Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 16s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Molecular Diagnosis Of Tuberculosis and Non-Tuberculous Mycobacteria: Evaluating Dual PCR Targeting IS6110 and rpoB Gene

Ashish Soni[1], Indu Sharma[1], Swati Soren[2], Jasna Shaji[3], Darshan Ambiga[3], Diksha Shindhe[4], Anjali Kukreti[5], Anushka Saini[5], Narotam Sharma[6, 7,] Ajay Singh[8], Ankita Singh[6, 9], Jagjeet Singh[6]

[1]NIMS Institute of Allied Medical Science and Technology, NIMS University, Jaipur, Rajasthan, India

[2]Department of Zoology, Jamshedpur Co-Operative College, Jamshedpur, Jharkhand, India

[3]Amity Institute of Virology and Immunology, Amity University, Noida, Uttar Pradesh, India

[4]Manipal Institute of Technology, MAHE, Manipal, Karnataka, India

[5]Department of Biotechnology, Dr. PDBH Government PG College, Kotduara, Uttarakhand, India

[6]DNA Labs -A Centre for Applied Sciences, East Hope Town, Dehradun Uttarakhand, India

[7]Department of Chemistry, Uttaranchal Institute of Technology, Uttaranchal University, Dehradun, Uttarakhand, India

[8]School of Applied and Life Sciences (SALS), Uttaranchal University, Dehradun, India

[9]Uttaranchal University of Technology, Uttaranchal University, Dehradun, Uttarakhand, India

Cite this paper as: Ashish Soni, Indu Sharma, Swati Soren, Jasna Shaji, Darshan Ambiga, Diksha Shindhe, Anjali Kukreti, Anushka Saini, Narotam Sharma, Ajay Singh, Ankita Singh, Jagjeet Singh, (2025) Assessment Of Management Strategies In Alcoholic Liver Disease Patients With Hypertension Or Diabetes Mellitus. *Journal of Neonatal Surgery*, 14 (16s), 960-965.

ABSTRACT

A sum of 100 clinical specimens was examined, showing that sputum had the highest positivity rates for MTB, with 13 of 34 samples testing positive for rpoB and 9 for IS6110. Significantly, differences among these markers were noted, indicating possible genetic variations or co-infections. Pus samples (n=26) demonstrated a low detection rate, with merely 6 samples testing positive for IS6110, while fistula samples (n=18) exhibited an equal distribution of rpoB and IS6110 positivity (7 cases each). Menstrual blood samples (n=9) presented distinct diagnostic challenges, showing rpoB positivity in 2 instances and IS6110 in 3 instances. In total, MTB was detected in 35 instances (70%), whereas NTM was verified in 15 instances (30%). These results highlight the importance of multi-gene molecular diagnostics for accurate distinction between MTB and NTM, decreasing the chances of misdiagnosis. The research shows that dual PCR is a strong and dependable diagnostic method, potentially enhancing TB case identification and focused treatment approaches. Additional validation involving varied populations and incorporation with next-generation sequencing is advised to improve diagnostic precision and public health strategies.

Keywords: *Dual PCR, IS6110, rpoB, MTB, NTM*

1. INTRODUCTION

The disease Tuberculosis (TB) which is caused by Mycobacterium tuberculosis (MTB) remains a tentative problem in terms of healthcare on a global scale. Health institutions reported an approximated number of 10.6 million new cases alongside 1.6 million deaths in 2021 [1]. Along with MTB, along with aid other non-tuberculous mycobacterium (NTM) are now becoming recognized as parasitic pathogens. These bacteria impact individuals suffering from immunosuppression or existing pathologies of the lung [2]. It is important to differentiate NTM on NTM so that the right treatments are given to the patients. This is due to NTM patients not having a favorable response towards known anti TB drugs [3].

The addition of molecular diagnostics has made identification of mycobacteria more precise and efficient. This is made possible because of PCR techniques. The IS6110 insertion sequence is known for not being present in different species of NTM [4]. The ribosomal binding protein (rpoB) on the contrary let us identify NTM as it differentiates non-tuberculous species [5].

By combining the two primers the dual PCR method achieves simultaneous amplification of both IS6110 and rpoB. This allows distinct identification of NTM and MTB from a single test. Research confirms that the method is sensitive and specific where infection of NTM and MTB coexist [6, 7]. The employment of Dual PCR as a primary diagnostic technique can

optimize TB case detection and prevent mislabeling patients with NTM infections, thus improving care for patients [8].

Despite the value of this study, previous research showed limitations in sample heterogeneity and population coverage. Consequently, further research should extend the verification of Dual PCR to different clinical and environmental samples with the aid of next generation sequencing (NGS) for detailed strain analysis [9]. The integration of molecular pathology and modern sequencing techniques can potentially improve the detection of drug-resistant strains and facilitate progress in anti-public health surveillance programs [10].

2. MATERIALS AND METHODOLOGY

Site of Implementation of Work

The entirety of the experimentation was conducted at DNA Labs CRIS Centre for Research and Innovative studies (Parent organization) of DNA Labs- A Centre for Applied Sciences (DLCAS), situated in East Hope Town, Laxmipur, Dehradun, Uttarakhand.

Samples Collection and Analysis

A total of 100 clinical specimens were analyzed, comprising pus, sputum, fistula and menstrual blood. The samples were analyzed using dual polymerase chain reaction (dPCR), targeting the rpoB gene and IS6110 gene. To differentiate **Mycobacterium tuberculosis (MTB) from non-tuberculous mycobacteria (NTM)**, a **dual PCR assay** was performed. The assay targeted the **rpoB gene**, which is specific for Mycobacterium tuberculosis complex, and the **IS6110 gene**, a widely used molecular marker for MTB detection. DNA extraction was performed from all clinical samples, followed by amplification using gene-specific primers. The PCR results were interpreted based on the presence or absence of amplification bands, allowing for precise identification of **MTB and NTM**. An analysis was performed on the D-PCR results of rpoB and IS6110 gene for pulmonary and extrapulmonary samples.

Processing of Clinical Samples

Clinical samples, such as pus, sputum, fistula and menstrual blood, were handled by **moving** them into centrifuge tubes. Lysis buffer was introduced, and the samples were centrifuged to collect cells for later DNA extraction.

Extraction of DNA and Amplification of Specific Genes

DNA was obtained through two distinct methods: the magnetic bead technique and the Norzen Biotech kit. In the magnetic bead technique, the procedure included incubation with proteinase K, then ethanol precipitation, column purification, and elution. The Norzen Biotech kit utilized a spin column extraction technique that depends on the attraction between nucleic acids and silica in the purification procedure. The duel polymerase chain reaction (PCR) was utilized to increase the specific DNA sequences of interest and was performed in BIO RAD T100 Thermal Cycler. Primers specifically created to target the repetitive sequences IS6110 and rpoB were synthesized and arranged following standard protocols. The PCR master mix comprised PCR buffer, dNTPs, MgCl2, primers, Taq polymerase, and the DNA template. The PCR cycle included phases for denaturation, annealing, extension, and a final extension phase. The primers employed in the dual PCR for IS6110 comprised the sequences 5’-CCTGCGAGCGTAGGCGTCGGT-3’ (F) and 5’-CTCGTCCAGCGCCGCTTCGG-3’ at 235bp [11, 12] as well as the primer that amplified the 136bp rpoB repetitive sequence.. The complementary strand arrangement is 5’-CGT ACG GTC GGC GAG CTG ATC CAA 3’ (F) and 5’-C CAC CAG TCG GCG CTT GTG GGT CAA-3’ (R) [13].

Agarose gel electrophoresis

Amplified DNA products were analyzed by agarose gel electrophoresis. The agarose gel was prepared by dissolving agarose in an electrophoresis buffer, casting the gel, and then loading the DNA samples. Electrophoresis was conducted to separate the DNA fragments according to their size. Following separation, the gel was stained and the DNA fragments were visualized under UV light for subsequent analysis [14, 15].

3. RESULT

The diagnostic utility of rpoB and IS6110 gene tests in a variety of biological samples, including sputum, pus, fistula, and menstrual blood, was investigated in a systematic review of 100 clinical cases. The cohort, which included 72 males and 28 females between the ages of 11 and 75, showed distinct patterns in gene testing across various sample types. 13 of the 34 sputum samples tested positive for rpoB, and nine tested positive for IS6110. Notably, these markers shared an inconsistency, showing rpoB-positive but IS6110-negative results in people who were 20, 41, 45, 49, 50, and 58 years old. Female sputum donors (n=9) had low positivity for rpoB (31 years) and isolated detection of IS6110 (33 and 53 years), suggesting potential sex-related variations in detection rates.

Pus samples (n=26) exhibited positivity only six times, mainly demonstrating rpoB negativity. Only a specific group of individuals, namely those who are 11, 19, 32, 35, 46, and 55 years old, exhibited the presence of the IS6110 gene. Notable differences in gene expression indicate the presence of environmental or genetic elements that affect the effectiveness of detection.

Fistula samples (n=18) exhibited intricate diagnostic characteristics; seven cases were positive for IS6110 and seven for

rpoB. The presence of rpoB positivity in older males (55, 58, and 62 years) indicates possible chronic or latent infections, while dual-negative instances were frequent among younger individuals (17, 31, and 32 years).

Menstrual blood samples (n=9) showed rpoB positivity in 2 cases (ages 20 and 30) and IS6110 positivity in 3 cases (ages 31, 35, and 43), requiring additional investigation into pathogen-host interactions in women of reproductive age.

In total, rpoB positivity was found in 29 patients, whereas IS6110 was identified in 22 instances. Men showed a greater prevalence of rpoB positivity, especially in those over 40, aligning with trends in chronic diseases. The comparatively lower detection rate in females, along with significant IS6110 positivity in menstrual blood, highlights the necessity for gender-focused diagnostic approaches.

These results emphasize the significance of multi-gene molecular tests to enhance diagnostic accuracy. The noted discrepancy between rpoB and IS6110 requires additional genomic and epidemiological studies to clarify host-pathogen interactions and enhance focused therapeutic approaches, especially in atypical sample types.

Among the **50 positive cases** (based on either rpoB or IS6110 detection) **MTB Complex cases** were **35 (70%) and NTM cases** were **15 (30%).**

Table I- MTB Complex and NTM Differentiation

Mycobacterial Species	No. of Positive Samples
M. tuberculosis Complex	35
NTM (MOTT)	15
Unidentified	50
Total	100

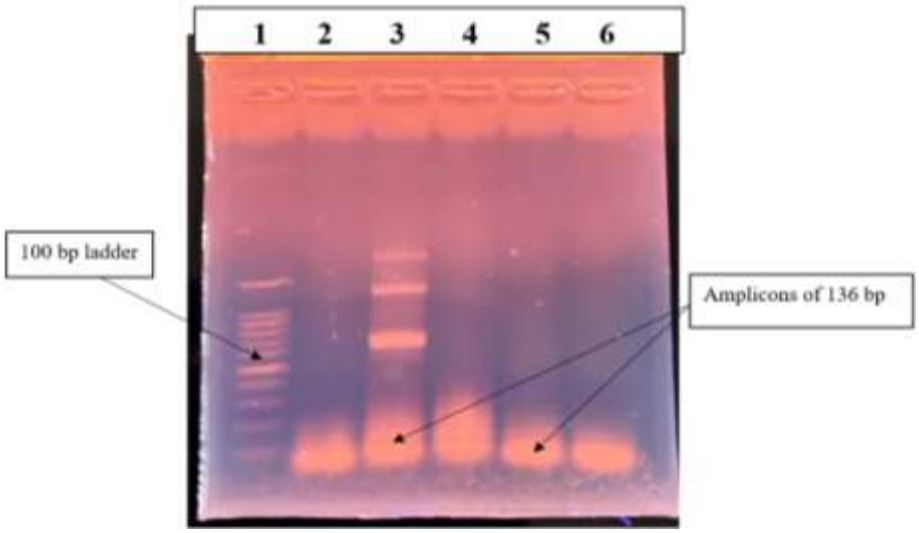


Figure: 1 D-PCR assay results performed with culture isolates of Non-mycobacterium tuberculous. Amplicons of size 136 bp were amplified from Non-mycobacterium tuberculous strain on lanes 3 and 5 by a single D-PCR. Lane: 1, ladder DNA (100-bp ladder).

A **D-PCR assay** was performed on all **15 NTM isolates** confirmed by the NAAT. The **235-bp DNA fragment** was amplified in **all MTB positive samples (35)**, while the **136-bp DNA fragment** was detected in **all 15 NTM isolates**.

Table II - Mycobacterium Isolate Identification by D-PCR

D-PCR (Base Pairs)	No. of Isolates Identified As
M. tuberculosis Complex (235bp)	35
NTM Specific (136bp)	15

This analysis confirms that the **rpoB and IS6110 gene tests with D-PCR assays, effectively differentiated MTB Complex from NTM strains.**

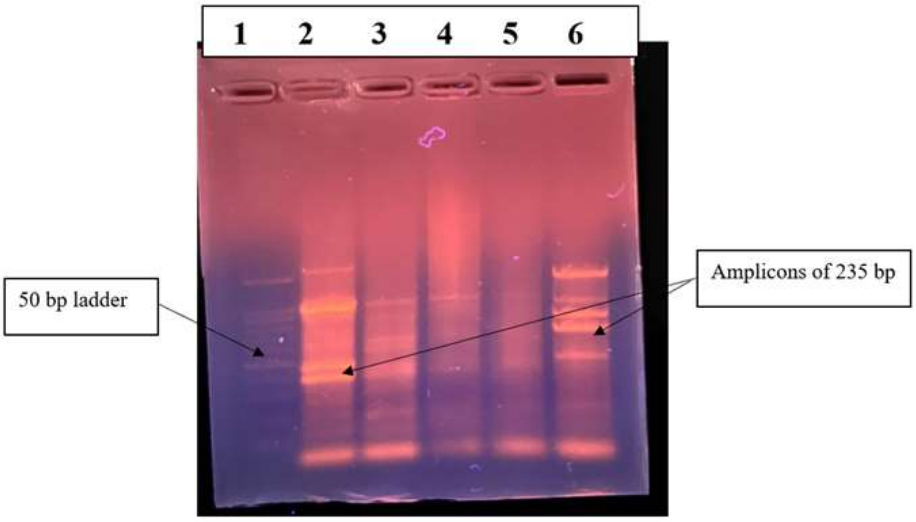
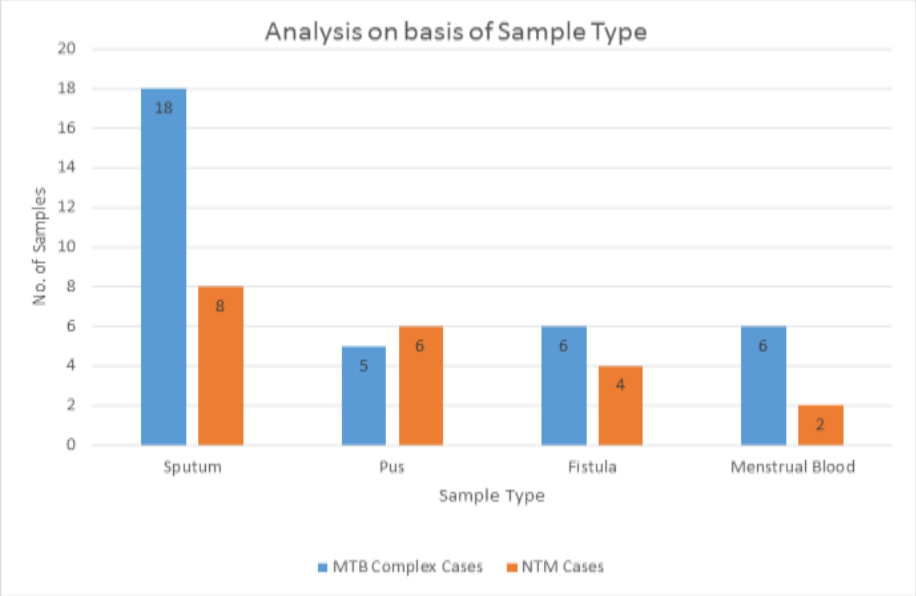


Figure: 2 D-PCR assay results performed with culture isolates of Mycobacterium tuberculosis. Amplicons of size 235 bp were amplified from Mycobacterium tuberculosis stain on lanes 2 and 6 by a single D-PCR. Lane: 1, ladder DNA (50-bp ladder).

Table III – MTB Complex and NTM Differentiation by Sample Type

Sample Type	MTB Complex Cases	NTM Cases
Sputum	18	8
Pus	5	6
Fistula	6	4
Menstrual Blood	6	2
Total	35	15



Graph I – Analysis of the MTB & NTM result on the basis of Sample Type

Our result can be concluded as Sputum is the best reliable sample type for MTB and NTM Cases as is shows the most positivity rate and other samples that include Pus, Fistula and Menstrual Blood samples had lower positivity rate.

4. DISCUSSION

This research shows the effectiveness of dual polymerase chain reaction (D-PCR) in distinguishing the Mycobacterium tuberculosis (MTB) from non-tuberculous mycobacteria (NTM) by employing rpoB and IS6110 markers. D-PCR has demonstrated itself to be a trustworthy and swift diagnostic method, showcasing exceptional specificity and sensitivity, whuch is consistent with earlier research (7, 8).

Sputum samples exhibited the highest positivity rate, validating their effectiveness for diagnosing MTB. Nonetheless, the identification of D-PCR in pus, fistula, and menstrual blood underscores its greater importance beyond traditional sputum examination (6). Quick identification of NTM is essential, as incorrect diagnoses may result in ineffective treatments (3).

In contrast to conventional cultural techniques, D-PCR provides quicker results and enhanced precision. Employing rpoB for the detection of NTM is essential for recognizing drug-resistant strains, facilitating prompt action against multidrug-resistant TB (16, 17). The addition of IS6110 enhances the accuracy in differentiating MTB (18).

Limitations involve the requirement for larger sample sizes and additional verification among various populations. Future research should incorporate next-generation sequencing (NGS) for improved strain characterization and evaluate the cost-effectiveness of D-PCR in low-resource settings (19). In conclusion, D-PCR offers a rapid, precise, and efficient substitute for traditional methods, enhancing early detection and focused treatment approaches for MTB and NTM infections.

5. CONCLUSION

Using IS6110 and rpoB gene markers, this study confirms that dual PCR is a useful method for differentiating Mycobacterium tuberculosis (MTB) from non-tuberculous mycobacteria (NTM). The results show how reliable and specific this method is in detecting MTB and NTM infections, especially in settings with limited resources where prompt diagnosis is essential for successful treatment.

This study, which was carried out on 100 clinical samples from Dehradun, Uttarakhand, shows that Dual PCR is a reliable diagnostic method. The importance of distinguishing between these mycobacterial types is highlighted by the fact that out of the samples under study, MTB was found in 35 cases and 15 were classified as NTM. While the detection of MTB and NTM in pus, fistula, and menstrual blood broadens the diagnostic options beyond conventional sputum analysis, the results show that sputum is still the most reliable sample type.

While the results are encouraging, additional research with a larger and more varied population is required to validate the findings due to the small sample size and constrained geographic scope. The effects of MTB and NTM will be better understood if this study is expanded to include more clinical and environmental samples. Additionally, combining dual PCR and next-generation sequencing (NGS) may improve strain characterization and our comprehension of drug resistance patterns, leading to more successful treatment approaches.

This strategy can significantly enhance TB surveillance, facilitate rapid identification of drug-resistant strains, and reduce the likelihood of misdiagnosing NTM infections by utilising cutting-edge sequencing technologies and molecular diagnostic techniques. These developments will ultimately support public health initiatives, guaranteeing prompt interventions and improved clinical results for individuals afflicted by mycobacterial infections.

6. ACKNOWLEDGEMENT

The experimental setup was carried out at DNA Labs-A Centre for Applied Sciences, East Hope Town, Dehradun, Uttarakhand, India with facility of BSL 2 with practices of BSL 3. Some of the samples were also received from Gautam Buddha Chikitsa Mahavidyalaya, Dehradun, Uttarakhand, India.

7. FUNDING SOURCE

Self-funded project

8. ETHICAL STATEMENT

All experiments and procedures were performed following the ethical guidelines of DNA Labs-A Centre for Applied Sciences, East Hope Town, Dehradun, Uttarakhand, India , and were approved by the laboratory ethical committee for medical research (Laboratory Consent Letter ref. no.: DLCA/2023/LAB-2/CL/203).

9. CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

[1] World Health Organization (WHO). (2022). *Global Tuberculosis Report 2022*. WHO Press.

[2] Daley, C. L., Iaccarino, J. M., Lange, C., Cambau, E., Wallace, R. J., Andrejak, C., ... & Griffith, D. E. (2020).

Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA guideline. *European Respiratory Journal*, *56*(1), 2000535.

[3] Forbes, B. A., Hall, G. S., Miller, M. B., Novak, S. M., Rowlinson, M. C., Salfinger, M., & Somosközi, Á. (2018). Practice guidelines for clinical microbiology laboratories: Mycobacteria. *Clinical Microbiology Reviews*, *31*(2), e00038-17.

[4] van Soolingen, D., Hermans, P. W., de Haas, P. E., Soll, D. R., & van Embden, J. D. (1993). Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *Journal of Clinical Microbiology*, *31*(8), 1961-1965.

[5] Kim, B. J., Math, R. K., Jeon, C. O., Yu, H. K., Park, Y. G., Kook, Y. H., & Kim, B. J. (2013). A PCR-based method for discrimination of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria using rpoB gene-derived primers. *Diagnostic Microbiology and Infectious Disease*, *75*(2), 175-176.

[6] Huang, W. C., Yu, M. C., Chen, H. Y., Hsu, H. L., & Jou, R. (2019). Evaluation of a multiplex real-time PCR for identification of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria. *Journal of Microbiological Methods*, *158*, 1-7.

[7] Singh, N., Jain, S., Goel, A., Bhargava, R., & Verma, R. (2021). Application of dual PCR for rapid identification of mycobacterial infections: An alternative to conventional methods. *Indian Journal of Medical Microbiology*, *39*(1), 72-78.

[8] Ryu, Y. J., Koh, W. J., Daley, C. L., & Kim, S. Y. (2022). Diagnostic advances for pulmonary tuberculosis and nontuberculous mycobacterial lung disease. *Expert Review of Molecular Diagnostics*, *22*(4), 373-389.

[9] Brown-Elliott, B. A., Philley, J. V., & Wallace, R. J. (2019). Current and emerging diagnostic approaches for nontuberculous mycobacterial infections. *Clinical Microbiology Reviews*, *32*(2), e00010-19.

[10] Koh, W. J., Stout, J. E., & Yew, W. W. (2020). Advances in the diagnosis and treatment of pulmonary disease due to *Mycobacterium avium complex*. *Respirology*, *25*(10), 1022-1035.

[11] Parekh, K. M., Inamdar, V., Jog, A., and Kar, A. (2006). A comparative study of the diagnosis of pulmonary tuberculosis using conventional tools and polymerase chain reaction. *Indian Journal of Tuberculosis*, *53*, 69-76.

[12] Tang, Y. W., Meng, S., Li, H., Stratton, C. W., Koyamatsu, T., and Zheng, X. (2004). PCR enhances acid-fast bacillus stain-based rapid detection of *Mycobacterium tuberculosis* . *Journal of Clinical Microbiology*, *42*(4), 1849-1850. https://doi.org/10.1128/JCM.42.4.1849-1850.2004.

[13] Kim, Bum-Joon, Seong-Karp Hong, Keun-Hwa Lee, Yeo-Jun Yun, Eui-Chong Kim, Young-Gil Park, Gil-Han Bai, and Yoon-Hoh Kook. Journal of clinical microbiology 42, no. 3 (2004): 1308-1312.

[14] Zhao, Y, (2019). Molecular detection of non-tuberculous mycobacteria using PCR and sequencing methods. *Diagnostic Microbiology and Infectious Disease*, *95*, 137–142. https://doi.org/10.1016/j.diagmicrobio.2019.03.003

[15] Goletti, D.,(2022). Current and future diagnostic approaches for tuberculosis: An overview of advances and challenges. *Expert Review of Molecular Diagnostics*, *22*, 115–130. https://doi.org/10.1080/14737159.2022.2040468

[16] Boehme, C. C., Nabeta, P., Hillemann, D., Nicol, M. P., Shenai, S., Krapp, F., ... & Perkins, M. D. (2011). Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine*, 363(11), 1005-1015. https://doi.org/10.1056/NEJMoa0907847

[17] Mokrousov, I., Narvskaya, O., Otten, T., Limeschenko, E., Steklova, L., & Vyazovaya, A. (2016). High prevalence of rifampin-resistant M. tuberculosis strains in a TB hospital in St. Petersburg, Russia: a retrospective study. *Journal of Clinical Microbiology*, 40(11), 4015-4018.

[18] Telenti, A., Imboden, P., Marchesi, F., Lowrie, D., Cole, S., Colston, M. J., ... & Bodmer, T. (1993). Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. *The Lancet*, 341(8846), 647-650. https://doi.org/10.1016/0140-6736(93)90444-W

[19] Koh, W. J., Chang, B., Jeong, B. H., Jeon, K., Kim, S. Y., & Kim, C. K. (2020). Clinical significance and treatment outcomes of pulmonary disease caused by Mycobacterium abscessus and Mycobacterium massiliense. *Clinical Infectious Diseases*, 70(5), 754-762. https://doi.org/10.1093/cid/ciz285