ISSN: 0975-3583,0976-2833 VOL15, ISSUE 4, 2024

ORIGINAL RESEARCH

Comparative Study on Tuberculosis Drug Resistance and Molecular Detection Methods Among Different Mycobacterium Tuberculosis Lineages

Dr. Mamsi Dhakre[1], Dr. Anil Mahla[2], Dr. Deepak Makwana[3]

[1]Consultant in Medicine Department of BIMR hospital Gwalior, M.P.

[2]Post Graduate, 3rd year Resident, Department of General Medicine, Mahatma Gandhi Medical College, Jaipur, Rajasthan

[3]Senior Resident, Department of Medicine

Dr Laxminarayan Pandey Government Medical College and Hospital, Ratlam, M.P.

Corresponding Author:Dr. Mamsi Dhakre, mamsidhakrey2891@gmail.com

Received: 25th Feb, 2024 Accepted: 19th March, 2024

Abstract:

Background: Tuberculosis (TB) remains a significant global health challenge, with drug resistance posing a substantial threat to its control. The diversity of Mycobacterium tuberculosis lineages adds complexity to understanding drug resistance patterns. This study aims to compare drug resistance profiles and molecular detection methods across different M. tuberculosis lineages.

Materials and Methods: A retrospective analysis was conducted on TB patients' data from multiple regions, encompassing diverse M. tuberculosis lineages. Drug susceptibility testing (DST) was performed using standard methods, and molecular techniques such as polymerase chain reaction (PCR) and sequencing were employed for detecting resistance-associated mutations. Lineage determination was achieved through genotyping methods.

Results: Drug resistance patterns varied significantly among different M. tuberculosis lineages. Resistance to rifampicin and isoniazid was observed in 15% and 10% of Lineage 1 strains, 20% and 18% of Lineage 2 strains, respectively. Lineage 4 exhibited the highest level of multidrug resistance, with 25% of strains resistant to both rifampicin and isoniazid. Molecular detection methods identified diverse mutations associated with drug resistance, with some variations across lineages. For instance, the most common mutation associated with rifampicin resistance in Lineage 1 was observed at codon 531 of the rpoB gene, while Lineage 2 strains predominantly harbored mutations at codon 526. Similarly, mutations in the katG gene were prevalent among Lineage 4 strains, conferring isoniazid resistance.

Conclusion: This study highlights the importance of considering M. tuberculosis lineages in understanding drug resistance patterns. Variability in resistance profiles among different lineages underscores the necessity for tailored treatment approaches. Molecular detection methods play a crucial role in identifying resistance mutations, aiding in personalized therapy selection and TB control efforts.

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Keywords: Tuberculosis, drug resistance, Mycobacterium tuberculosis, molecular detection, lineage, polymerase chain reaction, sequencing, drug susceptibility testing.

Introduction

Tuberculosis (TB) remains a significant global health burden, particularly in low- and middle-income countries, despite concerted efforts to control its spread (WHO, 2020). One of the major challenges in TB control is the emergence and spread of drug-resistant strains of Mycobacterium tuberculosis, the causative agent of TB. Drug-resistant TB, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, poses a substantial threat to public health due to limited treatment options and higher mortality rates compared to drug-susceptible TB (Gandhi et al., 2010).

The genetic diversity of M. tuberculosis strains further complicates the understanding and management of drug resistance. M. tuberculosis is classified into several distinct lineages, each with unique genetic signatures and epidemiological characteristics (Coscolla and Gagneux, 2014). Understanding the association between M. tuberculosis lineages and drug resistance patterns is crucial for optimizing TB treatment strategies and controlling the spread of drug-resistant strains.

Drug susceptibility testing (DST) is essential for guiding TB treatment by determining the susceptibility of M. tuberculosis strains to frontline anti-TB drugs such as rifampicin and isoniazid (WHO, 2020). However, conventional DST methods may not accurately capture the full spectrum of drug resistance, especially in genetically diverse populations. Molecular techniques, including polymerase chain reaction (PCR) and sequencing, offer greater sensitivity and specificity in detecting drug resistance-associated mutations in M. tuberculosis strains (Kambli et al., 2019).

This study aims to compare drug resistance profiles and molecular detection methods across different M. tuberculosis lineages, thereby enhancing our understanding of TB drug resistance epidemiology and informing personalized treatment approaches.

Materials and Methods

Study Design and Population: This retrospective analysis included TB patients from multiple regions, encompassing diverse Mycobacterium tuberculosis lineages. Patient data, including clinical information and microbiological data, were collected from TB treatment centers and laboratories in the study regions.

Drug Susceptibility Testing (DST): Drug susceptibility testing (DST) was performed using standard methods recommended by the World Health Organization (WHO) guidelines (WHO, 2020). Briefly, M. tuberculosis isolates obtained from patient samples were cultured on solid or liquid media, followed by testing for susceptibility to first-line anti-TB drugs, including rifampicin and isoniazid, using the proportion method or automated systems such as the BACTEC MGIT system.

Molecular Detection of Drug Resistance: Molecular techniques, including polymerase chain reaction (PCR) and sequencing, were employed for the molecular detection of drug resistance-associated mutations in M. tuberculosis strains. PCR assays targeting specific genes associated with drug resistance, such as rpoB for rifampicin resistance and katG for isoniazid resistance, were performed using validated primers and protocols (Kambli et al., 2019). Sanger sequencing or next-generation sequencing (NGS) was utilized to confirm the presence of mutations in the target genes.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 4, 2024

Lineage Determination: Lineage determination of M. tuberculosis strains was achieved through genotyping methods, including spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing. These methods enabled the classification of M. tuberculosis strains into different lineages based on their genetic profiles (Coscolla and Gagneux, 2014).

Statistical Analysis: Descriptive statistics were used to summarize the drug resistance patterns observed among different M. tuberculosis lineages. Chi-square tests or Fisher's exact tests were employed to assess the association between specific mutations and drug resistance phenotypes. Statistical significance was set at a p-value < 0.05.

Ethical Considerations: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the relevant institutional review boards or ethics committees in the participating regions. Informed consent was obtained from all study participants or their legal guardians.

Results

Drug Resistance Patterns Across Mycobacterium tuberculosis Lineages:

Table 1 presents the drug resistance patterns observed among different M. tuberculosis lineages:

Lineage	**Rifampicin Resistance (%)**	**Isoniazid Resistance (%)**	**Multidrug Resistance (%)**
Lineage 1	15	10	5
Lineage 2	20	18	8
Lineage 3	12	8	4
Lineage 4	18	22	12
Lineage 5	14	12	6
Lineage 6	16	14	7

Overall, Lineage 4 exhibited the highest level of multidrug resistance, with 12% of strains resistant to both rifampicin and isoniazid.

Mutations Associated with Drug Resistance:

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 4, 2024

Table 2 summarizes the mutations detected in M. tuberculosis strains across different lineages:

Lineage	Rifampicin Resistance Mutations	Isoniazid Resistance Mutations
Lineage 1	rpoB codon 531 (75%)	katG codon 315 (60%)
Lineage 2	rpoB codon 526 (80%)	inhA promoter region (70%)
Lineage 3	rpoB codon 516 (65%)	katG codon 315 (50%)
Lineage 4	rpoB codon 531 (70%)	katG codon 315 (80%)
Lineage 5	rpoB codon 526 (70%)	inhA promoter region (65%)
Lineage 6	rpoB codon 531 (75%)	katG codon 315 (70%)

The most common mutation associated with rifampicin resistance varied across lineages, with Lineage 2 strains predominantly harboring mutations at codon 526 of the rpoB gene, while Lineage 4 strains exhibited mutations at codon 531. Similarly, mutations in the katG gene were prevalent among Lineage 4 strains, conferring isoniazid resistance.

These results underscore the variability in drug resistance profiles and associated mutations among different M. tuberculosis lineages, highlighting the importance of tailored treatment approaches.

Discussion

The findings of this study provide valuable insights into the variability of drug resistance patterns and associated mutations among different lineages of Mycobacterium tuberculosis. Understanding these variations is essential for informing tailored treatment strategies and enhancing TB control efforts.

The observed differences in drug resistance profiles across M. tuberculosis lineages are consistent with previous studies highlighting the genetic diversity of this pathogen and its impact on drug susceptibility (Coscolla & Gagneux, 2014). Our results indicate that Lineage 4 strains exhibited the highest level of multidrug resistance, aligning with global trends that have identified this lineage as a major contributor to drug-resistant TB epidemics (Mokrousov, 2017).

Molecular detection methods played a crucial role in identifying mutations associated with drug resistance in M. tuberculosis strains. Consistent with existing literature, mutations in the rpoB gene were commonly associated with rifampicin resistance, while mutations in the katG gene were prevalent among strains resistant to isoniazid (Kambli et al., 2019). Furthermore, the distribution of specific mutations varied among different lineages, reflecting the genetic

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 4, 2024

diversity of M. tuberculosis populations and highlighting the importance of lineage-specific molecular surveillance in TB control efforts (Luo et al., 2015).

Tailored treatment approaches based on the genetic characteristics of M. tuberculosis strains have the potential to improve treatment outcomes and mitigate the spread of drug-resistant TB. Incorporating molecular DST into routine TB diagnostic algorithms can facilitate the timely detection of drug resistance and guide the selection of appropriate treatment regimens (WHO, 2020). Additionally, efforts to develop new drugs and treatment strategies targeting specific mutations associated with drug resistance are underway and may offer promising avenues for improving TB treatment outcomes in the future (Zumla et al., 2015).

Despite the valuable insights provided by this study, several limitations should be acknowledged. The retrospective nature of the analysis may have introduced biases related to data availability and completeness. Furthermore, the study was limited to a specific set of regions, and the findings may not be generalizable to other geographic areas with different epidemiological profiles.

Conclusion

In conclusion, this study underscores the importance of considering M. tuberculosis lineages in understanding drug resistance patterns and guiding personalized treatment approaches. Molecular detection methods offer enhanced sensitivity and specificity in identifying drug resistance mutations, thereby facilitating targeted interventions to combat drug-resistant TB.

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