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Review article

Current status of mycobacterial identification in clinical laboratories in Korea

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Abstract

Accurate identification of *the Mycobacterium tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) is crucial for effective patient management. With declining tuberculosis and rising NTM infections in South Korea, rapid diagnostics are essential. This review provides a comprehensive overview of current diagnostic methods for mycobacterial identification used in Korean clinical laboratories. The field has shifted from conventional methods, such as acid-fast bacilli staining, culture, and biochemical tests, toward rapid technologies. In Korea, immunochromatographic assays (ICA) targeting the MPT64 antigen are widely used to differentiate MTBC from NTM. For species-level NTM identification, laboratories employ advanced techniques including mycolic acid analysis (HPLC), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and diverse molecular assays. Prominent molecular diagnostics include real-time PCR, PCR-restriction fragment length polymorphism (RFLP), line probe assays (LPA), and DNA sequencing of genes like 16S rRNA and rpoB. These modern techniques offer significantly improved speed and accuracy, replacing traditional approaches in routine diagnostics. In South Korea, modern tools have supplanted conventional methods for mycobacterial identification. Real-time PCR and antigen detection are the primary assays for identifying MTBC in cultures. For NTM species, laboratories use a range of techniques including PCR-RFLP, HPLC, LPA, MALDI-TOF MS, and sequencing. Next-generation sequencing is poised to become a pivotal future tool, offering comprehensive species identification and simultaneous profiling of drug resistance. Those modern techniques will transform diagnostic and surveillance strategies for mycobacterial diseases.

Keywords: Diagnosis, Identification, Mycobacteria, *Mycobacterium tuberculosis* complex, Nontuberculous mycobacteria

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Introduction

Background

The family *Mycobacteriaceae* are aerobic, non-motile, gram-positive, rod-shaped bacteria that exhibit slow growth compared to other non-fastidious bacteria. It has an unusual cell-wall structure called lipid rich mycolic acid. It is widely distributed in various environments including soil, water, and animals [1].

Mycobacterium tuberculosis mainly affects the lungs and moves through the blood to other organs,

including the kidneys, spine, and brain. Tuberculosis caused by *M. tuberculosis* remains the second leading cause of death worldwide, although its incidence rate decreased by 8.7% between 2015 and 2022 [2]. The number of infections caused by *M. tuberculosis* in South Korea has been decreasing continuously, with an average of 7.2% per year since 2011 [3]. Nontuberculous mycobacteria (NTM) can cause infections in the lungs, skin, soft tissue, lymph nodes, and other organs [4]. Global meta-analyses have shown an increasing trend in NTM disease, with an overall rate increasing by 4% per year [5]. NTM infections have also increased in South Korea since the first cases of lung disease were reported in 1981 [6,7].

Rapid and accurate identification of mycobacteria is important for appropriate diagnosis and management. Traditionally, the identification of mycobacteria in clinical laboratories relied on conventional methods such as microscopic examination and biochemical tests. There have been many advances in diagnostic tests for mycobacterial identification and these new methods have replaced conventional methods based on biochemical tests [8].

Several diagnostic methods for mycobacteria have been introduced in clinical laboratories. These include immunochromatographic assays, polymerase chain reaction (PCR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), sequencing, and next-generation sequencing. The development of new diagnostic methods has greatly improved the diagnostic accuracy and turnaround time.

Objectives

It aims to provide an overview of various diagnostic methods used for the identification of mycobacteria in clinical laboratories.

Classification of mycobacteria

The family *Mycobacteriaceae* includes 216 mycobacterial species (202 validly published with the correct name and 14 synonyms) in the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/mycobacterium). Mycobacteria can be divided into two main groups based on their growth rates: slow- and rapid-growing organisms. These two groups have been clearly differentiated using 16S rRNA trees [9]. Gupta et al. [10] proposed the division of *Mycobacterium* into five genera based on the average amino acid identity of conserved protein families. These include *Mycobacterium* and four novel genera (*Mycobacteroides*, *Mycolicibacter*, *Mycolicibacillus*, and *Mycolicibacterium*) (Table 1). This taxon has been accepted as a valid taxon since it was first proposed in 2018. There is still much controversy regarding the use of new genera according to the newly proposed classification of mycobacteria. In fact, the authors of the 13th edition of manual of clinical microbiology used the single genus, *Mycobacterium*. However, we use the term *Mycobacteriaceae* to refer to only the five newly classified genera in this review.

In addition, mycobacteria also can be divided in two groups of *Mycobacterium tuberculosis* complex (MTBC) and NTM depending on their pathogenicity. MTBC is transmitted through air, whereas NTM is usually acquired from the environment, especially through a water source [11]. NTM was not recognized as

a pathogen until the 1930s; however, NTM have been recognized as important pathogens since the 1950s owing to respiratory infections that have occurred. The MTBC comprises 13 strain/species including *M. tuberculosis, M. bovis, M. caprae, M. africanum, M. pinnipedii, M. microti,* and other species [12]. It is a slow-growing group that requires more than 10–20 days to appear as a visible colony in culture. NTM can be classified into four groups, according to Runyon's recommendations. They were divided into slow-growing NTM (SGM) and rapid-growing NTM (RGM). SGM can be subdivided into three groups depending on the production of pigments in the light or dark: photochromogens (requiring light to form a pigment), scotochromogens (producing a pigment in the dark), and non-photochromogens (no pigment).

In South Korea, the most common species in SGM is *M. intracellulare*, followed by *M. avium*, *M. kansaii*, *M. gordonae*, and *Mycolicibacter terrae* complex [6]. The four most common RGM species are *Mycobacteroides abscessus, Mycolicibacterium fortuitum* complex, *Mycobacteroides chelonae*, and *Mycolicibacterium mucogenicum* [6].

Table 1. Classification of mycobacteria proposed by Gupta et al. [10] in 2018

Group	Genus	Clade	Species
Slow-growing	*Mycobacterium*	"Tuberculosis-Simiae" clade	*M. tuberculosis* complex, *M. avium* complex, *M. intracellulare*, *M. gordonae* clade, *M. kansasii* clade, *M. arupense*, *M. lentiflavum*, *M. simiae* clade, *M. scrofulaceum*, *M. parascrofulaceum*, *M. gastri*, *M. interjectum*, as well as several other slow-growing species
	Mycolicibacter	"Terrae" clade	*M. terrae* complex *(M. terrae, M. sinensis, M. senuensis, M. nonchromogenicus* etc.)
	Mycolicibacillus	"Triviale" clade	*M. koreensis, M. parakoreensis, M. trivialis*
Rapid-growing	*Mycobacteroides*	"Abscessus-Chelonae" clade	*M. abscessus* (*M. abscessus* subsp. *abscessus, bolletii, massiliense*), *M. chelonae*, *M. immunogenum*, *M. franklinii, M. salmoniphilum, M. saopaulense*
	Mycolicibacterium	"Fortuitum-Vaccae" clade	All rapid-growing mycobacterial species, except those from the "Abscessus-Chelonae" clade (*M. fortuitum, M. mucogenicum, M. peregrinum, M. septicum, M. goodii, M. phocaicum, M. conceptionense, M. porcinum, M. flavescens, M. phlei, M. senegalense, M. smegmatis* etc.)

Acid-fast bacilli (AFB) stain

Microscopic examination of AFB is used as a rapid screening method to detect *Mycobacteriaceae*; however, its overall sensitivity is lower than that of culture [8]. Three common methods for AFB staining are used in clinical laboratories: Ziehl–Neelsen, Kinyoun, and fluorochrome. Owing to the unique cell wall of mycobacteria, the red dye with carbol fuchsin or fluorochrome is retained even after decolorization with acid alcohol. Fluorochrome staining with auramine O or auramine-rhodamine is commonly used in clinical laboratories because of its high sensitivity and convenience [13].

According to a national survey in South Korea conducted in 2014, 84.3% of clinical laboratories used fluorescent staining as the primary staining method [14]. Additional Ziehl–Neelsen staining was performed in 62.7% of the laboratories to confirm the fluorescence staining results. In the 2024 report of the Korean Association of External Quality Assessment Service, most clinical microbiology laboratories used the Ziehl–Neelsen (53.4%) or fluorochrome (46.2%) methods for AFB smears (Table 2) [15].

Table 2. Current status of mycobacterial testing in clinical laboratories in South Korea

Methods		No.	%
AFB smear (n = 238)	Ziehl–Neelsen	127	53.4
	Fluorochrome	110	46.2
	Kinyoun	1	0.4
Culture - solid media (n = 123)	Ogawa	115	93.5
	Lowenstein-Jensen	8	6.5
Culture - liquid media (n = 117)	MGIT	109	93.2
	BacT/ALERT	7	6.0
	Others	1	0.9
Identification (n = 67)	PCR	31	46.3
	Rapid Ag Test	25	37.3
	DNA probe	8	11.9
	MALDI-TOF	1	1.5
	HPLC	1	1.5
	Sequencing	1	1.5

Cited from a report by the Korea Association of External Quality Assessment Services on acid-fast bacilli testing in Korea [15].
Abbreviations: AFB, acid-fast bacilli; PCR, polymerase chain reaction; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; HPLC, high-performance liquid chromatography.

Mycobacterial culture

Mycobacterial culture is essential for species identification and drug susceptibility tests, according to the recommendations of the World Health Organization (WHO). Many reports recommended the use of both liquid and solid media for mycobacterial culture to increase the detection rate [8,16-18]. Several solid media have been used to isolate *Mycobacteriaceae* from clinical specimens. Egg-based media, such as Lowenstein–Jensen (LJ) and Ogawa media, are commonly used and are more effective in the recovery of MTBC than NTM. Middlebrook 7H10 or 7H11 agar is used for antimicrobial susceptibility tests for *Mycobacteriaceae* and has the advantage of shorter growth time than egg-based media [19]. In general, the use of a liquid medium can reduce the turnaround time and has a higher detection rate than a solid medium, although the liquid culture exhibits a high contamination rate [20,21]. Three common commercial systems for liquid culture are the BACTEC MGIT 960 System (Becton-Dickinson), the BacT/ALERT 3D liquid culture system (bioMérieux), and VersaTREK (ESP culture system II; Trek Diagnostics, Inc.). BACTEC MGIT 960 System has shown to have the highest sensitivities and negative predictive values, both when used alone or in combination with LJ [20]. The BacT/ALERT 3D system also showed a better isolation rate than the LJ and MB7H10 media [21]. In South Korea, 82.9% of the laboratories use both solid and liquid media. Most of them use a combination of Ogawa and MGIT [14]. This finding is consistent with that of a survey conducted in 2024. Most laboratories used Ogawa media (93.5%) for solid culture and MGIT systems (93.2%) for liquid culture (Table 2) [15].

Mycobacterial identification

1) Biochemical test

Traditionally, the identification of *Mycobacteriaceae* was based on biochemical testing such as growth on niacin, TCH (thiophene-2-carboxylic acid hydrazide), nitrate, and 68°C catalase using colony. The niacin test is widely used to identify *M. tuberculosis*. *M. tuberculosis, M. simiae*, and some strains of *M. chelonae* accumulate large amounts of niacin because they lack the enzymes required for the conversion of niacin to ribonucleotides [22]. *M. tuberculosis* also exhibits properties that grow in TCH (10 mg/mL), nitrate reduction, and inactivate on 68°C catalase. Considerable time is required for the identification of *Mycobacteriaceae* using biochemical tests because a sufficient number of mycobacterial colonies are required. However, only a small number of mycobacterial species can be identified using biochemical tests. Conventional biochemical tests are rarely used to identify mycobacteria.

2) Mycolic acid (MA) analysis

MAs are 2-alkyl, 3-hydroxy long-chain fatty acids that can be used to identify *Mycobacteriaceae* by extracting MAs from the cell wall [23]. The major mycobacterial MAs include mycolates, ketomycolates, methoxymycolates, ω-carboxymycolates, and epoxymycolates. Mycobacteria can be distinguished by the length of their carbon chains in MAs, and the pattern of these degradation products is related to the species identification of the mycobacteria [24,25]. MAs can be detected using gas–liquid chromatography (GLC), thin-layer chromatography, and high-performance liquid chromatography (HPLC). HPLC offers high sensitivity and a broader range of compounds, but generally has a lower separation efficiency than GLC [26].

The Sherlock Mycobacteria Identification System (SMIS; MIDI Inc.) is a computer-assisted system that identifies mycobacterial species using a Microbial ID. The SMIS is based on the separation and quantification of MAs using chromatography. All peaks are classified by clustering based on their characteristic patterns, and the strains are identified by comparison with the patterns of the mycobacteria library. The SMIS includes approximately 1,200 species from aerobic libraries, including the MI7H10 library (a total of 31 species of mycobacteria). SMIS can accurately identify 75% of *Mycobacteriaceae* species using version 2.95, and additional identification is possible by measuring the relative peak height ratio and relative retention times [26].

HPLC is the main method used to identify mycobacteria at one tertiary hospital in South Korea. They constructed database for mycobacteria using 33 reference strains by themselves [27]. The proportions of MTBC and NTM were 87.6% and 12.4%, respectively [28].

3) Immunochromatography assay

An immunochromatographic assay (ICA) uses an antigen–antibody reaction to detect a causative pathogen. Lateral flow immunochromatography assays (LFAs) assays are widely used in clinical laboratories and offer the advantages of low cost, rapid reporting, and ease of use. LFAs have been introduced to discriminate between MTBC and NTM by using monoclonal antibodies against tuberculosis (TB) antigens [29]. *M.*

tuberculosis secretes more than 33 proteins.

The MPT64 (*Mycobacterium tuberculosis* protein 64) antigen, a 24kDa secretory protein, is one of the dominant proteins secreted only by the MTBC during growth [30]. There are several commercial MPT64 ICA kits, including SD Bioline™ TB Ag MPT64 (Standard Diagnostics, Abbott Inc.), STANDARD Q TB MPT64 Ag (SD Biosensor Inc.), Capilia™ TB-Neo (TAUNS Laboratories Inc.), and BD MGIT TBc Identification Test (TBc ID, Becton Dickinson) (Table 3).

The SD Bioline™ TB Ag MPT64 test is detectable in both solid and liquid cultures within 15 min. The sensitivities of the solid and liquid media were shown to be 99.4% and 96.7%, respectively, and increased in AFB-positive liquid cultures to 100% and 100%, respectively [31]. The sensitivities of Capilia™ TB-Neo and STANDARD Q TB MPT64 Ag provided by the manufacturer are 99.4% and 100%, respectively. In one study, the sensitivity of Capilia™ TB-Neo was 97%, and some false negatives were confirmed due to mutations in the *mpb64* gene [32]. The BD MGIT TBc Identification Test (TBc ID) is designed for the rapid identification of MTBC from the BD MGIT™ liquid culture. The sensitivity and specificity of the TBc ID test were shown to be 98.5% and 100%, respectively, compared to sequencing of the 16S rRNA gene [33]. LFAs using MPT64 antigen may be useful for discriminating MTBC from NTM. In South Korea, the rapid antigens test is used as the primary identification method for cultured mycobacteria in laboratories (44.3% in 2014 and 37.3% in 2024) [14,15]. However, it should be noted that the absence of MPT64 antigens or genetic mutations may result in false negative.

Table 3. Characteristics of immunochromatography assays for *M. tuberculosis* complex

Assay	Type	Target	Time to result	Media	Performance	Manufacturer, headquarters
SD Bioline™ TB Ag MPT64	Device	MPT64 Ag	15 min	Solid culture Liquid culture	Sensitivity: 98.6% Specificity: 100%	Abbott, Korea
STANDARD Q TB MPT64 Ag	Device	MPT64 Ag	10 min	Solid culture Liquid culture	Sensitivity: 100% Specificity: 100%	SD Biosensor, Korea
Capilia™ TB-Neo	Device	MPT64 Ag	15 min	Solid culture Liquid culture	Sensitivity: 99.4% Specificity: 100%	TAUNS Laboratories, Japan
BD MGIT™ TBc Identification Test	Device	MPT64 Ag	15 min	Only use AFB smear-positive BD MGIT™ tubes	Sensitivity: 98.5% Specificity: 100%	Becton Dickinson, USA

Abbreviations: MPT64, *Mycobacterium tuberculosis* protein 64; AFB, acid-fast bacilli.

4) MALDI-TOF MS

MALDI-TOF MS is a valuable tool for identifying bacteria isolated from clinical specimens. This method is based on species-specific protein spectrum fingerprints produced from microbial cell extracts [34]. The generated protein spectral fingerprints are used for final identification by comparison with the profiles in a database. In South Korea, three commercial systems are available for use in the clinical laboratories. These include the VITEK MS (bioMérieux), MALDI-TOF Biotyper Sirius (Bruker Daltonics), and MicroIDSys Elite (ASTA Inc.) (Table 4). These systems are faster, simpler, and cheaper than the other approaches. The use of MALDI-TOF MS for the identification of *Mycobacteriaceae* remains challenging owing to their cell wall and infectivity. Therefore, an effective inactivation and extraction step is required. The pretreatment process includes the addition of beads and/or reagents, although this varies slightly among the systems.

VITEK MS V3.0 MYCOBACTERIUM DATABASE, MBT Mycobacteria IVD software, and MycoDB v2.0 are the databases used to interpret all protein spectral fingerprints produced by VITEK MS, Biotyper, and MicroIDSys, respectively. According to the manufacturer's brochure, these databases contain 182, 39, and 71 mycobacterial species and subspecies entries in the MBT Mycobacteria IVD software, VITEK MS V3.2, and MycoDB V2.0, respectively.

Using VITEK MS V3.2, the identification rates of MTBCs and mycobacterial species reached high as 100% and 96.2%, respectively, on a solid medium [35]. In the case of the MBT Mycobacteria IVD software, the identification of NTM had an accuracy of 97% [36]. Song et al. [37] reported that the reliable identification rate of MicroIDSys was 93.5% using MycoDB v2.0-beta, although it was as low as 26.6% when using MycoDB v1.95s. Overall, MALDI-TOF MS is an accurate and reliable approach for identifying mycobacterial species. Currently, only one laboratory in South Korea uses MALDI-TOF MS to identify mycobacterial species.

Table 4. Characteristics of three MALDI-TOF MS systems for mycobacteria

Company	Vitek MS PRIME	MALDI Biotyper Sirius	IDSys LT®
	bioMérieux	Bruker Daltonics	ASTA
Panel	Disposable slide with 48 positions 192 isolates can be tested per run	Reuseable slide with 96 well	Disposable or reuseable 6 × 16 µFocus Plate
Criteria	99.9%: identification	2.300-3.000: Highly probable species identification	≥ 140: Acceptable species identification
	99.8%–90.0%: identification	2.000-2.299: Secure genus identification, probable species identification	< 140, ≥ 130: Need for confirmation
	89.9%–85.0%: identification	1.700-1.999: Probable genus identification	< 130: Not reliable identification
	84.9%–70.0%: no identification	0.000-1.699: Not reliable identification	
Database for mycobacteria	VITEK MS V3.2 MYCOBACTERIUM DATABASE (IVD) SARAMIS Knowledge Base V4.16 (RUO)	MBT Mycobacteria IVD software	MicroIDSys - MycoDB v2.0
	IVD: 39 FDA claimed and unclaimed species and subspecies including *M. tuberculosis* complex, *M. fortuitum* group, *M. abscessus, M. intracellulare* SARAMIS: more than 60 mycobacteria species	182 species entries including *M. abscessus, M. intracellulare, M. fortuitum, M. tuberculosis*	71 mycobacterial species including *M. fortuitum, M. abscessus, M. kansasii, M. avium, M. in tracellulare, M. chelonae, M. gordonae, M. szulgai*
Pretreatment step	1) 70% ethanol and 0.5 mm glass bead 2) 70% formic acid and 100% acetonitrile 3) VITEK MS-CHCA matrix	1) Inactivation reagent 2) Acetonitrile and formic acid mix 3) CHCA matrix	1) Silica beads, Sodium dodecyl sulfate (SDS), 70% ethanol 2) Acetonitrile and formic acid 3) CHCA matrix

Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; FDA, the U.S. Food and Drug Administration.

5) Molecular methods

(1) PCR

PCR assays are valuable for rapid and accurate detection of MTBC. They can provide results within a few hours and have high sensitivity and specificity for cultures and specimens. Many commercially available PCR-based methods have been used to detect MTBC in cultures and clinical specimens (Table 5).

Table 5. Commercial molecular assays for *M. tuberculosis* complex

Assay	Type	Gene	Target	Manufacturer, headquarters
Amplified Mycobacterium Tuberculosis Direct (AMTD) test	isothermal transcriptase-mediated amplification assay	16S rRNA gene	Detection of MTBC	Hologic, Canada
AdvanSure TB/NTM real-time PCR kit	Real-time PCR	IS*6110* insertion sequence for MTBC ITS regions for NTM	Differentiation TB/NTM	Invitros, South Korea
PowerChek MTB/NTM real-time PCR kit	Real-time PCR	IS*6110* insertion sequence for MTBC ITS regions for NTM	Differentiation TB/NTM	Kogenebiotech, South Korea
Anyplex MTB/NTM real-time PCR kit	Real-time PCR	IS*6110* and *mpb64* for MTBC 16S rRNA gene for NTM	Differentiation TB/NTM	Seegene, South Korea
Real-Q MTB & NTM kit	Real-time PCR	IS*6110* insertion sequence for MTBC ITS regions for NTM	Differentiation TB/NTM	BioSewoom, South Korea
NextGene MTB/NTM Detection Kit	Real-time PCR	IS*6110* and *mpb64* for MTBC 16S rRNA gene for NTM	Differentiation TB/NTM	EONE Biotech Co, South Korea
The NeoPlex TB/NTM Detection Kit	Real-time PCR melting curve analysis	IS*6110* insertion sequence for MTBC 16S rRNA gene for NTM	Differentiation TB/NTM Identification of five NTM	GeneMatrix, South Korea
COBAS TaqMan MTB	Real-time PCR fully-automated system	16S rRNA gene, esx gene	Detection of MTBC	Roche, Switzerland
COBAS MTB-RIF/INH	Real-time PCR fully-automated system	*rpoB* gene for rifampicin resistance *katG* gene and *inhA* promoter region for isoniazid resistance	Detection of resistance for rifampicin and isoniazid	Roche, Switzerland
Xpert MTB/RIF	Nested real-time PCR fully-automated system	*rpoB* gene for MTBC and rifampicin resistance	Detection of MTBC and Rifampicin resistance	Cepheid, U.S
Xpert MTB/RIF Ultra	Nested real-time PCR fully-automated system	IS*1081* and IS*6110* for MTBC *rpoB* gene for rifampicin resistance	Detection of MTBC and Rifampicin resistance	Cepheid, U.S
BD MAX MDR-TB	Real-time PCR fully-automated system	IS*6110* and IS*1081* insertion sequence *rpoB* gene for rifampicin resistance *katG* gene and *inhA* promoter region for isoniazid resistance	Detection of MTBC and Rifampicin and isoniazid resistance	Becton-Dickinson, U.S

Abbreviations: PCR, polymerase chain reaction; MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria; TB, tuberculosis.

The Amplified Mycobacterium Tuberculosis Direct test (Hologic) has been approved by the U.S. Food and Drug Administration (FDA) for use with both smear-positive and smear-negative clinical specimens. This method is based on ribosomal RNA amplification using an isothermal transcriptase-mediated amplification assay. The sensitivity and specificity were 91.2% and 98.9%, respectively, for all specimens, including smear-positive and smear-negative specimens [38]. AdvanSure TB/NTM real-time PCR kit (Invitros), Real-Q MTB & NTM kit (BioSewoom), Anyplex MTB/NTM Real-time Detection (Seegene), PowerChek MTB/NTM Real-time PCR kit (Kogenebiotech), and NextGene MTB/NTM Detection Kit (EONE BIOTECH Co., Ltd.) have been developed and are used in South Korea [39-41]. AdvanSure TB/NTM real-time PCR kit, PowerChek MTB/NTM Real-time PCR kit, and Real-Q MTB & NTM kit uses IS*6110* and ITS regions for the MTBC and NTM, respectively. The Anyplex MTB/NTM Real-time Detection Kit and NextGene MTB/NTM Detection Kit use the IS*6110* and *mpb64* regions for the detection of MTBC, and the 16S rRNA gene for the detection of NTM. The sensitivity and specificity of AdvanSure and PowerChek for MTBC were reported to be 96.9% and 98.5%, respectively [42]. The sensitivities of AdvanSure and PowerChek for NTM detection were lower at 81.5% and 88.9%, respectively, although their specificity was higher (99.6% and 98.7 %, respectively) [42]. The accuracy and sensitivity of Real-Q MTB & NTM kit was reported to be 98.8% and > 95%, respectively [40]. One false-negative result was obtained for *M. sediminis*. There has been a

report on the clinical performance of the NextGene MTB/NTM Detection Kit [41]. It was approved as Class III by the Ministry of Food and Drug Safety in South Korea and certified as Class C under the new European Union In Vitro Diagnostic Regulations. The NeoPlex TB/NTM Detection Kit (GeneMatrix) is a single-tube multiplex real-time PCR based on proprietary C-Tag™ technology. This assay simultaneously detects MTBC, NTM, and five common NTM species (*M. abscessus, M. massiliense, M. avium, M. intracellulare, M. kansasii*) using melting curve analysis.

The COBAS Amplicor test (Roche Diagnostics) is the first commercially available automated nucleic acid amplification system. Roche later replaced the system with a real-time PCR method, the COBAS TaqMan MTB, resulting in a significant improvement in specificity compared to the previous version [43]. The Cobas 5800/6800/8800 system automates all the processes, including nucleic acid extraction, purification, PCR amplification, and detection. A meta-analysis showed summary estimates of sensitivity and specificity of 80.8% (95%confidence interval [CI] 0.758–0.850) and 99.0% (95%CI 0.981–0.994), respectively, for respiratory specimens [44].

Xpert MTB/RIF is widely used to detect MTBC and rifampin (RIF) resistance in clinical specimens directly. The WHO recommends the use of Xpert MTB/RIF or Xpert Ultra as an initial test for adults and children with signs and symptoms of TB [45]. Automated nested real-time PCR is performed using a disposable cartridge with the GeneXpert Instrument System. Xpert MTB/RIF amplifies *rpoB* to identify MTBC and detect of rifampicin resistance. The sensitivity of Xpert MTB/RIF is 100% for smear-positive samples and 68% for smear-negative specimens [46]. Xpert Ultra was developed to overcome the limitation of low sensitivity in smear-negative specimens. This assay uses a 50 μL reaction tube and has two different multicopy amplification targets (IS*1081* and IS*6110*) for MTBC. Based on a meta-analysis, the sensitivity and specificity of Xpert Ultra for pulmonary TB were 90.9% and 95.6%, respectively, while those of Xpert MTB/RIF were 84.7% and 98.4%, respectively [47]. The BD MAX MDR-TB (Becton-Dickinson) is a fully automated real-time PCR system, and the target genes for MTBC are IS*6110* and IS*1081*. The sensitivities for smear-positive and smear-negative samples were 100% and 64.5%, respectively; however, the overall specificity was 100% [48]. The regions responsible for rifampicin and isoniazid resistance were *rpoB* and *inhA* promoter region/*katG* genes, respectively.

Previous reports have shown that 17.1% and 14.3% of clinical laboratories in South Korea use conventional and real-time PCR as the primary identification methods for MTBC from culture media [14]. A 2024 report showed that 40% of clinical laboratories used PCR to identify MTBC [15].

(2) DNA Chip

DNA chips confirm the expression of numerous DNA sequences. These are classified as cDNA chips and oligonucleotide chips. Oligonucleotide chip systems have been used to simultaneously identify mycobacterial species and detect drug resistance mutations. A DNA microarray biochip (CapitalBio Company Ltd.) was developed to identify 17 mycobacterial species by targeting the 16S rRNA [49]: *M. tuberculosis*, *M. intracellulare*, *M. avium*, *M. gordonae*, *M. kansasii*, *M. fortuitum*, *M. scrofulaceum*, *M. gilvum*, *M. terrae*, *M. chelonae/M. abscessus*, *M. phlei*, *M. nonchromogenicum*, *M. marinum/M. ulcerans*, *M. aurum*, *M. szulgai/M. malmoense*, *M. xenopi*, and *M. smegmatis*.

Commercial DNA chips are mostly used to detect resistance in *M. tuberculosis*. The TB-Biochip oligonucleotide microarray system (Engelhardt Institute of Molecular Biology) was designed to detect mutations associated with RIF resistance in mycobacteria. The sensitivity and specificity of this system was 80% and 100%, respectively, compared with conventional drug susceptibility testing results [50]. The CombiChip Mycobacteria™ Drug-Resistance Detection DNA chip (GeneIn, Inc.) was used to detect mutations associated isoniazid (INH) resistance in *katG* codon 315, *inhA*15, and RIF. The sensitivity of RIF resistance was reported to be 93.0% and 100% [51,52]. The sensitivities of INH resistance have been reported to be 71.4% and 84.1%, respectively [51,52]. BluePoint MTBDR (BioConcept Inc.) is another commercial assay designed to identify *M. tuberculosis* and determine its resistance to RIF and INH. The sensitivities of RIF and INH resistance in this system have been reported to be 100% and 97.1%, respectively [53]. However, few clinical laboratories in South Korea use chips for routine identification and susceptibility testing of mycobacteria.

(3) DNA probe assay

The DNA probe assay is a nucleic acid hybridization test based on the binding ability of complementary strands. This method is used for rapid identification of MTBC and NTM in colonies after cultivation. DNA probes are short, single-stranded nucleic acid sequences complementary to target sequences within the MTBC and are typically labeled with a fluorescent dye, chemiluminescent label, or enzyme. The Gen-Probe Rapid Diagnostic System (Gen-Probe; currently Hologic) was the first commercial molecular assay for *M. avium-M. intracellulare* complex (MAC), *M. kansasii*, and *M. gordonae*. Initially, ^{125}I-labeled DNA probes against rRNA were used. Later, they were changed to chemiluminescent (acridinium ester)-labeled DNA probes (AccuProbe MTBC) to overcome the limitations of the use of radioisotopes, and the results can be obtained within approximately 2–3 hours. The AccuProbe MTBC was approved by the FDA in 1990 for culture applications. The sensitivity and specificity of MTBC were 96.4% and 100%, respectively, in broth cultures [54]. It has been reported that the cross-reactivity of *M. terrae* and *M. celatum* with MTBC probes is due to temperature changes [55]. Recently, cross-reactivity of MAC probes with *M. aroiense*, *M. chimaera*, *M. colombiense*, and *M. paraense* has been reported [56].

(4) PCR-restriction fragment length polymorphism (RFLP)

PCR-RFLP is a technique used to detect DNA fragments from solid and liquid cultures using restriction enzymes. PCR restriction-enzyme analysis-*hsp65* using *BstEII* and *HaeIII* showed more accurate results (90.3%) than phenotype methods (77.9%), with few incorrect results (0.2%) for common mycobacterial species [57]. *RpoB* is commonly used for PCR-RFLP analyses [58]. The Myco-ID kit (M&D) identifies various *Mycobacteriaceae* by 360-bp *rpoB* gene amplification with the restriction enzymes *MspI* and *HaeIII* [59]. PCR-RFLP can be used to rapidly and accurately identify mycobacterial species.

(5) Line probe assay (LPA)

The LPA is a molecular diagnostic test based on PCR and DNA reverse hybridization techniques. It is commonly used in clinical laboratories because it can simultaneously identify 10–20 mycobacterial species. Several LPA assays are used in clinical practice (Table 6).

Table 6. Characteristic of line probe assays for the identification of nontuberculous mycobacteria

Assay	Manufacturer	Target	Intended use	Species
GenoType Mycobacterium CM/AS	Bruker	23 rRNA gene	Culture only	CM Ver 2.0 MTBC, *M. avium*, *M. intracellulare*, *M. chelonae*, *M. abscessus* complex, *M. fortuitum* group, *M. gordonae*, *M. scrofulaceum*, *M. szulgai*, *M. interjectum*, *M. kansasii*, *M. malmoense*, *M. marinum/M. ulcerans*, *M. xenopi*. AS Ver 1.0 *M. simiae*, *M. mucogenicum*, *M. goodii*, *M. celatum*, *M. smegmatis*, *M. genavense*, *M. lentiflavum*, *M. heckeshornense*, *M. szulgai/M. intermedium*, *M. phlei*, *M. haemophilum*, *M. kansasii*, *M. ulcerans*, *M. gastri*, *M. asiaticum*, *M. shimoidei*
GenoType CMdirect VER 1.0	Bruker	23 rRNA gene	Specimen only	MTBC, *M. avium*, *M. intracellulare*, *M. chelonae*, *M. abscessus* complex, *M. fortuitum* group, *M. gordonae*, *M. scrofulaceum*/ *M. intracellulare*, *M. szulgai*, *M. interjectum*, *M. kansasii*, *M. malmoense*, *M. marinum/M. ulcerans*, *M. xenopi*.
INNO-LiPA Mycobacter V2	Innogenetics	16S-23S rRNA spacer region	Specimen or culture	MTBC, *M. avium*, *M. intracellulare*, *M. chelonae* complex, *M. kansasii*, *M. xenopi*, *M. gordonae*, *M. genavense*, *M. simiae*, *M. marinum/M. ulcerans*, *M. celatum*, *MAIS*, *M. scrofulaceum*, *M. malmoense*, *M. haemophilum*, , *M. fortuitum* complex, *M. smegmatis*.
AdvanSure mycobacteria GenoBlot assay	Invitros	16S-23S rRNA spacer region	Specimen or culture	MTBC, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. abscessus*, *M. chelonae*, *M. kansasii*, *M. szulgai*, *M. gordonae*, *M. celatum*, . *marinum/M. ulcerans*, *M. simiae*, *M. lentiflavum/M. genavense*, *M. xenopi*, *M. smegmatis*, *M. malmoense*, *M. gastri*, *M. flavescennse*, *M. vaccae*, *M. fortuitum* complex, *M. terrae* complex
MolecuTech REBA Myco-ID	YD Diagnostics	*rpoB*	Specimen or culture	MTBC, *M. avium*, *M. intracellulare*, *M. scroflaceum*, *M. abscessus*, *M. massilience*, *M. chelonae*, *M. fortuitum* complex, *M. marinum/ulcerans*, *M. kansasii*, *M. genavense/simiae*, *M. celatum*, *M. terrae/nonchromogenicum*, *M. gordonae*, *M. szulgai*, *M. mucogenicum*, *and M. aubagnense*

Abbreviations: CM, common mycobacteria; AS, additional species; MTBC, *Mycobacterium tuberculosis* complex.

The INNO-LIPA Mycobacteria assay (Innogenetics) allows the identification of 17 different mycobacterial species in one strip. The test is based on nucleotide differences in the 16S-23S rRNA spacer region, and a positive reaction is determined by color changes in the band of a nitrocellulose strip. It is necessary to pay attention to cross-reactions with rare mycobacteria, although INNO-LIPA has high sensitivity (100%) and specificity (94.4%) [60]. The other LPA used is the GenoType Mycobacterium assay (Bruker) targeting the 23S rRNA gene. The assay is divided into two steps: common mycobacteria (CM) and additional species (AS) assays for NTM. CM ver2.0 can identify more than 20 mycobacterial species, including MTBC. AS ver1.0 is complementary to CM and allows the identification of more than 15 clinically important but less common NTM (Table 6). The sensitivity and specificity of these assays were 97.9% and 92.4% for CM and 99.3% and 99.4% for AS, respectively [61]. Another GenoType CM*direct* Ver1.0-LPA (Bruker) kit can detect and differentiate between MTBC and NTM species directly from decontaminated sputum specimens. Although this kit is similar to the GenoType Mycobacterium CM, it differs in the fact that specimens can be used; however, it cannot distinguish *M. scrofulaceum* and *M. intracellular*e. The AdvanSure Mycobacteria GenoBlot assay (Invitros) was developed in South Korea. The AdvanSure Mycobacteria GenoBlot assay can detect 70 species of *Mycobacteriaceae* and identify 24 species by targeting the 16S-23S rRNA spacer region.

Compared with 16S rRNA and *rpoB* gene sequencing, the common NTM species were correctly identified at 100 %, and the overall concordance rate was 89.7% [62]. This assay showed a high concordance rate (90%) when used to directly detect and identify mycobacteria in clinical specimens [62]. MolecuTech REBA Myco-ID (YD Diagnostics) is a reverse blot hybridization line-probe assay designed for the rapid identification and differentiation of MTBC and 21 NTM by targeting the *rpoB* gene. This kit enables the automation of post-PCR steps such as hybridization and washing with the HybREAD480 system. This kit differentiated *M. abscessus* from *M. massiliense*; however, *M. lentiflavum* strains were misidentified as *M. gordonae*. The accuracy of MolecuTech REBA Myco-ID was reported to be 94.3% for 192 clinical *Mycobacteriaceae* strains [63].

(6) Sanger sequencing

Sequencing is considered the gold standard for the identification of *Mycobacteriaceae*. The 16S rRNA gene is the most common target in bacteria because it is present in all bacteria and contains both conserved and variable regions. However, the 16S rRNA gene is inadequate for mycobacterial identification due to its high sequence similarity [64]. Several other genes, such as the 16S-23S rRNA gene internal transcribed space (ITS) region, *rpoB*, *gyrB*, *hsp65*, and *sodA* have been used to identify mycobacterial species. The ITS is a spacer sequence located between the 16S and 23S rRNA genes. It is good to analyze because the sequence size of the ITS is small (only 200–330 bp), although this depends on the species. However, the use of the ITS gene alone may be limited in distinguishing closely related NTM [65]. Partial *hsp65* gene using 440-bp is useful for differentiating *M. marium* from *M. ulcerans* and *M. gastri* from *M. kansasii* complex because it is more variable than the 16S rRNA gene; however, *M. simiae* is indistinguishable from *M. genavense* [64]. The *rpoB* gene encodes the β subunit of the bacterial RNA polymerase. A partial *rpoB* gene between 342 and 723 bp is used for mycobacterial identification, although its total length is approximately 3,600 bp [64,66].

M. chelonae was clearly distinguished from *M. abscessus* and *M. mucogenicum* by using the *rpoB* gene [64,66]. *M. simiae* was distinguished from *M. genavense* using *rpoB*. For RGM, *rpoB* genes showed higher discrimination power than 16S rRNA and *recA* genes, with a higher bootstrap value and greater divergence in phylogenetic tree analysis [67]. It is impossible to identify all NTM using only one specific gene. It would be preferable to use a few genes simultaneously to accurately identify NTM. One laboratory routinely uses sequence analysis to identify MTBC and NTM cases in South Korea.

(7) Next-generation sequencing (NGS)

NGS can be used to sequence millions of fragments simultaneously and offers a much higher throughput. Whole-genome sequencing (WGS) can provide information about the entire bacterial genome, including drug resistance. WHO has published the catalogue of MTBC mutations associated with drug resistance to serve as a global standard for interpreting WGS data [68]. There are several WGS tools, such as GenTB, TB Profiler, PhyResSE, CASTB, and Mykrobe. These were introduced in the WHO guidelines and can be used to report the resistance profiles and lineages of clinical isolates [69]. Deeplex Myc-TB (GenoScreen) is a commercial assay based on targeted deep sequencing for the detection of MTBC, NTM, and resistance genes in clinical samples. This assay can detect the genotyping and spoligotyping of MTBC strains and can

identify more than 100 species of NTM and resistance mutations to 15 anti-drugs with a turnaround time of less than 48 h [70]. We believe that NGS will play an important role in the diagnosis and surveillance of drug resistance in TB.

Conclusion

In this review, we introduced common diagnostic methods for the detection and identification of *Mycobacteriaceae*. There have been many advances in diagnostic tests for mycobacterial identification, and these new methods have replaced conventional methods based on biochemical tests. In South Korea, real-time PCR and antigen detection are the main tools used to identify MTBC in cultures. At present, PCR-RFLP, HPLC, LPA, MALDI-TOF, and sequencing are used for the identification of NTM in Korea. We believe that NGS will play an important role in the near future in identifying mycobacteria and detecting resistance genes in clinical laboratories.

Ethics statement

It is not a human population study; therefore, approval by the institutional review board or the obtainment of informed consent is not required.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Data availability

This review article does not involve the generation or analysis of new datasets. All data supporting the findings are derived from previously published studies, which are appropriately cited within the manuscript.

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