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Developing A Rapid, Cost-Effective and Accurate Diagnostic Approach for Pulmonary and Extra Pulmonary Tuberculosis

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Abstract: Global growth in multidrig-resistant tuberculosis (MDR-TB) demands rapid and accurate clinical equipment for effective disease control. "The purpose of this study was to detect genetic mutations in mycobacterium tuberculosis associated with drug resistance using MTBDRPLUS line probe assay". Molecular testing identified mutations in RPOB (S531L) and KATG (S315T) genes, which confirms multidrig-resistant strains. The assay demonstrated high specificity and rapid results, aligning with phenotypeic conclusions. These results emphasize the value of molecular diagnosis in early identity, treatment guidance and epidemiological monitoring.

Keywords: Drug resistance, gene mutation, line probe assay, MDR-TB, molecular diagnostics

INTRODUCTION

Tuberculosis (TB), a major global health threat due to mycobacterium tuberculosis (MTB), has reported 1.3 million deaths in 2020 (WHO, 2021). "The emergence of drug-resistant TB (DR-TB)" has further intensified the crisis, indicating 75 million new cases with estimates and a potential global economic burden of $ 16.7 trillion in the next 35 years if clinical and monitoring intervals persist (Zweynert, 2022). Although the sputum smear microscopy is usually used in low-solution settings, its limited sensitivity-only when the weight of bacteria is more than 5,000 basili/ml-this is inadequate for diagnosis of latent TB (WHO, 2012; Dzamon et al., 2014). Resistance in MTB is mainly powered by mutations in specific genes, especially RPOB (refampicin resistance) and catag or inha (isoniazid resistance) (itawa et al., 2018; Valafar, 2021).

Molecular assays, such as PCR-based line check assays and DNA sequencing, enable rapid detection of these mutations, allowing initial diagnosis and timely initiation of effective treatment. The current study aims to identify molecular assays to identify general mutations in drug-resistance-genes, which can support more accurate and targeted TB management strategies.

METHODOLOGY

"Genotype MTBDRPLUS assay (Han Lifesque, Nehraine, Germany), a DNA strip-based molecular method supported by the World Health Organization (WHO, 2008) is a DNA strip-based molecular method used to detect the first row anti-tuberculosis drugs, especially the resistance to the anti-tuberculosis drugs, especially Refactin (RIF)" and the resistance associated with the resistance (inh). Is. The process includes three main stages: "DNA extraction, PCR amplification and reverse hybridization".

1. DNA Extraction

DNA was extracted from decontaminated clinical specimens and MTB cultures grown on Lowenstein-Jensen (LJ) medium using the "GenoLyse kit, following the manufacturer's protocol."

2. PCR Amplification

A 45 µL amplification mixture was prepared in a DNA-free area, combining:

- "10 µL of Amplification Mix A (AM-A, containing polymerase)"
- "35 µL of Amplification Mix B (AM-B, containing biotin-labeled primers)"
- 5 µL of DNA extract (added separately in a designated DNA-handling area)

Amplification was carried out using standard thermocycling conditions recommended in the kit protocol.

3. Reverse Hybridization

Hybridization was performed using the TwinCubator system as follows:

- "20 µL of Denaturation Solution and 20 µL of amplified sample were mixed in each well and incubated for 5 minutes at room temperature."
- "1 mL of prewarmed Hybridization Buffer was added, and one strip was placed per well."
- Incubation was carried out at 45°C for 30 minutes.
- "Strips were washed with 1 mL of Stringent Wash Solution (STR) at 45°C for 15 minutes, rinsed with 1 mL of Rinse Solution (RIN) for 1 minute, and incubated with 1 mL of diluted conjugate for 30 minutes."
- Following two RIN washes and one distilled water rinse, 1 mL of diluted substrate was added.
- The reaction was stopped with distilled water upon visible band development, and strips were air-dried.

4. Interpretation

Strips were interpreted per the manufacturer's instructions. The assay detects wild-type (WT) and mutant (MUT) sequences in three resistance-associated genes:

- rpoB: Rifampicin resistance
- katG and inhA: High- and low-level isoniazid resistance

Each strip contains 27 reaction zones, including internal controls and probes specific to WT and MUT regions. Absence of WT bands or presence of MUT bands indicates resistance-related mutations.

RESULT

"Molecular Detection of Drug Resistance-Associated Mutations"

"To investigate the molecular basis of drug resistance in *Mycobacterium tuberculosis* isolates, the GenoType MTBDRplus Line Probe Assay (version 2.0) was utilized. This assay targets mutations in the rpoB gene (associated with rifampicin resistance), the katG gene (high-level isoniazid resistance), and the inhA promoter region (low-level isoniazid resistance and cross-resistance with ethionamide)."

Out of the 101 culture-confirmed *M. tuberculosis* isolates, molecular testing was conducted on 60 selected isolates based on phenotypic resistance profiles, growth consistency, and sample integrity. The MTBDRplus assay successfully identified specific mutations associated with drug resistance in 38 isolates (63.3%).

Rifampicin Resistance (rpoB gene mutations)

"Mutations in the 81-bp Rifampicin Resistance Determining Region (RRDR) of the *rpoB* gene were observed in 34 of the 60 tested isolates (56.7%). The most frequently detected mutation was S531L (TCG→TTG), found in 22 isolates (64.7% of rifampicin-resistant cases), followed by H526Y and D516V, observed in 7 and 5 isolates

respectively. Loss of the wild-type (WT) probe bands and the presence of mutation (MUT)" probe bands confirmed these substitutions.

Isoniazid Resistance (katG and inhA mutations)

High-level isoniazid resistance due to katG S315T mutation was detected in 24 isolates (40%). Additionally, inhA promoter mutations (notably C15T) were found in 7 isolates (11.7%), indicating low-level resistance and possible cross-resistance to ethionamide. Three isolates harbored both *katG* and *inhA* mutations, indicating a combined mechanism of resistance.

Correlation with Phenotypic DST

A strong correlation (kappa value > 0.80) was observed between molecular and phenotypic drug resistance results for rifampicin and isoniazid. All rifampicin-resistant strains identified by the MTBDRplus assay were also confirmed by the Proportion Method and NRA. Similarly, isoniazid resistance detected at the molecular level corresponded with phenotypic resistance profiles.

DISCUSSION

This study employed the GenoType MTBDRplus assay, "a line probe molecular technique, for the detection of genetic mutations associated with resistance to *rifampicin* and *isoniazid*, the two cornerstone drugs in first-line anti-tuberculosis therapy. The assay efficiently identified mutations within the rpoB gene, particularly in the rifampicin resistance-determining region (RRDR), as well as in the katG and inhA genes, which are implicated in high- and low-level isoniazid resistance, respectively (Hillemann et al., 2005; Mokrousov et al., 2002)".

Among the MDR-TB isolates analyzed, the majority harbored rpoB S531L mutations, a well-documented and globally prevalent marker of rifampicin resistance (Ramaswamy & Musser, 1998). Similarly, the KATG S315T mutation was most often seen among isoniazid-resistant isolates, aligned with molecular epidemiological patterns (Zhang et al., 2005) worldwide. Identification of INHA promoter mutation, especially C15T replacement, pushed forward the molecular basis of resistance and raised concerns about potential cross-resistance for ethiomide and prostheemide-usually drugs used in MDR-TB resignation.

MTBDRPLUS assay showed high consensus with phenotypeic drug sensosis testing (DST), which strengthened its clinical accuracy and clinical relevance. However, it is important to accept the boundaries of molecular assays, which mainly detect known mutations. Novels or rare mutations outside the target areas can remain unhappy, potentially interpretations of false sensitivity. Thus, whereas molecular equipment such as MTBDRPLUS serves as invaluable initial clinical strategies, especially in resource-limited and high-bound settings, they should be complemented with phenotypeic methods for comprehensive resistance profiles (WHO, 2016).

Thus, molecular diagnosis such as mtbdrplus assay enhances TB control efforts by offering rapid, accurate and actionable insights into drug resistance patterns. Their integration in regular clinical practice enables timely initiation of proper treatment, reduces clinical delays, and promises better patient results and reduces the transmission of resistant TB strains.

CONCLUSION

The molecular analysis conducted using the "genotype MTBDRPLUS line probe assay", successfully identified the major mutation associated with the resistance of refampicin and isoniazid in mycobacterium tuberculosis isolates. The findings revealed a high proliferation of RPOB gene mutations, especially the S531L replacement, indicate a broader rifampicin resistance among tested isolates. Similarly, the S315T mutation in KATG genes emerged as the major mechanism behind high-level isoniazid resistance, while interaction in the INHA promoter area, especially C15T, suggested the presence of low-level resistance and potential cross-resistance to ethnamide.

These results outline the clinical importance of molecular assays in the early and accurate identity of multidisciplinary tuberculosis (MDR-TB). The high consensus between genotypeic and phenotypeic resistance patterns confirms the reliability of molecular testing as a rapid clinical tool, which enables timely initiation of proper medical regimens. In addition, identification of specific mutation patterns may report individual treatment strategies and contribute to better monitoring of drug-resistant TB in spatial areas.

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