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A Comparative Analysis Of Phenotypic, Genotypic And Non-Commercial Assays In Drug Susceptibility Testing Of Mycobacterium Tuberculosis

Anupurba Roy Chowdhury[1], Dr. Jitendra Malviya (Professor)[2]

Department of Microbiology

[1,2]Sunrise University, Alwar, Rajasthan

ABSTRACT

The increase in drug resistant TB demands accurate and timely clinical approach. This study compared the first and second power anti-TB drugs to detect resistance to phenotypic, genotypic assays. Genotypic tests offered rapid results, while phenotypic methods covered wide profiles. Among the isolates, there were 37 MDRs and 1 XDR. Conclusions emphasize a combination of clinical methods for early identification and improvement of TB control, especially in resource-limited settings.

Keywords: Diagnostic methods, drug susceptibility testing, genotypic assay, , phenotypic methods

INTRODUCTION

A major global health concern tuberculosis (TB) has reported about 1.3 million deaths in 2020 (WHO, 2021) due to mycobacterium tuberculosis (MTB). Mycobacterium tuberculosis complex (MTBC), which includes both human and animal pathogens, presents important clinical and treatment challenges, especially in the context of increasing resistance (Thone and Williams, 2019). “The emergence of drug-resistant forms such as multidrug-resistant (MDR) and massive drug-resistant (XDR) TB has accelerated the public health burden (WHO, 2016). In order to control effective treatment and transmission, it is necessary to find an initial and accurate detection of drug resistance. The Drug Sensitivity Test (DST) methods are broadly classified into phenotypic and genotypic assays.”

Phenotypic methods, such as ratio method and absolute concentration method, are widely used but time consuming. Genotypic methods, like genotype MTBDRPLUS assay, offer rapid detection of resistance-that of resistance--- mutation in genes such as RPOB, KATG, and Inha (Hazbón et al., 2006).

METHODOLOGY

A cross-sectional study to assess the sensitivity of the drug in mycobacterium tuberculosis (MTB) is separated from suspected pulmonary and extrapulmonary TB patients. Clinical samples (sputum, pus, body fluids) were collected, decontaminated using the method of modified Petroff’s method, and the Ziehl-Nelson for acid-fast bacilli was examined by staining. Cultures were grown on the Lowenstein-Jensen (LJ) medium and through biochemical trials M. Known as tuberculosis.

Drug susceptibility testing (DST) was performed using three methods:

1. **Phenotypic Methods**

- Proportion Method (PM): Used to assess resistance to "first-line drugs (INH, RIF, EMB, STR)" by comparing colony counts on drug-supplemented media with those from drug-free controls.
- Absolute Concentration Method (ACM): Used to determine MICs for first- and second-line drugs (OFX, KAN) by evaluating bacterial growth on drug-containing media.

2. **Genotypic Method**

- **GenoType MTBDRplus Assay**: A molecular method to detect mutations in "*rpoB*, *katG*, and *inhA* genes associated with RIF and INH resistance." DNA was extracted, amplified by PCR, and hybridized to probes on nitrocellulose strips.

"The performance of GenoType MTBDRplus assays was compared to the Proportion Method. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and agreement (kappa statistic)" were analysed for each method.

RESULT

Phenotypic Assays:

- "The Proportion Method (phenotypic) was used as a reference standard for both first-line and second-line drug susceptibility testing (DST)". This method is widely adopted and involves assessing bacterial growth in the presence of antibiotics to determine resistance.
- "The Absolute Concentration Method was also used for first-line DST, focusing on the concentration of drugs required to inhibit bacterial growth."
- Results from the Proportion Method for first-line drugs "(e.g., INH, RIF, SM, and EMB)" showed different resistance patterns, such as resistance to single drugs and multiple drugs "(e.g., SM + RIF, SM + EMB, and SM + INH + RIF + EMB). These findings are tabulated and illustrated in Table 1".

Table 1: First- Line drug resistance pattern in Absolute concentration method

Resistance Pattern in First-Line Drugs	Absolute Concentration Method
Resistance to 1 drug - INH	5
RIF	5
SM	5
EMB	2
Resistance to 2 drugs	4
SM+RIF	1
SM+EMB	1
Resistance to 4 drugs - SM+INH+RIF+EMB	31
Sensitive	39

Genotypic Assays:

- The MTBDRplus Line Probe Assay (genotypic) was employed to detect mutations in "the rpoB, katG, and inhA genes, which are associated" with resistance to rifampicin and

isoniazid. This method provides a faster alternative for detecting drug resistance, as it does not require bacterial culture.

- The genotypic assay yielded rapid results, identifying rpoB mutations (linked to rifampicin resistance) and katG mutations (linked to isoniazid resistance). It proved valuable in detecting multidrug-resistant tuberculosis (MDR-TB) strains and understanding drug resistance patterns. The results from the MTBDRplus assay were compared to the Proportion Method (Table 2), showing similar resistance patterns between both methods.

Table 2: First-Line DST results by MTBDRplus assay in Comparison with Proportion method

Drug Susceptibility Pattern	Proportion method	MTBDRplus assay
Susceptible to RIF and INH	47	50
RIF-Mon resistant	8	4
INH-Mon resistant	9	9
MDR (Resistant to RIF and INH)	37	38

Comparison of Methods:

- A direct comparison was made between the Proportion Method (phenotypic), MTBDRplus Line Probe Assay (genotypic), and non-commercial assays (Nitrate Reductase Assay and CRI test).
- In the study, results from the Proportion Method and MTBDRplus Line Probe Assay were compared and shown to correlate for first-line drug resistance. The results were also contrasted with the findings from the Nitrate Reductase Assay and CRI test for identifying MDR and XDR strains.
- The MTBDRplus Line Probe Assay showed high sensitivity in detecting mutations responsible for rifampicin and isoniazid resistance, while the Nitrate Reductase Assay provided a quicker alternative for detecting XDR-TB.
- MDR strains of M. tuberculosis were identified using all three methods (phenotypic, genotypic, and non-commercial), with the MTBDRplus Line Probe Assay providing quicker results for gene mutations associated with drug resistance.
- XDR-TB strains were identified using the Proportion Method. The study found that out of 37 MDR strains, only one strain was identified as XDR.

DISCUSSION

The current study successfully employed and compared three major DST functioning, namely, phenotypic, genotypic and non-commercial assays for evaluating first-row in MTB isolates and drug resistance of the second line. The traditional ratio method, used as a phenotypic gold standard, detected resistance patterns for multiple drugs, reflects resistance to all four first-power “drugs (inh, rif, EMB, SM) with 31 isolates”. In comparison, the complete concentration method, another phenotypic assay, showed concurrent results for these multidrug-resistant (MDR) strains, confirmed its utility in regular screening (Palomino et al., 2007).

“On the other hand, genotypic MTBDRPLUS assay identified mutations in RPOB, KATG and Inha Jean, which offers rapidly detection of resistance to RIF and INH”. These results were largely corresponding to phenotypic results, supporting the established reliability of genotypic

assays in detection of major resistance markers (Talenti et al., Hilman et al., 2007). However, genotypic assays cannot always detect low normal mutation or resistance arising from the Flox mechanisms, outlining the complementary role of phenotypic DST.

Therefore, phenotypic methods are important for confirmation, genotypic assays provide speed and uniqueness, while non-commercial tests are useful in low-resources settings. A combined approach detects time of TB drug resistance at times and accurately.

CONCLUSION

The study successfully performed and compared the first row of phenotypic, genotypic and non-commercial assays and the second line and the second line DST. The ratio method acts as a reference standard, while the MTBDRPLUS line probe assay provided rapid results for genotypic detection, test offered for quick screening of MDR and XDR strains. These methods simultaneously contribute to a comprehensive approach to testing the sensitivity of the drug in tuberculosis.

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