American Journal of Biochemistry and Biotechnology

Original Research Paper

Analysis of the Utility of MPT64, Drug Susceptibility Testing and Tuberculosis/Nontuberculous Mycobacteria Reverse Transcription Polymerase Chain Reaction for Tuberculosis Diagnosis and Efficiency Compared with Traditional Culture

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Article history
Received: 22-07-2024
Revised: 12-09-2024
Accepted: 27-09-2024

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Abstract: Defining the molecular mechanisms underlying the dormant state of *Mycobacterium tuberculosis* (MTB) is essential for developing more effective treatments for tuberculosis (TB) infection. We investigated the positivity and resistance rates of TB tests on 6,174 samples referred to a single hospital. Of these, 342 cases (5.5%) were confirmed as positive by acid-fast bacilli staining, 243 cases (71.1%) were detected as MTB cultured on *Mycobacterium* growth indicator tube medium and 3% Ogawa solid medium, and 341 cases (99.7%) and 234 cases (68.4%) were cultured on either *Mycobacterium* Growth Indicator Tube or 3% Ogawa solid medium, respectively. For the 341 samples detected as positive in both media, an antigen MPT64 rapid test was performed, confirming TB in 333 cases (97.7%), and 279 cases (81.8%) were analyzed using TB/nontuberculous mycobacteria real-time reverse transcription polymerase chain reaction (RT-PCR). Among the 145 cases confirmed as TB, drug-susceptibility tests showed resistance rates of 23 (15.9%) to isoniazid, 11 (7.6%) to rifampicin, 10 (6.9%) to ethambutol and 10 (6.9%) to rifabutin. Liquid culture testing, despite its longer duration, yielded more accurate results than the relatively rapid RT-PCR. A combination of traditional culture, MPT64, and RT-PCR tests used for infection confirmation was the most efficient method in increasing the TB confirmation rate. This information may aid in developing more precise diagnostic methods for TB.

Keywords: Tuberculosis Culture, MPT64 Protein, Reverse Transcription Polymerase Chain Reaction, Antibiotic Susceptibility Testing, Antibiotic Resistance Testing

Introduction

Tuberculosis (TB) is a fatal respiratory disease caused by *Mycobacterium tuberculosis* (MTB). Among the 38 member countries of the Organization for Economic Cooperation and Development, South Korea ranks first in TB incidence and third in mortality (Bagcchi, 2023). According to the data reported and analyzed for patients with TB in South Korea, the number of newly reported TB cases in 2022 was 16,264 (31.7 cases per 100,000 population), indicating an 11% decrease (2,071 cases) from 2021 (18,335 cases; 35.7 cases per 100,000 population) (Lee *et al*., 2023). Furthermore, among patients with TB showing resistance to rifampicin or multidrug-resistant TB (MDR-TB), 29 cases exhibited resistance to one or more quinolone drugs and 172 cases showed resistance to both rifampicin and isoniazid (Statistics Korea, 2022). Since its peak in 2011, with 39,557 newly reported cases of TB, a continuous annual decrease of 7.8% has resulted in a 58.9% decrease over the past 11 years (Statistics Korea, 2022).

According to the World Health Organization, approximately 10.6 million people worldwide contracted TB in 2021, with an incidence of 134 cases per 100,000

population. Among all patients with TB, 6.7% were human immunodeficiency virus-infected. Geographically, the majority of TB cases in 2021 were distributed as follows: Southeast Asia (45%), Africa (23%), Western Pacific (18%), Eastern Mediterranean (8.1%), the Americas (2.9%) and Europe (2.2%) (Bagcchi, 2023).

Furthermore, the worldwide record of MDR-TB/Rifampicin-Resistant TB (RR-TB) cases in 2021 showed approximately 450,000 cases, representing a 3.1% increase from 437,000 cases in 2020. This increase was attributed to the impact of the coronavirus disease 2019 pandemic on TB incidence, which increased overall between 2020 and 2021. In 2021, approximately 191,000 deaths (ranging from 119,000-264,000) were attributed to MDR/RR-TB (Ohkado and Kato, 2022).

Diagnosis and testing for TB and drug-resistant TB are essential steps in the TB treatment pathway and must be rapidly and accurately carried out. Recently, a significant transformation has been observed in the TB diagnostic landscape with the introduction of highly specific and sensitive rapid molecular tests that depart from the reliance on traditional microscopy and culture methods. TB diagnosis is defined as bacteriological confirmation by culture, molecular testing, and smear microscopy. Rapid molecular diagnostic tests for drug resistance have become increasingly important in clinical practice.

In 2021, a total of 6.4 million people worldwide were newly diagnosed with TB; of whom, 5.3 million (83%) had pulmonary TB, leading to an increase in bacteriologically confirmed cases from 59% in 2020 to 63% in 2021. The highest proportions were observed in Europe and America, whereas similar levels were observed in other regions (WHO, 2022). Most TB infections are latent or inactive, with TB affecting approximately 15 million individuals, making it one of the leading causes of death worldwide. TB incidence and mortality have been declining as socioeconomic conditions have improved, with the mortality rate decreasing from 23% in 2000 to 16% in 2017 (WHO, 2018). However, by the third quarter of 2023, patients with TB aged ≥65 years increased by 5.0% compared with that in the previous year. During the same period, the number of those acquiring TB aged ≥80 years increased by 7.8%, whereas a slight increase of 0.1% was observed in the reported cases for all age groups. Cases among those <50 years of age decreased, with a reduction of 13.7 and 13.5% among teenagers and those in their thirties, respectively. Although approximately 85% of patients have pulmonary TB, only 30% of those exposed to TB are infected. Moreover, only 10% of infected individuals manifest symptoms, with the rest becoming latent or inactive carriers. The increase in patients with TB aged ≥65 years underscores the importance of raising awareness about TB screening (Myeong-ji, 2023).

TB presents with a variety of clinical symptoms, including coughing lasting more than 2 weeks, sputum production, fever, chest pain, neurological symptoms, and loss of appetite. Diagnosis based solely on chest radiography is challenging and obtaining samples for testing can be difficult in several cases. Acid-Fast Bacillus (AFB) smear tests conducted for TB diagnosis have recently increased in frequency; however, they have decreased sensitivity as they cannot distinguish nontuberculous mycobacteria (NTM) infections, which are becoming more prevalent (ATS, 2000; JAMA, 1999; Park *et al*., 2009).

NTM infections, a subset of which is utilized as part of the differential diagnosis in immunocompromised patients with difficult-to-treat musculoskeletal infections, have significant clinical importance. Currently, molecular tests cannot fully replace conventional smear and culture methods; however, they can serve as useful adjunct tests, particularly for diagnosing *Mycobacterium marinum* infections (Jeon *et al*., 2022).

The use of automated liquid culture media (*Mycobacterium* Growth Indicator Tube [MGIT] 960) for AFB culture testing, typically taking 2-6 weeks, poses a significant challenge as it often delays TB confirmation (Kanchana *et al*., 2000). Recently, nucleic acid amplification tests (polymerase chain reaction [PCR]), which are more sensitive than AFB smear tests requiring three or more examinations for accuracy and faster than traditional culture tests, have been introduced for TB confirmation. Moreover, drug-susceptibility testing methods are increasingly important for determining the efficacy of certain drugs (Griffith *et al*., 2007). Molecular diagnostic tests for TB involve the amplification of specific deoxyribonucleic acid or ribonucleic acid sequences unique to MTB using PCR, offering higher sensitivity and specificity than traditional TB tests. While molecular diagnostic tests are costlier than AFB smears or culture tests, they offer favorable reproducibility and provide results within 24-48 h (JAMA, 2009).

Recently, molecular diagnostic methods, such as MTBDRplus (Hain Lifescience GmbH, Nehren, Germany), loop-mediated isothermal amplification, line probe assay, Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA), and whole genome sequencing, have enabled simultaneous diagnosis and identification of mutations related to anti-TB drugs (Iwamoto *et al*., 2003; WHO, 2013). Diagnostic analyses using Xpert® and TB-PCR showed similar sensitivity and specificity of over 95% for smear-positive samples. Among these, Xpert® analysis demonstrated higher sensitivity for smear-negative specimens, shorter processing time, and significantly superior performance in predicting rifampicin resistance compared with TB-PCR analysis (Son *et al*., 2022).

TB is curable with a 6-month treatment regimen if anti-TB drugs are consistently administered after early diagnosis. However, failure of initial treatment due to inappropriate drug prescriptions can result in lower cure rates. Therefore, susceptibility testing for anti-TB drugs is essential for appropriate diagnosis and treatment, making

the development of rapid diagnostic methods crucial. Drug resistance refers to the resistance to drugs that inhibit growth, enabling the organism to survive and grow even at concentrations that can hinder growth. Factors contributing to drug resistance include inappropriate prescriptions, treatment methods, and treatment interruptions (Park, 2011). One of the epidemiological indicators for evaluating the performance of national TB control programs is the prevalence and trends of drug-resistant TB. The emergence of drug-resistant TB strains is a major cause of treatment failure. As treatment and management of patients require specialized care, diagnosing drug-resistant TB based on drug-susceptibility results allows for the prescription of appropriate anti-TB drugs (Espinal *et al*., 2001; Sharma, 2009). Patients who have had contact with MDR-TB, with a history of previous treatment, or originate from regions with a high MDR-TB prevalence can be predicted for susceptibility to anti-TB drugs (Wright *et al*., 2009; Nathanson *et al*., 2010). However, in the United States, 60% of patients with MDR-TB do not have identifiable risk factors, indicating the necessity of drug-susceptibility testing for all patients (Bloch *et al*., 1994). In contrast, according to the guidelines of the Korea Disease Control and Prevention Agency for TB treatment, drug-susceptibility testing is recommended for the initial isolate of all patients with TB and should be repeated in cases where the TB culture remains positive after more than 3 months of treatment or if clinical treatment failure is suspected (Griffith *et al*., 2007).

This study aimed to evaluate the most rapid and accurate test for the diagnosis of TB among respiratory specimens that tested positive during the antimicrobial-susceptibility testing process at the Microbiology Laboratory of Hallym University Hospital in Gyeonggi-do. Additionally, we aimed to assess the utility of antimicrobial-susceptibility testing methods, including mycobacterial culture media and molecular diagnostic tests, to determine the efficiency of the laboratory workflow. This study also sought to evaluate the current status of MTB and NTM and assess rapid testing methods to contribute to shortening the duration of TB treatment.

Materials and Methods

The specimens used in this study were selected from patients who visited the Microbiology Laboratory at Hallym University Hospital in Gyeonggi-do, South Korea, with suspected TB and underwent TB testing between January 1, 2021, and December 31, 2021. A total of 6,174 specimens were collected. However, the study's reliance on sample results from a single hospital limits the reliability and generalizability of its findings to a broader population. For more thorough insights, future studies will need to include a broader, multi-center investigation. By collecting data from diverse patient populations and medical environments through multi-center studies, the reliability and applicability of the findings can be further enhanced.

In this study, the MTB diagnostic method involved preprocessing the specimens using the N-acetyl-L-cysteine-sodium hydroxide (NaOH-NALC) method. MTB was diagnosed if the specimens were positive in both AFB Smear Examination (Ziehl-Neelsen stain) and AFB Culture (BACTEC MGIT 960 liquid culture tubes [Becton Dickinson] and 3% Ogawa agar plates [Korean Institute of Tuberculosis, Cheongju, Korea]), as well as in either the rapid immunochromatographic test (MPT64 TB-specific antigen [Ag] test [Standard Diagnostics, Yongin, Korea]) or AdvanSure MTB/NTM Reverse Transcription (RT)-PCR (Anyplex Plus MTB/NTM Detection [Seegene, Seoul, South Korea]). If diagnosed as MTB, an antimicrobial susceptibility test was then performed.

Among patients who tested positive in the acid-fast staining test, antimicrobial-susceptibility testing and molecular diagnosis by PCR were performed to compare positivity rates. Additionally, MPT64 and MTB/NTM tests were conducted to assess the potential utility of these methods.

This study was approved by the Institutional Review Board (IRB) of Hallym University Sacred Heart Hospital, South Korea (IRB No. 2023-02-002-001). The requirement for informed consent was waived by the IRB as the statistics of examinations conducted by medical institutions were used retrospectively and patients' personal information was not used.

Sample Preprocessing

Samples were processed using the NaOH-NALC method. Initially, an equal volume of NaOH-NALC-sodium citrate solution was added to the sample, followed by gentle vortexing for 15-30 seconds to fully liquefy the sample. If a mucous-like consistency was visually observed, NALC powder (30-35 g) was added and the mixture was homogenized. After stabilizing for 15-20 min, phosphate buffer (pH 6.8; 50 mL) was added and centrifuged at 3,000× g for 15 min. Subsequently, for aerosol stabilization, the mixture was vortexed for 5 min before inoculating the sediment onto the MGIT culture medium (BD BBL Mycobacteria Growth Indicator Tube; Becton Dickinson, Sparks, MD, USA).

AFB Smear Examination

After digestion/decontamination, concentration, and resuspension of the sediment, the mixture was mixed well using a pipette, and two to three drops were spread onto a clean, dry slide. The smears were subsequently allowed to dry completely. For fixation of the organisms, the slide was heated using a slide warmer at 65-75°C for 2-3 h, with caution to avoid ultraviolet exposure. If only one to two AFB were detected in the entire smear and suspected to be positive, a re-evaluation was performed using another smear from the same patient. Smears suspected to be positive by fluorescence staining were reconfirmed using Ziehl–Neelsen staining.

AFB Culture Test

For the culture, the same preprocessing as that for the AFB smears was performed. Processed samples were inoculated into BACTEC MGIT 960 liquid culture tubes (Becton Dickinson) and 3% Ogawa agar plates (Korean Institute of TB, Cheongju, Korea) using the same method. MGIT culture tubes were incubated at 37°C for 6 weeks, while 3% Ogawa agar plates were incubated at 37°C for 8 weeks. If AFB growth was observed in one or more liquid or solid media, the culture was determined to be positive. Additionally, if no growth was detected in the BACTEC MGIT 960 system for up to 6 weeks, it was considered negative.

Rapid Immunochromatographic Test

In cases where positive growth was observed in the MGIT culture, clusters were identified from the sample for TB organism identification using the MPT64 TB-specific antigen (Ag) test (Standard Diagnostics, Yongin, Korea). A small number of clusters were collected and inserted into the TB Ag MPT64. The kit was examined after approximately 15 min. If two red lines were visible (control and sample lines), the samples were considered TB-positive. If only one red line was visible (control line), it was interpreted as NTB.

AdvanSure MTB/NTM RT-PCR

The preprocessed specimens were examined using the Anyplex Plus MTB/NTM Detection (Seegene, Seoul, South Korea) system. Interpretation of the results was based on three channels (MTB, mycobacteria, and internal control), each corresponding to the carboxyfluorescein, carboxy rhodamine, and cyanine-5 wavelengths. A cycle threshold (CT) value <35 was considered positive. If the CT value was equal to or less than that of mycobacteria, MTB was interpreted as TB-positive. If the CT value was greater than that of mycobacteria, it was considered a possible simultaneous infection of TB and NTM. CT values of <35 were interpreted as NTM.

Antimicrobial Susceptibility Testing

For patients who were TB-positive, known concentrations of antimicrobial agents were added to the cultured samples of the isolated bacteria, and bacterial growth was observed. Resistance was determined if the strain isolated from the patient grew in the control group but not in the medium supplemented with antimicrobial agents. Conversely, if growth occurred in both the control group and the medium with antimicrobial agents, the cells were interpreted as susceptible.

Traditional drug-susceptibility testing is the standard method used by TB research institutions. This method involves setting critical concentrations of drugs that can inhibit the growth of standard strains, such as H37Rv, or drug-resistant strains isolated from patients without treatment. Susceptibility was determined if >20 bacterial colonies (1%) grew at these concentrations.

Rapid drug-susceptibility testing using a line probe assay (MolecuTech REBA MTB-MDR; YD Diagnostics, Yongin City, South Korea) relies on the differences in nucleotide sequences between the wild-type and mutant alleles of the target genes (*rpoB*, *katG*, *inhA*, and *ahpC*). Genetic amplification experiments were conducted to determine isoniazid/rifampicin resistance. Rifampicin resistance in TB bacteria and MTB infection in the samples was determined using genetic extraction and RT-PCR.

Statistical Analysis

Data processing and statistical analyses were conducted using electronic medical records from the hospital's digital comprehensive medical information system, specifically the Order Communication System, known as RefoMax. Secondary data analysis was performed using IBM SPSS Statistics (version 29.0; IBM Corp., Armonk, NY, USA) to confirm the utility of the antimicrobial-susceptibility testing culture media and molecular diagnostic tests to determine the efficiency of the laboratory workflow. We investigated the current status of MTB and NTM and the correlation between rapid testing methods.

Results

AFB Smear and Culture Examination

From January 1 to December 31, 2021, a total of 6,174 specimens were received for examination. Among these, 342 (5.5%) were positive for AFB, and 5,832 were negative. The distribution of isolated specimens was as follows: Sputum, 284; Bronchoalveolar Lavage (BAL) fluid, 41; pleural fluid, 10; tissue, five; and urine, two. Among the 342 positive AFB smear specimens, 243 (71.1%) showed growth in both MGIT liquid medium and 3% Ogawa solid medium. Furthermore, 341 specimens (99.7%) grew in either MGIT or Ogawa medium alone and 234 specimens (68.4%) grew in the Ogawa medium alone, indicating a significantly higher detection rate in the MGIT medium. The time to cluster formation, represented by time to detection, averaged 15 days and 22 h (Table 1).

Table 1: Smear test for acid-fast bacilli and positive rates on various culture media

	Acid-fast bacilli smear tests (%)		*Mycobacterium tuberculosis* positive (%)	
	Positive	Negative	MGIT (liquid type)	3% Ogawa (medium type)
	342 (5.5%)	5,832 (94.5%)	341(99.7%)	234(68.4%)
Total	342		341	

MGIT, *Mycobacterium* Growth Indicator Tube

Rapid Immunochromatographic Test and AdvanSure TB/NTM RT-PCR

Among the 341 specimens (99.7%) that tested positive in the MGIT liquid medium, 333 (97.7%) were determined to be TB-positive using the SD Bioline TB Ag MPT64 rapid test (Standard Diagnostics) for rapid Ag detection. Eight (2.3%) specimens tested negative. Among these eight specimens, AdvanSure TB/NTM RT-PCR (LG Life Science, Seoul, Korea) identified four cases of MTB and four cases of TB/NTM. When the 341 TB-positive specimens (99.7%) in the MGIT liquid medium were analyzed using AdvanSure TB/NTM RT-PCR, 279 (81.8%) and 62 (18.2%) returned positive and negative, respectively (Fig. 1).

MTB Drug Susceptibility Test

The results of the drug-susceptibility testing for anti-TB drugs were as follows: Resistance to isoniazid was observed in 23 cases (15.9%), rifamycin in 11 cases (7.6%), ethambutol in 10 cases (6.9%) and rifabutin in 10 cases (6.9%) (Table 2).

Among confirmed patients with TB, men demonstrated predominance (100; 69.0%) over women (45; 31.0%). The average age distribution indicated a higher prevalence of individuals in their 70s (33; 22.8%) and 50s (28; 19.4%) than that in other age groups, regardless of sex. Meanwhile, among the 31 patients who showed resistance to drug-susceptibility testing, men demonstrated predominance (22; 71.0%) over women (9; 29.0%) and the average age distribution across sexes revealed a higher occurrence of individuals in their 50s (8; 25.8%) and 70s (7; 22.5%) than that in other age groups (Table 3).

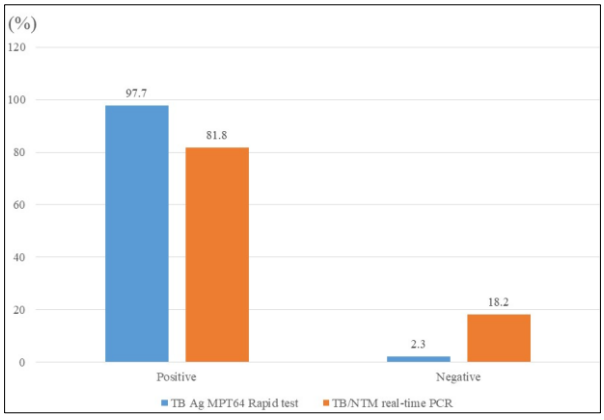

Fig. 1: Results of SD Bioline TB Ag MPT64 rapid test and AdvanSure TB/NTM RT-PCR test on samples that tested positive in *Mycobacterium* Growth Indicator Tube liquid medium; RT-PCR, reverse-transcription-polymerase chain reaction

Table 2: Results of *Mycobacterium tuberculosis* drug-susceptibility test

	Susceptibility	Resistance (%)
INH 0.2 (isoniazid)	122	23(15.9)
INH 1.0 (isoniazid)	131	14(9.7)
RIF (rifamycin)	134	11(7.6)
SM (streptomycin)	139	6(4.1)
EMB (ethambutol; ETB)	135	10(6.9)
KM (kanamycin; KAN)	143	2(1.4)
CPM (capreomycin)	143	2(1.4)
PTH (prothionamide)	144	1(0.7)
CS (cycloserine; CCS)	145	0(0)
PAS (p-aminosalicylicacid)	145	0(0)
OFX (ofloxacin)	141	4(2.8)
PZA (pyrazinamide)	141	4(2.8)
RBT (rifabutin; RFB)	135	10(6.9)
MOX (moxifloxacin; MXF)	141	4(2.8)
AMK (amikacin)	143	2(1.4)
LIN (linezolid; LZD)	145	0(0)
LEV (levofloxacin; LVX)	141	4(2.8)

Table 3: Distribution of patients who underwent antimicrobial-susceptibility tests

		MTB (%)	Tuberculosis resistance (%)
Sex	Male	100(69.0)	22(71.0)
	Female	45(31.0)	9(29.0)
Age group (years)	20	10(6.9)	2(6.5)
	30	6(4.1)	2(6.5)
	40	16(11.0)	4(12.9)
	50	28(19.4)	8(25.8)
	60	25(17.2)	3(9.7)
	70	33(22.8)	7(22.5)
	80	25(17.2)	4(12.9)
	90	2(1.4)	1(3.2)

MTB, *Mycobacterium tuberculosis*

The distribution of drug resistance showed no extensive drug-resistant TB (XDR-TB), which is resistant to at least one fluoroquinolone and one of the injectable drugs (amikacin, kanamycin, or capreomycin), as well as bedaquiline or linezolid. Five cases (3.4%) of pre-XDR-TB, defined as resistant to all fluoroquinolones, including isoniazid and rifampicin, were recorded. MDR-TB, which is resistant to the two most important first-line drugs, isoniazid and rifampicin, accounted for seven cases (4.8%). Specifically, RR-TB was found in two cases (1.4%) and isoniazid-resistant TB accounted for the highest distribution, with 15 cases (10.3%). Additionally, streptomycin resistance was observed in two cases (1.4%), while 114 specimens (78.7%) showed susceptibility to all anti-TB drugs (Fig. 2).

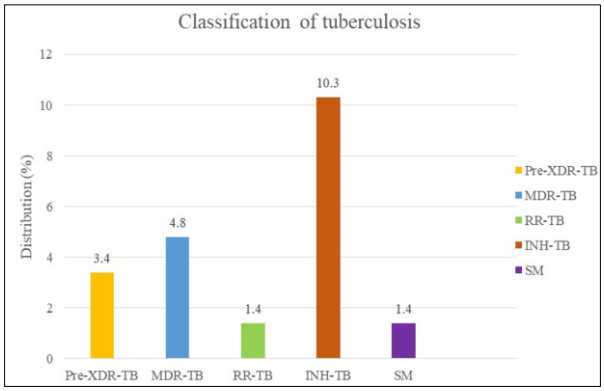

Fig. 2: Distribution of antimicrobial resistance; INH, isoniazid; MDR, multidrug-resistant; RR, rifampicin-resistant; SM, streptomycin; TB, tuberculosis; XDR, extensive drug-resistant

Discussion

TB continues to be a global health issue with a higher prevalence in developing nations than in advanced nations, although its incidence rate has been steadily declining (Dye *et al*., 1999; Kim, 2006). Delayed diagnosis and inappropriate treatment in clinical settings can lead to the emergence of drug-resistant TB bacteria, making the early detection of TB, especially through molecular diagnostics, critically important for effective treatment and prevention of transmission in clinical practice (Jung *et al*., 2008).

Pulmonary diseases caused by TB and NTM present challenges in differentiation based solely on various clinical symptoms or radiographic findings. In clinical practice, it is common to encounter cases in which TB treatment is initiated based on positive AFB staining, only to later identify NTM upon culture, leading to discontinuation or alteration of medication (Ryu *et al*., 2016).

In this study, among the 6,174 samples confirmed as TB-positive by AFB staining, positive TB results were observed in 342 cases (5.5%). A limitation of AFB staining is its inability to differentiate between viable and non-viable organisms in the sample and distinguish between different mycobacterial species. Therefore, in such cases, confirmation should be obtained through additional cultures or PCR testing. However, PCR testing may yield false positives in individuals taking anti-TB medications or with a history of TB. Positive samples were predominantly obtained from sputum and BAL fluid, indicating a higher false-positive rate in these samples than in tissues or urine.

Of the 342 positive samples in the AFB smear test, 341 (99.7%) and 234 (68.4%) showed positive results when cultured on liquid and solid media, respectively. This indicates that liquid media have a higher sensitivity than solid media. However, one sample that did not grow in the liquid medium grew in the solid medium. Therefore, concurrent testing in both types of media is essential for accurate diagnosis.

Shortening the culture time for AFB is clinically significant. Solid media often require approximately 4 months for culture and drug-susceptibility testing, which can pose difficulties in treating patients suspected of having drug-resistant TB. Liquid media can expedite the decision-making process for treatment by reducing culture time. However, in the MGIT system, which uses liquid media, many nutritional components facilitate the proliferation of contaminating organisms (Yi *et al*., 2000), leading to a higher contamination rate than that of solid media (Tortoli *et al*., 1999; Yun-mi and Myeong-hee, 2000; Lee *et al*., 2003). Moreover, doubling the concentration of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin can result in more than a twofold decrease in contamination rates (Peres *et al*., 2011).

Of the 341 samples confirmed to be positive in liquid media, the MPT64 Ag test yielded 333 positive results (97.7%). For the remaining eight samples that tested negative, TB/NTM PCR testing revealed four cases of MTB and four cases of mixed MTB and NTM. The positivity rate decreased compared with that in previous studies, where all samples tested positive in the MPT64 Ag test (Lee *et al*., 2012).

Furthermore, of the 341 liquid media-positive samples, TB-PCR testing showed a positivity rate of 279 (81.8%), which was less accurate than that of the liquid media culture results. Despite the improvement in accuracy and reduced turnaround time due to the advancement of TB-PCR testing, the positivity rate of the liquid medium culture is more reliable than that of the TB-PCR results. Therefore, confirming TB solely by PCR testing remains a challenge. To diagnose MTB, a combination of investigations should be carried out. These include chest radiography, AFB smear tests, cultures, and rapid tests, such as PCR; all these methods yield swift results and both the results and clinical presentation should be examined to confirm MTB diagnosis (Bang *et al*., 2011).

In this study, susceptibility testing for anti-TB drugs was conducted in 145 cases identified as TB. The results showed resistance to isoniazid in 23 cases (15.9%), rifampicin in 11 cases (7.6%), ethambutol in 10 cases (6.9%) and rifabutin in 10 cases (6.9%). The distribution of antimicrobial resistance in this study revealed negativity for XDR-TB, while pre-XDR-TB was observed in five cases (3.4%), MDR-TB was found in seven cases (4.8%) and RR-TB was identified in two patients (1.4%). Isoniazid-monoresistant TB, displaying resistance to isoniazid alone, was the most prevalent, with 15 cases (10.3%). Additionally, resistance to streptomycin was observed in two cases (1.4%), while 114 cases (78.7%) showed susceptibility to all anti-TB drugs.

As 60% of patients with drug-resistant TB in the United States do not have specific risk factors, susceptibility testing for all patients with TB remains necessary (Bloch *et al*., 1994). Therefore, the United States mandates susceptibility testing for all patients with TB, including those undergoing initial treatment (Wright *et al*., 2009; Nathanson *et al*., 2010). Similarly, according to the guidelines of the Korean Centers for Disease Control and Prevention for TB treatment, susceptibility testing for anti-TB drugs is recommended for all patients with TB based on the first isolated strain, and repeat testing is advised if the culture remains positive or clinical treatment failure is suspected, even after 3 months of treatment (Joint Committee for the Development of Korean Guidelines for Tuberculosis, 2011). However, varying practices among hospitals or attending physicians regarding susceptibility testing make it difficult to compare drug resistance rates temporally, institutionally, or regionally (Jeong *et al*., 2005). Future research, including approaches to fundamental aspects of MTB resistance and clinical intervention, is necessary (Goossens *et al*., 2020).

Conclusion

Targeted resistance profiling provides new strategies aimed at acquiring or preventing drug-resistant TB strains, accelerating bacterial eradication and consequently reducing the duration of infection and the risk of acquiring drug resistance. These novel approaches complement the ongoing research on treatment-shortening strategies.

The accurate identification of NTM in cases of suspected TB has significant implications for patient management and treatment. NTM lung diseases require different treatment approaches compared with TB and misdiagnosis can lead to inappropriate treatment and potentially harmful side effects. Moreover, NTM infections are often chronic and require long-term treatment, which can pose serious economic and social effects on patients and their families. Therefore, molecular identification of NTM in cases of suspected TB is essential for the accurate diagnosis and management of patients. In the future, efforts should focus on reducing errors in results due to contamination and liquid media characteristics, as well as developing diagnostic methods for more precise diagnoses.

Acknowledgment

We express our gratitude to the staff of the microbiology laboratory at Hallym University Hospital in Gyeonggi-do.

Funding Information

This research received no external funding.

Author's Contributions

Ho Keun Choi and Ga Yeon Kim: Contributed substantially to the conception and design of this study.

Tae Soung Kim and Jae Kyung Kim: Made substantial contributions and acquired and analyzed the data.

All the authors have read and agreed to the published version of the manuscript.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Hallym University Sacred Heart Hospital (No. 2023-02-002-001). Patient consent was waived owing to the fact that the statistics of examinations conducted by medical institutions were used retrospectively and patients' personal information was not used.

Conflict of Interest

The authors declare no conflicts of interest.

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