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Detection of Rifampicin Resistance in Pulmonary Tuberculosis by Molecular Methods in a Tertiary Care Hospital

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Abstract: ***Introduction: Tuberculosis is the 2nd major cause of death and a significant public health concern among infectious diseases. India contributes to over 25% of the world's tuberculosis cases. Effective disease control depends on the early and rapid diagnosis of tuberculosis (TB) and the identification of antibiotic resistance. The CBNAAT and the LPA (Line Probe Assay) have been approved by the WHO for the rapid diagnosis of DRTB. Materials and Methods: A prospective study done in the department of Microbiology, GMC, Ongole. Sputum samples were collected from 1600 Presumptive TB and 350 Presumptive DR - TB patients attending at GGH, Ongole. CBNAAT was done on all sputum samples to detect tuberculosis and its RIF resistance. FL - LPA was done for CBNAAT - positive samples to detect RIF resistance mutation in the rpoB gene along with INH resistance mutations in the katG and in hA genes. SL - LPA was done for FL - LPA positive samples to detect FQ resistance mutations in gyrA and gyrB genes along with SLI drugs resistance mutations in rrs and eis genes. Results: Among 1600 presumptive TB samples, 197 (12.3%) positives for MTB, all were sensitive to Rifampicin by CBNAAT and rpoB gene mutation not detected by FL - LPA. Among 350 presumptive DRTB samples, 76 (21.7%) positives for MTB, 8 (10.5%) showing Rifampicin resistance by CBNAAT. Out of 76 CBNAAT MTB detected samples FL - LPA detected rpoB gene mutation in 10 samples (8 same as detected in CBNAAT and also in other 2 samples). Out of 10 RR samples, SL - LPA detected gyrB gene mutation showing FQ resistance in one sample. Conclusion: The current study suggests that while CBNAAT is thought to be the best approach for identifying MTB and detecting rifampicin resistance, it is important to keep in mind that resistance to isoniazid monotherapy and second - line drugs is also very prevalent. In these situations, LPA is more important.***

Keywords: MTB, CBNAAT, LPA, RIF resistance

1. Introduction

A major global public health concern, tuberculosis (TB) affected 1.3 million people in 2022 alone and affected 10.6 million more; 167, 000 of these deaths involved HIV - co - infected people [1]. More than 80 percent of tuberculosis cases and deaths happened in low - and middle - income nations [2]. Twenty - seven percent of tuberculosis cases worldwide are reported from India. More accurate molecular diagnostics and increased access to treatment have been made towards the goal by 2025.

Due to gaps in diagnosis and treatment, multidrug - resistant or rifampicin - resistant tuberculosis (MDR/RR - TB) presents serious difficulties to TB control efforts. MDR - TB is a term used to describe resistance to the 2 most potent first - line anti - TB medications, isoniazid and rifampicin. Of the computed 410, 000 MDR/RR - TB cases worldwide in 2022, only 176, 600 were found and 175, 650 underwent treatment [3].

The Xpert MTB/RIF assay also provides information about potential rifampicin resistance through the detection of mutations in an 81 - base pair region of the rpoB gene, which confers approximately 96% of rifampin resistance in MTBC [4].

An alternative method for identifying mycobacteria from culture isolates that employ hybridization - based probes is to use line probe assays. These assays use probes that focus on nucleotide variations in the 23S and/or 16S rRNA regions. Analytical specificity and sensitivity for LPA are usually >90% and outcomes can be obtained in 4 - 6 hours [4].

Therefore, the current research was undertaken to evaluate the occurrence of TB and to determine Rifampicin resistance by employing CBNAAT and LPA.

2. Material and Methods

Study Design and Place: A Prospective study was conducted in the Government Medical College's microbiology department in Ongole, Andhra Pradesh, India.

Inclusion criteria

1) Suspected patients of pulmonary TB and pulmonary DRTB
2) All age groups and both sexes.
3) Patients who have given informed consent.

Exclusion criteria

1) Patients who have not given consent

2) Suspected patients of extrapulmonary TB and extrapulmonary DRTB

Study period, sample collection, and processing

A prospective study was done in the Department of Microbiology, GMC, Ongole for a period of 4 months (October 2022 to January 2023). Early morning Sputum samples were collected from 1600 Presumptive TB and 350 Presumptive DR - TB patients attending at GGH, Ongole. CBNAAT was done on all sputum samples to detect tuberculosis and its RIF resistance. All CBNAAT - positive samples are transported in a cold chain to DFUL&TRC, Nellore. which is operated by DFIT for FL - LPA and SL - LPA.

- FL - LPA was done for CBNAAT - positive samples to detect RIF resistance due to mutation in rpoB gene as well as INH resistance due to mutations in katG and inhA genes
- SL - LPA was done for FL - LPA positive samples to detect FQ resistance due to mutations in gyrA and gyrB genes as well as SLI drug resistance due to mutations in rrs and eis genes.

3. Results

Among 1600 presumptive TB samples, 197 (12.3%) positives for MTB. Out of 197 MTB detected patients 150 (76.2%) were males and 47 (23.8%) were females. All were sensitive to Rifampicin by CBNAAT and rpoB gene mutation not detected by FL - LPA.

Among 350 presumptive DRTB samples, 76 (21.7%) positives for MTB. Out of 76 MTB detected patients 63 (82.9%) were males and 13 (17.1%) were females. Out of 76 CBNAAT MTB detected samples FL - LPA detected rpoB gene mutation in 10 samples (8 same as detected in CBNAAT and also in other 2 samples). Out of 10 RR samples, SL - LPA detected gyrB gene mutation showing FQ resistance in one sample.

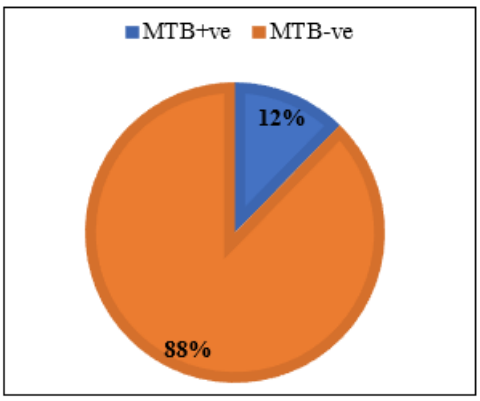

Figure 1: Results-Presumptive TB Samples- CBNAAT Tests (1600)

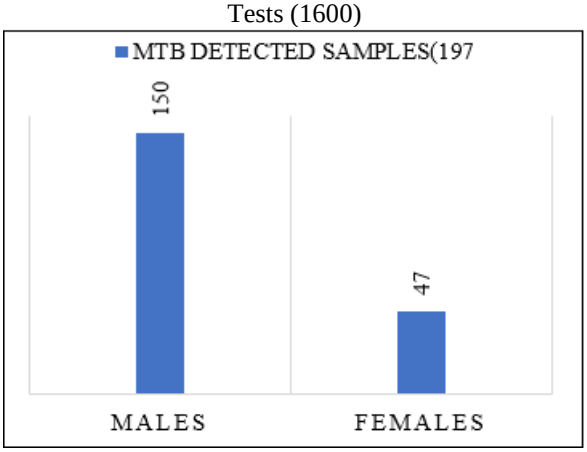

Figure 2: MTB Detected Samples (197)

Table 1: Presumptive TB samples - 1600

CBNAAT	MTB detected samples - 197	All are sensitive to Rifampicin
FL – LPA	not detected rpoB, katG, and inhA gene mutations.	
SL - LPA	not done for all 197 MTB samples as RIF and INH resistance were not detected in first - line LPA.	

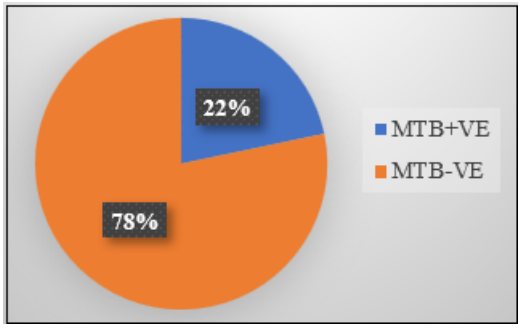

Figure 3: Results-Presumptive DR TB Samples - CBNAAT Tests (350)

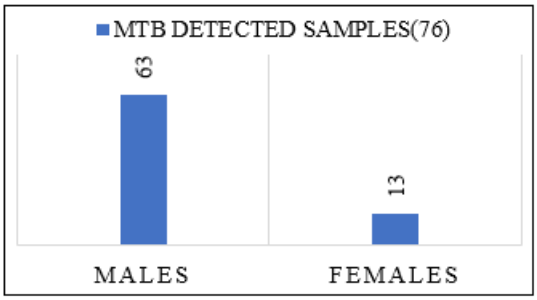

Figure 4: MTB Detected Samples (76)

Table 2: Presumptive DR TB Samples – 350

CBNAAT	MTB detected samples - 76	8 (10%) were showing resistance to RIF
FL - LPA (for all 76 samples of MTB)	10 (11.7%) samples were showing rpoB gene mutation due to RIF resistance. **(NOTE: 8 samples showing RIF resistance in FL - LPA showed RIF resistance in CBNAAT + 2 samples showing RIF resistance in FL - LPA were not shown RIF resistance in CBNAAT)**	katG and inhA genes mutation due to INH resistance not detected.
SL - LPA (for all 10 FL - LPA mutation detected samples)	One sample showed gyrB gene mutation due to resistance to Lfx and low - level Mfx. gyrA gene mutation was not detected for all FL - LPA mutation detected samples.	eis and rrs genes mutation due to SLI drugs resistance not detected.

4. Discussion

Molecular technologies such as LPA and CBNAAT are the most significant technologies to identify mutations in the inhA, katG, and rpoB genes, the LPA test might identify both INH and RIF resistance, but the CBNAAT can only identify RIF resistance.

The greatly shortened detection turnaround time is one of the primary and most evident benefits of using the CBNAAT. This test can reduce the time to diagnosis (TAT) to two to three hours while simultaneously detecting rifampin resistance. The rifampin resistance determining region (RRDR), an 81 - bp hotspot region covering codons 507 to 533, is the target of these molecular techniques designed to target the rpoB gene. DNA sequencing has identified over 50 mutations in this region thus far, however, the only known mutations that cause increased RIF resistance are point mutations at codons 526 or 531.

In the present study, Presumptive DRTB patients showed more MTB positivity rate (of 21.7%), which was similar to the research performed by J. Vijayalakshmi et. al (5). Male predominance is higher, the age group most affected was 41 - 60 yrs. followed by 20 - 40yrs which was similar to the research performed by J. Vijayalakshmi et. al (5).

Rifampicin resistance was detected in presumptive DRTB patients and it was more in males. Syed Beenish Rufai et al. 's study revealed similar outcomes (6).

In the present investigation, it was observed that Rifampicin resistance was detected in 10% of samples in CBNAAT. Similar outcomes were noted in research performed by Wadhwa et al. In the FL - LPA, 11.7% resistance to RIF was noted in the present study and 26% resistance to RIF was noted in the study by Vishal Wadhwa et al.

The study by S. Uma Devi et al. found that 6.82% of RIF resistance was detected by CBNAAT, whereas the RIF resistance detection rate by LPA was 14.7%. It was found in this study that LPA was superior to CBNAAT in terms of efficacy in detecting RIF resistance. The study conducted by Syed Beenish Rufai et al. revealed similar outcomes (6).

5. Conclusion

Promising molecular technologies for drug resistance detection include CBNAAT and LPA. The current study suggests that while CBNAAT is thought to be the best approach for identifying MTB and detecting rifampicin resistance, it is important to keep in mind that resistance to isoniazid monotherapy and Second - line medications are also widely used. In these situations, LPA is more important.

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Source of funding

None

Conflict of Interest

None

Ethical approval

The Institutional Ethics Committee approved the research.

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