	Skin and soft tissue, bone and keratitis	Insertion of prosthetic devices, including prosthetic heart valves, lens implants, artificial knees and hips
<i>M. marinum</i>	Slow grown, photochromogenic	Fingers, hands, elbows, knees or soft tissues (pool and aquarium granuloma), osteomyelitis, and arthritis	Solid-organ and hematopoietic stem cell transplant recipients or those on anti-TNF treatment
<i>M. ulcerans</i>	Slow grown	Buruli ulcer - indolent, with progressively necrotic lesions, causing ulcers and nodules, possibly affecting the bones	Skin trauma followed by the contact with contaminated soil or water
<i>M. haemophilum</i>	Fastidious, requires grown factors such as hemine, hemoglobins or iron	Multiple skin lesions or ulcers, abscesses, fistulas, osteomyelitis, cervical lymphadenitis	HIV patients, solid organ transplantation, bone marrow transplantation, and prolonged steroid use
<i>M. celatum</i>	Slow grown	Lung, lymphnodes and disseminated infections	Contact with ferrets, pigs and deer, suggesting a zoonotic disease



**Figure 2.** Macrolide resistance mechanism in *M. abscessus* Group. A. The exposition to macrolide antibiotics, induce the expression of *erm(41)* gene, producing a methyltransferase enzyme, that prevent the binding of antibiotic with 23S rRNA. B. In some isolates, the *erm(41)* have a 274 bp deletion, resulting in inactivating the gene expression, and allowing the use of macrolides.

of the aforementioned gene that is amplified and digested by two restriction enzymes BstE II and Hae III. According to the enzyme restriction pattern, the species are determined using an algorithm that presents several microbial profiles from published scientific data (<http://app.chuv.ch/prasite/index.html>). The main limitation of this technique is the fact that some species share the same profile and new species do not yet have the described restriction profile.<sup>1</sup> In house PCR methods standardization represent a challenge for the laboratories, since the different species present high similarity between genomes, making it difficult to choose gene targets that can be used to differentiate between them.

Currently, there are commercial PCR based kits as Genotype Mycobacterium CM™ (Hain, Lifescience, Germany), SpeedOligo™ (Thermo Fisher, Massachusetts, USA), and Q Gene Mycobacteria (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan). The last two methods include a nucleic acid chromatography of DNA-tagged primers and DNA-DNA hybridization on a membrane strip. The chromatography replaces the electrophoresis stage. All commercial PCR methods require fewer colonies, as well as requiring less microorganism manipulation, easy execution, and high ability to distinguish species that are not possible by biochemical methods.<sup>1</sup> However, a limited number of species profile in is identified. Costa-Alcalde *et al.*<sup>13</sup> analyzed the performance in distinguish species through Genotype Mycobacterium CM, comparing with results of sequency of *rpoB* gene and had a low agreement of 62,8%. Ramis *et al.*<sup>14</sup> evaluated the speed oligo detection capacity for detecting mycobacteria, previously sequenced in the *rpoB* and *hsp65* region and obtained a 93.5% of agreement. Chikamatsu *et al.*<sup>15</sup> analyzed Q Gene Mycobacteria with 340 types strains and clinical isolated and had an agreement of 99,4%. Furthermore, some still are expensive, limiting the laboratories that can use them.

More recently, the GENEDIA MTB/NTM™ detection kit (Green Cross Medical Science Corp., Chungbuk, Korea), was available for use. This is a multiplex RT PCR to differentiate

MTB and NTM samples, which consists of detecting the internal spacer region IS6110 for *M. tuberculosis* and the gene *rpoB* to NTM. The study of Shin *et al.*<sup>16</sup> analyzed 687 sputum samples, comparing the culture result with the kit's performance and observed 99.7% of specificity for NTM species, however, an extremely low sensitivity of 23.2% was observed. In addition, this method is not able to differentiate the MTN species.

Through genome sequencing, Matsumoto *et al.*<sup>17</sup>, developed a database of multiple locus sequence typing (MLST) based on 184 genes from 7,547 genomic profiles and. The authors describe the identification of 100 samples in 10 minutes, by using sequencing by Illumina MiSeq instrument and 200 samples with ONT MinION. Despite the genome sequencing being highly sensitive and specific, these methods are yet very expensive and inaccessible to most clinical microbiology laboratories.

#### Identification of NTM by MALDI TOF (Matrix Assisted Laser Ionization and Desorption)

MALDI TOF is an identification technique that is based on laser bacterial cell disruption, allowing protein ionization, which is pulled into a detection channel by vacuum. The period these proteins take during flight (TOF) result in different peaks profile, allowing for identification according to genus and species.<sup>18</sup> For many bacterial species, direct identification of the colony is already possible; however, for identification of *Mycobacterium* spp., prior extraction is necessary due to the thicker and more resistant wall, which makes it difficult to expose ribosomal proteins that are used for identification. In addition, databases are already being updated for the introduction of new species.<sup>19</sup> The MALDI TOF methodology has revolutionized microbiology with regard to the rapid and accurate identification of microorganisms. The big issue is that, despite the very low cost to perform the test, the equipment acquisition and maintenance has a high cost, limiting the tool to large laboratories.



## HPLC - High Performance Liquid Chromatography

HPLC is a method of separating chemical compounds in solution to identify and quantify each component in a solution. For mycobacteria identification use, the analysis of the mycolic acids presents in the microorganism's wall is made, thus determining their profile, which is compared with a library of reference strains profiles. It is a fast, practical and relatively low-cost technique when compared to genomic methods. However, it yet has some limitations such as the presence of few species in the database (average of 25 species), besides, it often confuses complexes and, for these reasons, it hasn't been used in the clinical routine.<sup>5,11,12</sup>

## Monoclonal antibodies-based identification

Chuensirikulchai *et al.*<sup>20</sup> developed a biosensor that uses monoclonal antibodies specific for Ag85B. Ag85B is the most secretory protein of mycobacterial antigens, which is directly linked to the formation of the cell wall and shows molecular differences between species. This method was able to distinguish between MTB and NTM, however, the distinction among NTM species is not available until now. The use of Ag85B as a diagnostic method is innovative and are under improvement, not being used by clinical laboratories until now.

## CONCLUSION

NTM are microorganisms with great biochemical, phenotypic and genotypic similarity, which makes their correct identification so difficult. These microorganisms have been neglected for years due to their slow course of infection and consequent diagnostic difficulties, and due to be considered a colonizing or contaminating of culture. Due to the elevation of the vulnerable population, reports of affected patients by NTM have been increasing. Compared to other bacterial groups, we have few studies about the drugs efficiency, sensitivity test cutoffs and resistance researches. With new high precision molecular techniques such as next generation sequencing and MALDI-TOF, a major revolution in this genus is expected like species reclassification, grouping of some and even the studies of new species.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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## AUTHORS' CONTRIBUTIONS

NCR drafted and wrote all the manuscript with input from CHA, VCRC and NFGC. AJSD, TRG and RG developed clinical and microbiological discussions on the subject. All authors read and approved the final manuscript.

## REFERENCES

1. Ministry of Health, H. S. S. ed. (2008). *National Manual for the Laboratory Surveillance of Tuberculosis and others Mycobacterias*. Brasília Available at: [http://bvsms.saude.gov.br/bvs/publicacoes/manual\\_vigilancia\\_laboratorial\\_tuberculose.pdf](http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_laboratorial_tuberculose.pdf).
2. Falkinham, J. O. (2015). *Environmental Sources of Nontuberculous Mycobacteria*. *Clin. Chest Med.* 36, 35–41. doi: 10.1016/j.ccm.2014.10.003
3. Honda, J. R., Knight, V., and Chan, E. D. (2015). *Pathogenesis and Risk Factors for Nontuberculous Mycobacterial Lung Disease*. *Clin. Chest Med.* 36, 1–11. doi: 10.1016/j.ccm.2014.10.001
4. Henkle, E., and Winthrop, K. L. (2015). *Nontuberculous Mycobacteria Infections in Immunosuppressed Hosts*. *Clin. Chest Med.* 36, 91–99. doi: 10.1016/j.ccm.2014.11.002
5. Forbes, B. A., Hall, G. S., Miller, M. B., Novak, S. M., Rowlinson, M.-C., Salfinger, M., et al. (2018). *Practice Guidelines for Clinical Microbiology Laboratories: Mycobacteria*. *Clin. Microbiol. Rev.* 31, e00038-17. doi: 10.1128/CMR.00038-17
6. Prevots, D. R., and Marras, T. K. (2015). *Epidemiology of Human Pulmonary Infection with Nontuberculous Mycobacteria: A Review*. *Clin. Chest Med.* 36, 13–34. doi: 10.1016/j.ccm.2014.10.002
7. Guglielmetti, L., Mougari, F., Lopes, A., Raskine, L., and Cambau, E. (2015). *Human infections due to nontuberculous mycobacteria: the infectious diseases and clinical microbiology specialists' point of view*. *Future Microbiol.* 10, 1467–1483. doi: 10.2217/fmb.15.64
8. Carneiro, M. D. S., Nunes, L. de S., David, S. M. M. de, and Barth, A. L. (2017). *Lack of association between rrl and erm(41) mutations and clarithromycin resistance in Mycobacterium abscessus complex*. *Mem. Inst. Oswaldo Cruz* 112, 775–778. doi: 10.1590/0074-02760170080
9. Carvalho, N. F. G. de, Pavan, F., Sato, D. N., Leite, C. Q. F., Arbeit, R. D., and Chimara, E. (2018). *Genetic correlates of clarithromycin susceptibility among isolates of the Mycobacterium abscessus group and the potential clinical applicability of a PCR-based analysis of erm(41)*. *J. Antimicrob. Chemother.* 73, 862–866. doi: 10.1093/jac/dkx476
10. Kasperbauer, S. H., and De Groote, M. A. (2015). *The Treatment of Rapidly Growing Mycobacterial Infections*. *Clin. Chest Med.* 36, 67–78. doi: 10.1016/j.ccm.2014.10.004
11. van Ingen, J. (2015). *Microbiological Diagnosis of Nontuberculous Mycobacterial Pulmonary Disease*. *Clin. Chest Med.* 36, 43–54. doi: 10.1016/j.ccm.2014.11.005
12. Griffith, D. E., Aksamit, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., et al. (2007). *An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases*. *Am. J. Respir. Crit. Care Med.* 175, 367–416. doi: 10.1164/rccm.200604-571ST
13. Costa-Alcalde, J. J., Barbeito-Castañeiras, G., González-Alba, J. M., Aguilera, A., Galán, J. C., and Pérez-Del-Molino, M. L. (2019). *Comparative evaluation of the identification of rapidly growing non-tuberculous mycobacteria by mass spectrometry (MALDI-TOF MS), GenoType Mycobacterium CM/AS assay and partial sequencing of the rpoB gene with phylogenetic analysis as a reference method*. *Enferm. Infecc. Microbiol. Clin.* 37, 160–166. doi: 10.1016/j.eimc.2018.04.012
14. Ramis, I. B., Cnockaert, M., Von Groll, A., Mathys, V., Simon, A., Tortoli, E., et al. (2015). *Evaluation of the Speed-Oligo Mycobacteria assay for the identification of nontuberculous mycobacteria*. *J. Med. Microbiol.* 64, 283–287. doi: 10.1099/jmm.0.000025
15. Chikamatsu, K., Aono, A., Kawai, A., Hata, H., Iwamoto,

- to, T., Igarashi, Y., et al. (2019). Evaluation of Q Gene Mycobacteria: A novel and easy nucleic acid chromatography method for mycobacterial species identification. *J. Microbiol. Methods* 163, 105657. doi: 10.1016/j.mimet.2019.105657
16. Shin, S., Yoo, I. Y., Shim, H. J., Kang, O. K., Jhun, B. W., Koh, W. J., et al. (2020). Diagnostic Performance of the GENEDIA MTB/NTM Detection Kit for Detecting Mycobacterium tuberculosis and Nontuberculous Mycobacteria With Sputum Specimens. *Ann. Lab. Med.* 40, 169–173. doi: 10.3343/alm.2020.40.2.169
17. Matsumoto, Y., Kinjo, T., Motooka, D., Nabeya, D., Jung, N., Uechi, K., et al. (2019). Comprehensive subspecies identification of 175 nontuberculous mycobacteria species based on 7547 genomic profiles. *Emerg. Microbes Infect.* 8, 1043–1053. doi: 10.1080/22221751.2019.1637702
18. Cao, Y., Wang, L., Ma, P., Fan, W., Gu, B., and Ju, S. (2018). Accuracy of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Mycobacteria: a systematic review and meta-analysis. *Sci. Rep.* 8, 4131. doi: 10.1038/s41598-018-22642-w
19. Brown-Elliott, B. A., Fritsche, T. R., Olson, B. J., Vasireddy, S., Vasireddy, R., Iakhiaeva, E., et al. (2019). Comparison of Two Commercial Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Systems for Identification of Nontuberculous Mycobacteria. *Am. J. Clin. Pathol.* 152, 527–536. doi: 10.1093/ajcp/aqz073
20. Chuensirikulchai, K., Laopajon, W., Phunpae, P., Api-ratmateekul, N., Surinkaew, S., Tayapiwatana, C., et al. (2019). Sandwich antibody-based biosensor system for identification of Mycobacterium tuberculosis complex and nontuberculous mycobacteria. *J. Immunoassay Immunochem.* 40, 590–604. doi: 10.1080/15321819.2019.1659814