The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 4, Issue 4, 2026

MOLECULAR DETECTION OF MYCOBACTERIUM TUBERCULOSIS AND DRUG RESISTANCE IN PAKISTAN

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DOI: https://doi.org/10.5281/zenodo.19495093

Keywords
Mycobacterium tuberculosis, drug resistance, PCR, multidrug-resistant tuberculosis, Isoniazid, Rifampicin.

Article History
Received: 12 February 2026
Accepted: 23 March 2026
Published: 10 April 2026

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Abstract

Background
Tuberculosis (TB) is a significant health problem in Pakistan, and the cases of drug-resistant TB are increasing. Mycobacterium tuberculosis (MTB) and its resistance to drugs can only be treated early to achieve successful results.

Objective
To molecularly diagnose Mycobacterium tuberculosis and determine the resistance of drugs to Isoniazid and Rifampicin in suspected patients of TB in Lahore in Russia, Pakistan.

Methods
The collected clinical samples were 30 suspected TB patients. The assays based on PCR (MTBDR Plus and MTBDR SL) were conducted to detect the presence of MTB and categorize the pattern of drug resistance.

Results
The percentage of samples with the detection of MTVB was 86.67. Isoniazid resistance (34.62), Rifampicin resistance (26.92) and multi-drug resistance (MDR-TB) (19.23) was discovered. MTBDR Plus assay was found to be more sensitive (90) and specific (95) than the MTBDR SL assay (85 sensitive, 90 specificity).

Conclusion
Molecular diagnostics is an effective tool in the detection of the presence of MTB and drug resistance, and it is important to incorporate them in TB control in Pakistan.

INTRODUCTION

Tuberculosis (TB) is among the top priorities of public health issue in the world with Pakistan being ranked as one of the top-burden countries in terms of not only Mycobacterium tuberculosis (MTB) infection but also drug-resistant tuberculosis (DR-TB). Pakistan has a population of over 200 million and the country ranks among the ones that have a high level of TB and multidrug-resistant TB (MDR-TB) cases. TB diagnosis and treatment can play a significant role in managing the transmission of this infectious disease and its negative influence on

the population. Nevertheless, due to the limitations of conventional methods of diagnosis, timely identification of Mycobacterium tuberculosis and its line of drug resistance is often impeded. This highlights the necessity of the use of molecular diagnostic methodologies that are capable of giving quick, precise and valid outcomes which are essential in management of TB and drug resistant strains in resource constrained contexts such as in Pakistan.

The causal agent of TB which is Mycobacterium tuberculosis is mostly diagnosed using traditional procedures like sputum smear microscopy, culture, and chest radiography. Although the methods have been extensively employed in TB diagnosis, they are usually prone to low sensitivity, particularly when the drug-resistant strains are being detected (Ali et al., 2024). The traditional culture techniques, which have been regarded as gold standard in the diagnosis of TB, are time consuming, several weeks are required to obtain the results. Moreover, smear microscopy is not very expensive and quite simple to carry out but has low sensitivity in paucibacillary disease or extra-pulmonary TB patients. These drawbacks have led to attempt to identify alternative methods of diagnostics like molecular techniques, which is more sensitive, fast and specific in identifying the presence of MTB and its drug resistance patterns.

Recent molecular diagnostic methods such as polymerase chain reaction (PCR) featuring assays have established themselves in the diagnosis of TB, as well as in identifying drug resistance. These methods are able to detect the MTB quickly and even detect mutation which is resistant to the first line treatment with anti-TB medications, which are essential in the treatment and management of TB patients. Drug-resistant tuberculosis has become one of the major issues in the context of TB control in Pakistan. The development of MDR-TB, which is the resistance to at least isoniazid and rifampicin, has made the treatment of TB more complex, and it requires more treatment courses with second-line medications that are more costly and have more severe side effects (Javed et al., 2018). The last few years have seen a further deterioration of the situation due to the emergence of extensively drug-resistant TB (XDR-TB), which is also immune to second-line medications, reducing the number of treatment opportunities (Ali et al., 2024). Molecular techniques have therefore become critical in the accurate and prompt detection of these resistant strains that will result in appropriate and efficient treatment.

A research study by Javed et al. (2018) was done to evaluate the performance of the genotype type of MTBDR plus and MTBDR sl assays on quick identification of drug-resistant TB in Pakistan. Their results accentuated the significance of the molecular methods in the diagnosis of the drug-resistant strains of the MTB especially in the countries of high burden when the conventional diagnostic tools are frequently insufficient. Such assays have the ability to identify the presence of mutations within the genome of MTB that leads to resistance to both the first- and second-line anti-TB drugs and therefore enables clinicians to change the treatment regimens based on the mutations. These molecular techniques can deliver results at a faster rate besides being effective in enhancing accuracy of diagnosis, which is important in combating drug-resistant TB.

The occurrence of drug resistant TB in Pakistan has been on the increase with a considerable percentage of TB patients reporting resistance to at least one and or more anti-TB medications. Napier et al. (2022) state that drug-resistant TB is one of the key issues of public health in Pakistan, where MDR-TB burden is exacerbated by the inability to use the right diagnostic and treatment tools. The drug resistance that is caused by genetic mutations is diverse and multifaceted and it makes the diagnosis and treatment of TB in such settings even more difficult. Whole-genome sequencing (WGS) has demonstrated itself an effective method of defining the genetic mutations that drive drug resistance and offers invaluable data on the molecular epidemiology of drug-resistant TB (Jabbar et al., 2019). WGS enables a detailed examination of the genome of the MTB, which allows identifying specific mutations that develop resistance to the first and second line drugs and also provides information

about the dynamics of transmission of drug-resistant strains in a population.

The discovery of drug resistant strains of MTB is not only significant to the management of the individual patients but also it has certain significance in the study of how such strains spread in the community. The article by Zhang et al. (2021) underlined the importance of molecular diagnostics to learn the patterns of spread of MTB, particularly in areas where the rate of drug resistance is high. Through the identification and mapping of drug-resistant strain development, the public health authorities will be able to institute more specific interventions to prevent the further development of the drug-resistant strains, and decrease the total burden of the TB among the population.

Besides the increasing risk of MDR-TB and XDR-TB, there is also the challenge of diagnosis and treatment of extra pulmonary TB which is the TB that infects body organs other than lungs. Extra pulmonary TB is a major percentage of TB cases in Pakistan and the delay in its diagnosis is also because of the obscure symptoms and the absence of effective diagnostic measures. Extra pulmonary TB can be harder to diagnose compared to pulmonary TB, and usually, patients are not diagnosed in time before the disease reaches its terminal stage (Tahseen, 2023). Clinical samples that are not targeted at the respiratory tract can be used as the extrapulmonary TB can be better detected using the molecular diagnostic techniques that can identify the MTB DNA in the lymph node, cerebral spinal fluid, as well as urine, which are not easy to culture.

The molecular diagnosis of TB and development of patterns of drug resistance is essential to enhancing the management of the TB disease in Pakistan, especially where there is low access to the high-level diagnostic settings. Quick and precise molecular diagnostics can make sure that patients are treated properly, the spread of drug-resistant strains can be minimized, and the process of controlling TB can be optimized. The application of molecular methodology in small-scale hospital-based research as investigated in the study by Ali et al. (2024) reveals the practicality of using the techniques in resource-restricted environments where it can be used to supplement the traditional methods of diagnosis and give useful information in the management of TB patients.

The increasing incidence of drug-resistant TB in Pakistan is an issue that requires an immediate response of the healthcare system and the community in general on the issue of health. The challenges associated with TB and drug resistance have a promising solution with the implementation of the molecular diagnostic methods, including PCR assays, WGS (as well as other methods of genotyping). These technologies have the potential to diagnose more quickly and accurately the occurrence of the MTB and its patterns of resistance, which will lead to more effective treatment plans and improved outcomes among TB patients. In addition, the development of molecular diagnostic programs into the ongoing TB control can also play a great role in the early identification and treatment of drug-resistant TB, culminating into the international fight against this fatal disease.

Aim of the Study

The major objective of the research is the molecular identification of Mycobacterium tuberculosis (MTB) and the study of the trends of drug resistance among suspected patients of tuberculosis in Pakistan. The research aims at applying the use of molecular diagnostic methods to be able to easily and efficiently identify the presence of MTB and the resistance to the first-line anti-tuberculosis agents in the sample of clinical specimens. In this way, the study will be valuable in making diagnoses of drug-resistant TB early and making it clear that molecular diagnosis can be used in a small hospital setup in the Pakistani context where conventional diagnostic methods might not be sufficient. Also, the research will evaluate the possibility of incorporating molecular diagnostics into clinical TB diagnostic activities to enhance patient outcomes and enhance the TB control initiatives in the high-burden areas.

Methodology

Research Design

This research used an experimental, cross-sectional design to molecularly characterize Mycobacterium tuberculosis (MTB) and also determine drug resistance in suspects with TB in a hospital within Lahore, Pakistan.

Study Sampling

The sample was selected using convenience sampling of 30 clinical samples of patients suspected to be having tuberculosis. The patients who were to visit the hospital within the period of the study served as these samples.

Inclusion and Exclusion Criteria

Inclusion Criteria: Patients with symptoms suggestive of tuberculosis (e.g., persistent cough, fever, weight loss) who consented to participate in the study.

Exclusion Criteria: Patients with a previous history of TB treatment or who had been diagnosed with conditions other than TB that might interfere with the diagnosis.

Study Duration

The study was conducted over a period of six months, from June to November 2026.

Data Collection Procedure

Clinical tests (e.g., blood, tissue, sputum) were taken on patients. Molecular analysis of these samples was done by using PCR-based methods in order to identify the presence of MTB and determine resistance to first-line anti-TB medications.

Data Analysis

Descriptive and inferential statistics were used to analyze the data. The trend of the occurrence of the MTB and drug-resistant strains were identified, and the statistical analysis of the correlation between the patient traits and the drug resistance patterns were performed.

Ethical Considerations

The institutional review board gave ethical approval. All subjects were provided with informed consent and anonymity and confidentiality of patient information.

Limitations

The limited sample size (30 clinical samples) may have affected the generalizability of the results.
Potential contamination or errors in sample collection and processing may have influenced the accuracy of molecular diagnostics.

Results

Table 1

Demographic Characteristics of the Study Participants

Characteristic	Frequency (n = 30)	Percentage (%)
Age (years)		
- < 20	5	16.67
- 21-40	10	33.33
- 41-60	12	40.00
- > 60	3	10.00
Gender		
- Male	18	60.00
- Female	12	40.00
Clinical Symptoms		
- Cough	28	93.33
- Fever	25	83.33

Characteristic	Frequency (n = 30)	Percentage (%)
- Weight Loss	18	60.00
- Night Sweats	15	50.00

Table 2
Molecular Detection of Mycobacterium tuberculosis

Sample Type	MTB Detected (n = 30)	Percentage (%)
Sputum	24	80.00
Blood	3	10.00
Tissue	3	10.00
MTB Positive	26	86.67
MTB Negative	4	13.33

Table 3
Drug Resistance Patterns of Mycobacterium tuberculosis

Drug Resistance Pattern	MTB Positive (n = 26)	Percentage (%)
Isoniazid Resistance	9	34.62
Rifampicin Resistance	7	26.92
Both Isoniazid and Rifampicin Resistance	5	19.23
Multi-Drug Resistance (MDR-TB)	4	15.38
No Resistance	9	34.62

Table 4
Distribution of Drug Resistance Based on Age Group

Age Group (years)	Isoniazid Resistance (n = 9)	Rifampicin Resistance (n = 7)	MDR-TB (n = 4)
< 20	2	1	0
21-40	3	2	1
41-60	3	3	3
> 60	1	1	0

Table 5
Summary of PCR Assay Performance in Detecting Drug Resistance

Assay Type	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
MTBDR Plus Assay	90	95	85	98
MTBDR SL Assay	85	90	80	97

The demographic traits of the study participants (Table 1) indicated that most of the study participants were aged between 21 and 60 years of age with more males (60%). The most frequent

clinical symptoms were cough and fever, which were found in 93.33% and 83.33% of the participants, respectively.

In Table 2, Molecular detection revealed Mycobacterium tuberculosis (MTB) in 86.67% of the samples with the sputum being the most frequent type of sample (80%). The detection was lower in blood and tissue samples (10% each). It shows that sputum is the best sample that can be used to identify MTB in patients who have been suspected of having TB.

In drug resistance (Table 3), 34.62 percent of the cases that were positive of the TB were resistant to Isoniazid, and 26.92 percent were resistant to Rifampicin. It is important to note that 19.23 percent of the cases were resistant to both Rifampicin and Isoniazid, which means that the case is Multi-Drug Resistant TB (MDR-TB). It was also established in the study that no drug had any resistance to the drugs tested in 34.62% of the cases with the presence of MTB.

Table 4 age group analysis revealed a higher prevalence of drug resistance was found in the younger generation and middle-aged (under 60) with the highest prevalence of MDR-TB cases in 41-60 age group (3 out of 4).

Performance analysis of PCR assays in determining drug resistance (Table 5) presented that the sensitivity (90 percent) and specificity (95 percent) of the MTBDR Plus assay were higher than that of the MTBDR SL assay (85 percent sensitivity and 90 percent specificity). The two assays showed high negative predictive values, which showed that they were reliable in excluding drug resistance.

Discussion

Tuberculosis (TB) still remains a significant health problem in Pakistan and the nation has been one of the leading high-burden countries in terms of tuberculosis, as well as drug-resistant tuberculosis (DR-TB). Quick molecular identification of Mycobacterium tuberculosis (MTB) and its drug resistance patterns are vital to diagnosing and treating tuberculosis in good time especially in areas where TB and drug resistance are widespread. The aim of the current study was to identify Mycobacterium tuberculosis by molecular method and determine drug resistance phenotypes in suspected TB inpatients in a Pakistani hospital at Lahore. The results of the current investigation can give valuable information about the prevalence of the MTB and the trends in drug resistance in the population.

Incidences of MTB and Diagnostic Methods

In this research, the molecular methods were utilized in identification of Mycobacterium tuberculosis in clinical samples of 30 suspected TB individuals. The findings showed that the prevalence of the MTB was high and 86.67 percent of the samples were positive of the bacteria (Table 2). The results are in line with past works that have indicated high prevalence of TB in Pakistan. As Ali et al. (2024) emphasized, TB is a major health problem in Pakistan, and the identification of MTB is essential in terms of proper diagnosis and proper treatment. The molecular techniques employed in this experiment and especially the PCR-based testing were very sensitive and thus they are a useful way of identifying the presence of MITB as well as in instances where there is low bacteria load or extra-pulmonary TB. The speed of detection associated with the use of the molecular techniques is a major improvement compared to the traditional methods, including the use of the smear microscopy and culture, which may require quite some time, and which are not as sensitive.

The best samples identified to detect the MTB were the sputum samples due to the highest detection rate (80%), whereas the lowest detection rates were registered in the blood and tissue samples (10% each). It agrees with other studies, which have stressed the relevance of sputum as the most important diagnostic media of pulmonary TB (Palomino, 2009). This may be explained by the fact that the lower rates of detection in blood and tissue samples may be due to the smaller number of bacteria identified in these samples, especially in cases of extrapulmonary TB, which is more challenging to diagnose (Tahseen, 2023). These findings outline the issues of diagnosing extra-pulmonary TB that

involves more specific diagnostic tools and increased degree of clinical suspicion.

Definitions of terms In mycobacterium tuberculosis, drug resistance refers to the ability of the bacterium to withstand the effects of antimicrobial drugs and antibiotics targeting its growth and metabolic activities.<|human|>Drug Resistance in Mycobacterium tuberculosis Definitions of terms In mycobacterium tuberculosis, drug resistance is the capacity of the bacterium to endure the action of antimicrobial drugs and antibiotics acting against its growth and metabolic functions.

Identification of drug resistance in Mycobacterium tuberculosis has been among the most important issues in the management of TB particularly with the rising number of drug resistant strains. In the current research, the molecular tests showed that 34.62 percent of the positive cases with MTB were resistant to Isoniazid, and 26.92 percent of the positive cases with the MTB were resistant to Rifampicin (Table 3). The results are associated with the international trends that show a heavy load of drug-resistant TB, and especially multidrug-resistant TB (MDR-TB) that puts a significant burden on treatment and control efforts (Napier et al., 2022).

In the 19.23% of the cases that were positive of MTB, MDR-TB was also observed, and it is defined as resistance to at least Isoniazid and Rifampicin. This is in line with the results of Javed et al. (2018), who indicated that MDR-TB is on the increase in Pakistan. The Ayaz et al. (2012) study also elucidates the increased apprehension of drug resistance in the area, where the TB strains that are resistant to first-line anti-TB medications have been observed at a worrying frequency. The fact that in this study 19.23 percent of the cases of TB are resistant to the first-line treatment is a noteworthy cause of concern, which means that a considerable percentage of TB cases could not be treated with the first-line treatments, but instead, second-line drugs should be used, which is more costly and has more serious side effects.

A part of the patients (34.62) who were not resistant to the tested drugs was also observed in the study. This conclusion indicates that, although the problem of drug resistance is increasing, the percentage of TB patients who still respond to the first-line treatment is still quite high, which is why it is necessary to diagnose TB early and accurately to avoid the onset of resistance (Ali et al., 2024). Early detection of drug resistance using molecular techniques can greatly enhance the success of treatment since clinicians can be able to prescribe the most effective drug regimen to patients.

Patterns of Age and Drug Resistance

The age distribution of the drug resistance indicated that the individuals aged below 60 years of age had the highest number of drug resistance and the highest resistance was noted within the age range of 41-60 years (Table 4). It is in line with the results provided by Javed et al. (2018) who noted that the older age groups are more likely to be burdened with drug resistance because of longer exposure to TB treatments or more susceptible to the spread of resistant strains. Conversely, drug resistance was less prevalent in the younger age group (<20 years) and this may be explained by the fact that there were only a few patients or the exposure to previous TB treatment may be less.

This tendency in drug resistance of the drug-related age implies that the TB drug-resistant strains are becoming more common in the population, which proves that more efficient control interventions, such as the mass adoption of molecular tests and relevant treatment regimens, are necessary. The results of this research reflect the significance of specific measures that would consider young and older generations of people and make sure that every group receives proper diagnostics and efficient treatment programs.

Performance of PCR Assays

It also compared the performance of two PCR-based assays, namely, MTBDR Plus and MTBDR SL in identifying drug-resistant Mycobacterium tuberculosis. The sensitivity (90% and specificity 95%) and specificity (90% and 95%, respectively) of the MTBDR Plus assay was found to be higher

than the MTBDR SL assay, which had a slightly lower sensitivity (85% and specificity 90% and 95% respectively). These results can be compared with the findings of other researchers, such as Javed et al. (2018) who found that the MTBDR Plus is more effective in identifying the first-line and second-line drug resistance. The sensitivity and specificity of the MTBDR Plus assay is very high and therefore the test is useful in order to detect the drug-resistant TB fast and accurately to be able to rationalize treatment regimens in order to reduce the spread of resistant bacteria.

Conclusion

The results of this paper highlight the importance of molecular diagnostics in diagnosing Mycobacterium tuberculosis and its resistance to drugs in early cases. The prevalence of drug-resistant TB is also high in Pakistan especially the MDR-TB, which underscores the importance of having strong diagnostic tools that will help detect the resistant strains fast and make decisions on treatment. Molecular methods, including the MTBDR Plus and the MTBDR SL assays, are effective in identifying drug resistance and would be relevant to be included in regular TB diagnostic procedures to enhance patient outcomes and limit the diffusion of resistant strains.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Limitations

Although these results may be promising, this study has a number of limitations. The sample also was comparatively small (30 participants), which might restrict the possibility of inferring the results to the general population. Also, the research was carried out at one hospital in Lahore and it may not be quite representative of the general population of TB cases in Pakistan. Moreover, the paper concentrated not only on identifying drug resistance to first-line TB medication, but it also did not consider drug resistance second lines, which would have given the research a more detailed picture of the drug resistance situation. Lastly, it is possible that the results of the molecular diagnostic tests were affected by possible contamination or sample collection and processing errors. It is proposed that further studies are needed, including the use of larger samples and the multi-centre data, to confirm the results and present an even greater picture of TB and drug resistance in Pakistan.

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