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Prevalence of Rifampicin resistance detected by TrueNat assay in suspected pulmonary cases in a teritiary care hospital, Kurnool

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ABSTRACT

Background: Tuberculosis (TB) remains a major public health challenge in India, with the emergence of drug-resistant strains further complicating disease control. Early detection of rifampicin resistance is essential for prompt initiation of appropriate therapy and interruption of transmission. Rapid molecular diagnostic tools such as TrueNat have enhanced TB detection, particularly in decentralized and resource-limited settings.
Aim: To determine the prevalence of rifampicin resistance among suspected pulmonary tuberculosis patients using the TrueNat assay in a tertiary care hospital.
Materials and Methods: A prospective observational study was conducted from April 2024 to January 2026 in the Department of Microbiology in collaboration with Department of Pulmonology in a teaching hospital. Adult patients with clinical suspicion of pulmonary TB were included in the study. Sputum sample collected from suspected cases were processed with Acid fast staining by Ziehl Neelsen method and TrueNat assay for detection of Mycobacterium tuberculosis and Rifampicin resistance.
Results: A total of 2527 sputum samples were screened during the study period, of which 184 (7.28%) were positive for tuberculosis by TrueNat assay. Male predominance was observed, with 101 (54.9%) males and 83 (45.1%) females among confirmed cases. Rifampicin resistance was detected in 5 (2.7%) cases, while 179 (97.3%) cases were found to be rifampicin-sensitive.
Conclusion: The study demonstrated a moderate TB positivity rate and a relatively low prevalence of rifampicin resistance. TrueNat proved to be a rapid and effective tool for early diagnosis and resistance detection. Continuous molecular surveillance is essential to strengthen TB control strategies and achieve national TB elimination targets.

Keywords*: Tuberculosis, Rifampicin resistance, TrueNat, Pulmonary TB, Molecular diagnosis, Drug-resistant TB.*

INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality worldwide. According to the World Health Organization (WHO) Global Tuberculosis Report 2024, an estimated 10.6 million people developed TB globally in 2023, corresponding to an incidence rate of approximately 133 cases per 100,000 population **[1]**. Despite global control efforts, TB caused about 1.3 million deaths among HIV-negative individuals and an additional 300,000 deaths among people living with HIV, highlighting its continued public health significance **[1]**.

India carries the highest TB burden globally. As per WHO estimates, India accounted for nearly 27% of global incident TB cases, with approximately 2.8 million new cases annually. The estimated TB incidence rate in India in 2023–2024 was about 187 cases per 100,000 population **[1,2]**. According to the India TB Report 2024, TB mortality remains substantial

despite improved treatment access and expanded diagnostic services under the National TB Elimination Programme (NTEP) **[2,15]**. The transition from the Revised National Tuberculosis Control Programme (RNTCP) to NTEP has strengthened case detection, standardized treatment protocols, drug-resistance surveillance, and decentralized molecular diagnostics across the country **[2,15]**.

The emergence of drug-resistant tuberculosis has further complicated TB control efforts. Globally, over 4,10,000 individuals developed drug-resistant TB in 2022, with India contributing a significant proportion of the global burden **[1,2]**. India accounts for nearly one-fourth of the global multidrug-resistant TB (MDR-TB) burden, with MDR-TB prevalence estimated at 2.5–3.9% among new cases and 13–16% among previously treated cases **[2,13]**. Rifampicin-resistant TB (RR-TB) is defined as resistance to rifampicin detected by phenotypic or genotypic methods, with or without resistance to other anti-TB drugs **[13]**. Multidrug-resistant TB (MDR-TB) refers to resistance to isoniazid and rifampicin **[3,13]**, while extensively drug-resistant TB (XDR-TB) strains are MDR-TB and involves additional resistance to fluoroquinolones and at least one of the three second line injectable drugs (capreomycin, kanamycin and amikacin). **[4,13]**.

The emergence of MDR-TB and RR-TB is largely attributed to inappropriate treatment regimens, poor drug adherence, substandard drug quality, and premature interruption of therapy, leading to increased community transmission **[13,14]**. Drug-resistant TB is associated with prolonged therapy, higher treatment costs, increased toxicity, and lower treatment success rates compared to drug-susceptible TB **[3,4]**.

Rifampicin acts by inhibiting bacterial RNA polymerase and is highly effective against both intracellular and extracellular bacilli. Mutations in the rpoB gene lead to rifampicin resistance, making it a reliable surrogate marker for MDR-TB in most clinical settings **[4,11]**. Conventional culture-based drug susceptibility testing (DST), though considered the reference standard, requires several weeks to yield results **[5,12]**.

Sputum smear microscopy, though widely used in India, has limitations including low sensitivity and the requirement of a high bacillary load (approximately 10,000 bacilli/ml) for detection. The advent of rapid molecular diagnostic techniques such as TrueNat and CBNAAT has significantly improved TB detection and rifampicin resistance identification, even in low bacillary load specimens **[6,7,10]**.

TrueNat MTB/RIF is a chip-based real-time PCR platform developed in India, capable of detecting *Mycobacterium tuberculosis* complex DNA and rpoB gene mutations within 1–2 hours **[6]**. The World Health Organization endorsed TrueNat for TB diagnosis and rifampicin resistance detection in 2020, and it has been incorporated into the National TB Elimination Programme for decentralized use **[7,16]**. Its portability, minimal biosafety requirements, and suitability for peripheral laboratories make it particularly valuable in resource-limited settings **[6,7,16]**.

In view of the persistent TB burden and the growing concern of drug resistance, assessing the prevalence of rifampicin resistance among suspected pulmonary TB patients in tertiary care settings is essential for understanding local resistance patterns and guiding effective treatment strategies aligned with national TB elimination goals.

MATERIALS AND METHODS

Study Design and Setting

This is a prospective observational study conducted in the Department of Microbiology in collaboration with Department of Pulmonology, Viswabharathi Medical College and General Hospital. The study was done between April 2024 to January 2026 after obtaining approval from the Institutional Ethics Committee.

Study Population

The study population comprised of patients with clinical features suggestive of pulmonary tuberculosis such as cough> 2 weeks, fever, weakness, weight loss, hemoptysis in accordance with national tuberculosis diagnostic guidelines. [17] All the suspected patients were advised to collect two sputum samples and undergo radiological examination.

Patients with signs and symptoms of extrapulmonary tuberculosis, already receiving anti-tubercular therapy for more than two weeks prior to sample collection, Patients who were unable to provide an adequate specimen were excluded from the study.

Sample Collection and Processing

Sputum samples were collected following standard recommendations under the National Tuberculosis Elimination Programme (NTEP) guidelines.[17] Patients were instructed to provide two sputum samples, one at the spot and an early morning sample. Samples were collected in sterile, wide-mouthed, leak-proof containers. A minimum volume of 2–3 mL was considered adequate. Care was taken to ensure that the specimen contained mucopurulent material rather than saliva.

Specimens were transported to the microbiology laboratory within one hour of collection. In case of delay, samples were stored at 2–8°C and processed within 24 hours as per WHO laboratory biosafety recommendations.[18] All handling of specimens was performed under appropriate biosafety precautions.

Ziehl–Neelsen (ZN) Staining for Acid-Fast Bacilli
Direct smear microscopy was performed using the Ziehl–Neelsen (ZN) staining method for detection of acid-fast bacilli (AFB) following standard WHO and RNTCP/NTEP guidelines. [19]

A thin smear was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was flooded with strong carbol fuchsin and gently heated until steaming for five minutes. After rinsing with water, decolorization was carried out using 20% sulfuric acid until the smear appeared pale pink. The slide was then counterstained with methylene blue for one minute, washed, air-dried, and examined under oil immersion (100× objective).

Smears were graded according to standard WHO grading guidelines. [19] The presence of red, slender, beaded bacilli against a blue background was considered positive for AFB.

Detection of Mycobacterium tuberculosis and Rifampicin Resistance by TrueNat
All sputum samples from suspected case of pulmonary tuberculosis were subjected to molecular testing using the TrueNat MTB/MTB-RIF assay for rapid detection of Mycobacterium tuberculosis complex and rifampicin resistance as recommended by national guidelines. [20]

Principle of TrueNat
TrueNat is a chip-based real-time polymerase chain reaction (RT-PCR) assay. The test involves automated DNA extraction from processed sputum samples followed by amplification of MTB-specific genetic targets. Rifampicin resistance is detected by identifying mutations in the rpoB gene, which encodes the β-subunit of RNA polymerase. [21] Amplification and detection occur on a microchip platform with real-time fluorescence monitoring. Results are generated within approximately 1–2 hours and reported as MTB detected/not detected and rifampicin sensitive/resistant. [20]

Comparison of TrueNat with CBNAAT
TrueNat offers several operational advantages compared to Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), including portability, battery-operated functionality, lower infrastructure requirements, suitability for decentralized settings, and reduced turnaround time. It can be deployed in peripheral laboratories with minimal biosafety infrastructure. [22]

However, certain limitations exist. TrueNat requires sequential testing (initial MTB detection followed by rifampicin resistance testing). Sensitivity may be reduced in specimens with very low bacillary load. Additionally, confirmatory culture and drug susceptibility testing remain necessary in selected cases. [17]

Importance of the Present Study
Rifampicin resistance is a critical marker of multidrug-resistant tuberculosis and has significant implications for patient management and public health. Early detection facilitates timely initiation of appropriate treatment regimens and reduces community transmission of resistant strains.

Tertiary care hospitals serve as referral centers for complicated and previously treated cases, making them important sites for surveillance of drug resistance patterns. Determining the prevalence of rifampicin resistance among suspected pulmonary tuberculosis patients in these settings provides essential epidemiological data and supports evidence-based treatment strategies aligned with national TB elimination goals.

RESULTS
A total of 2527 samples were screened during the study period from April 2024 to January 2026 as shown in Table 1.

Table 1: Number of samples screened year wise

Year/Period	Number of samples screened
April 2024 – Dec 2024	864
Jan 2025 – Dec 2025	1455
Jan 2026	208
Total	2527

Year-wise distribution of test results (Table 2) revealed that during the study period, 2527 samples were screened. 184 sputum samples were positive by TrueNat assay, 163 sputum samples were positive by acid fast staining and 22 patients showed the radiological evidence of tuberculosis.

Table 2: Year wise Distribution of all test results

Year/Period	Total number of samples	No. Of samples positive by TrueNat	No. Of cases positive by ZN staining	No. Of samples positive by Chest x-ray
2024	864	69	61	12
2025	1455	109	98	10
2026	208	06	04	0
Total	2527	184	163	22

Rifampicin resistance detected by Truenat assay (Table 3) showed that the majority of cases were Rifampicin-sensitive (179 cases, 97.3%), while only 5 cases (2.7%) were found to be Rifampicin-resistant. Rifampicin resistance was seen in all newly diagnosed cases of tuberculosis.

Table 3: Rifampicin resistance detected by Truenat Assay

Resistance status	Frequency	Percentage (%)
Rifampicin-sensitive	179	97.3
Rifampicin-Resistant	5	2.7
Total	184	100

Gender-wise distribution showed a male predominance, with 101 males (54.9%) and 83 females (45.1%) as shown in table 4.

Table 4: Gender wise distribution of positive samples

Gender	Frequency	Percentage (%)
Male	101	54.9
Female	83	45.1

DISCUSSION

Tuberculosis continues to remain a major public health problem globally including India despite sustained control efforts **[1,2]**. In the present study, 2527 samples were screened over 21 months, of which 184 (7.28%) were positive. This positivity rate aligns with findings from other tertiary-care-based Indian studies reporting rates between 5–15% **[8]**.

Male predominance (54.9%) observed in this study is consistent with global epidemiological patterns **[1,2,9]**. Biological, behavioral, and socio-environmental factors have been implicated in higher TB prevalence among males **[9]**.

Rifampicin resistance was detected in 2.7% of confirmed cases, which corresponds with national estimates of 2–4% among new TB cases in India **[2,13]**. Early identification of rifampicin resistance is critical under current DR-TB management guidelines to ensure timely initiation of appropriate second-line regimens **[13,14]**. Mutations in the rpoB gene serve as a surrogate marker for MDR-TB, allowing rapid detection through molecular methods **[4,11]**.

TrueNat has demonstrated high sensitivity and operational feasibility in decentralized settings **[6,10]**. WHO endorsement and incorporation into national TB diagnostic algorithms have strengthened India's strategy toward universal drug susceptibility testing **[7,16]**.

CONCLUSION

The present study demonstrated a tuberculosis positivity rate of 7.28% among clinically suspected pulmonary TB patients in a tertiary care hospital setting. A clear male predominance was observed, consistent with national epidemiological trends. Rifampicin resistance was detected in 2.7% of confirmed cases, indicating a relatively low prevalence of drug resistance in the study population. The use of TrueNat enabled rapid detection of both *Mycobacterium tuberculosis* and rifampicin resistance, facilitating timely initiation of appropriate therapy. Continuous surveillance using rapid molecular diagnostics is essential to strengthen TB control efforts and support national TB elimination goals.

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