



Original Article

Prevalence Of Mycobacterium Tuberculosis And It's Rifampicin Resistance In Respiratory Sample In A Tertiary Hospital

Shivam Mudgal¹, Ashutosh Rawat², Ritu Jain³, Sanjana Jain⁴

¹Student M.Sc. Medical Microbiology, Department of Microbiology, Santosh Medical University, Ghaziabad, Uttar Pradesh, India

²Head of Department of Microbiology, Santosh Medical University, Ghaziabad, Uttar Pradesh, India

³Assistant Professor, Department of Microbiology, Santosh Medical University, Ghaziabad, Uttar Pradesh, India

⁴Student M.Sc. Medical Anatomy, Department of Anatomy, Santosh Medical University, Ghaziabad, Uttar Pradesh, India

OPEN ACCESS

Corresponding Author:

Shivam Mudgal

Department of Microbiology, Santosh Medical University, Ghaziabad, Uttar Pradesh, India
Email: ss5814594@gmail.com

Received: 25-09-2026

Accepted: 05-10-2026

Available online: 09-10-2026

ABSTRACT

Background: Tuberculosis remains a major problem for public health even though people are still working hard to control and stop it. The disease is still one of the main reasons people get sick and die from infections around the world, and India plays a big role in the overall number of cases and deaths from TB globally. The growing number of cases of Mycobacterium tuberculosis that are resistant to rifampicin has become a big problem for managing and controlling the disease effectively (1). Getting a diagnosis early and correctly is important so that treatment can start quickly and the disease doesn't spread to others. Regular smear tests usually don't detect tuberculosis well, especially when the infection isn't seen in the smear tests (2).

Aim: Prevalence of Mycobacterium tuberculosis and it's rifampicin resistance in respiratory samples in a tertiary hospital

Methods: Respiratory samples taken from patients who are suspected of having pulmonary tuberculosis will first be stained using the Ziehl-Neelsen method, and then tested with TrueNat to check both samples that show bacteria under the microscope and those that don't. The TrueNat MTB test will be used to detect Mycobacterium tuberculosis, and the TrueNat MTB-RIF Dx test will be used to check for rifampicin resistance in samples that test positive for tuberculosis. The information about the participants' health conditions will be gathered and studied using statistical methods.

Result The study used 133 respiratory samples to test for Mycobacterium tuberculosis and to check for resistance to rifampicin. Out of 133 samples, 7 samples tested positive using Ziehl-Neelsen staining, which is about 5.26% of the total. The TrueNat test found Mycobacterium tuberculosis in 18 samples, which makes up 13.53% of the total. This group included all 7 samples that were positive under the ZN method. Out of the 18 samples that tested positive with TrueNat, 11 samples showed negative results with ZN staining but positive results with TrueNat, showing that the molecular method detected more cases than the traditional microscopy method.

Rifampicin resistance was found in 3 (16.67%) of the 18 samples that tested positive with TrueNat.

Keywords: Tuberculosis, Mycobacterium tuberculosis, Rifampicin resistance, TrueNat, Smear-negative tuberculosis, Molecular diagnosis, MDR-TB.

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INTRODUCTION

Tuberculosis, or TB, is a contagious disease caused by the bacteria Mycobacterium tuberculosis. Even though there are

good treatments and programs in place to control it, TB is still a big problem for public health around the world. [1,2] It is one of the main infectious diseases that cause illness and death, especially in poorer countries.[1]

The increasing number of cases of tuberculosis that is resistant to drugs, especially multidrug-resistant tuberculosis (MDR-TB), is causing major problems in efforts to control tuberculosis. Rifampicin resistance is important in clinical settings because rifampicin is one of the strongest first-line medicines used to treat tuberculosis, and being resistant to it is often linked to multidrug-resistant TB. Patients who have infections caused by rifampicin-resistant strains usually need longer treatment with second-line drugs, which are more expensive, not as effective, and cause more side effects. So, quickly finding out if someone is resistant to rifampicin is important for starting treatment on time and stopping the spread of the disease.[6]

Pulmonary tuberculosis is the most common type of TB and helps spread the disease to people around us. [2,4] India has a big share of the world's TB cases, with many new cases found each year. [1,3] Things like not having enough food, living in crowded areas, being poor, bad living conditions, and having HIV make TB harder to stop and cause it to spread more.[5] Early diagnosis of tuberculosis is important because it helps reduce the spread of the disease and improves the chances of successful treatment.[1,2] Traditional methods like Ziehl–Neelsen smear microscopy are commonly used since they are easy to perform and cost-effective, but they are not very effective at detecting the bacteria, especially when there are few bacteria present.

Molecular tests such as TrueNat can quickly and accurately identify *Mycobacterium tuberculosis* and also detect resistance to rifampicin, which helps in diagnosing and treating tuberculosis sooner and more effectively. [6,7]

MATERIALS AND METHODS

2.1 Study Design

Cross-Sectional study

2.2 Study Setting

The present study, entitled “Prevalence of *Mycobacterium tuberculosis* and its Rifampicin resistance in respiratory samples in a tertiary hospital” will be conducted in department of Microbiology, Santosh Medical College and Hospital, Ghaziabad

2.3 Study population

Patient comes to the hospital with signs that might mean they have tuberculosis in their lungs, like prospective cough for two weeks or more, coughing up blood, unexplained losing weight, having fever at night, or feeling very hot without a clear reason. The doctor asks for a sample from the lungs, such as sputum, induced sputum, or bronchoalveolar lavage, to check for tuberculosis bacteria.

2.4 Inclusion Criteria

- All patients
- Both Male and Female
- Symptoms suggestive of pulmonary tuberculosis (e.g., productive. Cough ≥ 2 weeks,
- hemoptysis, unexplained weight loss

2.5 Exclusion Criteria

- Immunocompromised patients
- Any Patient having respiratory infection other than tuberculosis

2.6 Methodology

Specimen Collection

Each patient gave two sputum samples:

- One sample was taken in the early morning.
- Another sample was collected at a regular time.

The samples were put into clean, sealed, wide-mouthed containers so they could be tested under a microscope. For molecular testing, sputum samples were gathered in sterile Falcon tubes that work with Truenat testing

Smear Microscopy

All the sputum samples were tested using Ziehl–Neelsen staining to look for acid-fast bacilli. Smears were made on clean, grease-free glass slides, let dry in the air, then heated to fix them. They were stained first with carbol fuchsin, then washed with 20% sulfuric acid to remove excess stain, and finally stained again with methylene blue to make the bacteria easier to see. A microscope was used with a 100x oil immersion lens to look at the sample, and the acid-fast bacilli were assessed

based on the guidelines from the National Tuberculosis Elimination Programme

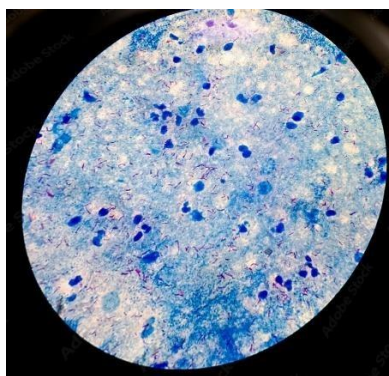


Fig 1 Positive smear

Molecular Detection

Both smear-negative and smear-positive samples were processed further with the Truenat MTB Plus test as instructed by the manufacturer. The processed samples were combined with lysis buffer and then underwent DNA extraction using the Truenat Sample Preparation Kit. The DNA that had been cleaned was taken from the collection tube and put into the Truenat MTB microchip, which already had dried PCR testing materials inside. The PCR amplification happened instantly on the Truelab analyzer with a protocol that was already set up. The results were either labeled as “MTB detected” or “MTB not detected,” and the amount of bacteria was shown in colony-forming units per milliliter (CFU/mL).

After finding MTB in the samples, they were further tested with the Truenat MTB-RIF Dx test. This test helps identify if the bacteria are resistant to rifampicin by checking for mutations in the *rpoB* gene.

RESULT

The study used 133 respiratory samples to test for *Mycobacterium tuberculosis* and rifampicin resistance. Out of 133 samples, 7 samples (5.26%) tested positive using Ziehl–Neelsen staining, and 18 samples (13.53%) came back positive with the TrueNat assay. All the samples that tested positive for ZN were also positive when tested using the TrueNat assay. Out of the 18 samples that tested positive with the TrueNat assay, 11 (which is about 61.11%) came back negative when checked using ZN staining, but positive with the TrueNat test. This shows that molecular testing methods are more sensitive than traditional microscopy techniques. Rifampicin resistance was found in 3 (16.67%) of the 18 samples that tested positive with TrueNat.

Table 1: Distribution of Samples According to Diagnostic Method

Diagnostic method	Positive cases	Percentage
Zn staining	7	5.26%
TrueNat Assay	18	13.53%
Rifampicin resistance	3	16.67%

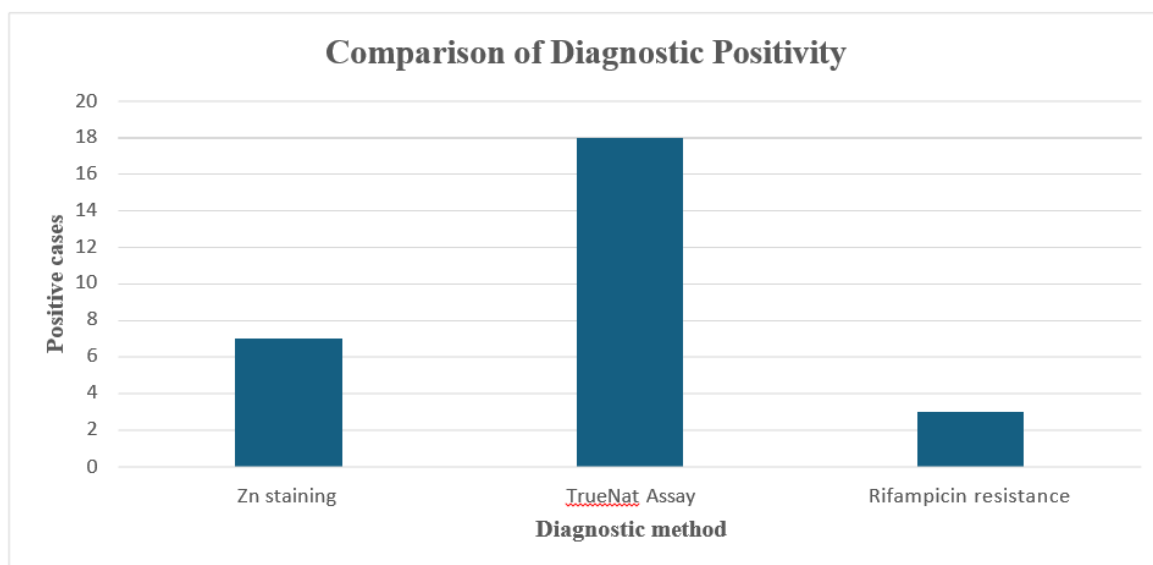


Figure 2: Comparison of positivity detected by ZN staining, TrueNat assay, and rifampicin resistance.

Out of the 18 samples that tested positive with TrueNat, 11 of them (which is 61.11%) that are negative by ZN smear microscopy. This shows that the TrueNat test found more cases of *Mycobacterium tuberculosis* that were not seen with the regular ZN staining method, proving that molecular tests are more sensitive.

Table 2: Detection of MTB by TrueNat Assay in ZN Smear Negative Samples

Parameter	No. of samples (n=18)	Percentage
Zn staining negative	11	61.1%
TrueNat positive	18	100%

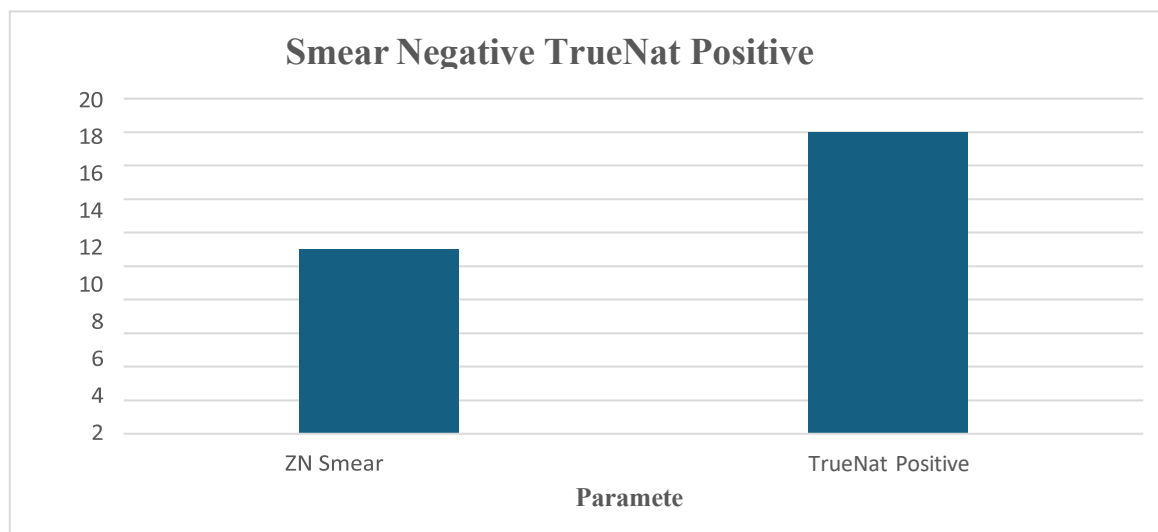


Figure 3: Detection of *Mycobacterium tuberculosis* by TrueNat assay among ZN smear-negative respiratory samples.

The test's ability to correctly find true positive cases was 38.89%, meaning it detected that percentage of all actual positive cases. This low sensitivity means the test might miss some positive cases. The test had 100% specificity, meaning it correctly found every case where the condition wasn't present and didn't mistakenly say it was present when it wasn't. This shows that the test is very good at correctly identifying when someone does not have the disease. The positive predictive value (PPV) was 100%, meaning every sample that tested positive was actually positive. So, a positive test result is probably very reliable. The negative predictive value was 91.27%, meaning that 91.27% of the tests that came back negative were actually correct, but a small number of negative results could still be cases where the real result was positive. The test showed very good ability to correctly identify negative cases and accurately confirm positive results, which means it's very dependable for confirming when someone is positive. However, its lower ability to detect all actual positive cases means it isn't very effective at finding every true positive.

Table 3: Diagnostic Accuracy of ZN Staining Compared with TrueNat Assay for Detection of *Mycobacterium tuberculosis*

Parameter	Percentage
Sensitivity	38.89%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	91.27%

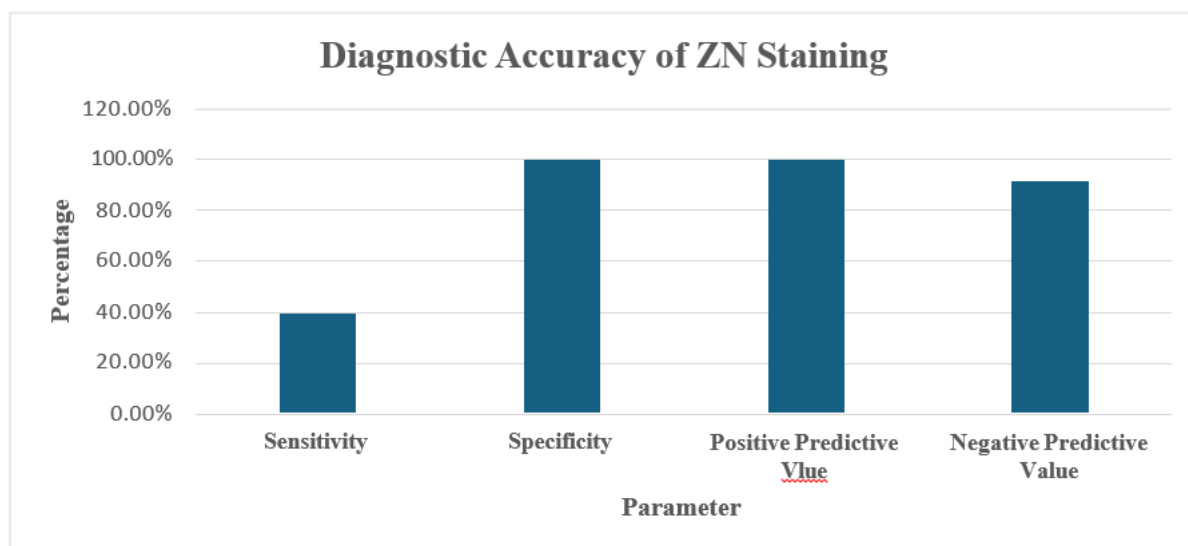


Figure 4: Diagnostic performance of ZN staining using TrueNat assay as reference method.

Out of the 18 samples that tested positive with TrueNat, 15 of them (which is about 83.33%) were found to be sensitive to rifampicin, while 3 of them (about 16.67%) were resistant to it. Most of the cases where *Mycobacterium tuberculosis* was found to be positive showed sensitivity to rifampicin, but a smaller number of cases had drug resistance.

Table 4: Distribution of Rifampicin Resistance Among TrueNat-Positive Samples

Result	Rifampicin resistance (n=18)	Percentage
Resistant	3	16.6%
Sensitive	15	83.4%

Rifampicin Resistance (n=18)

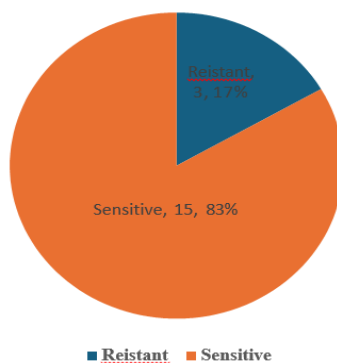


Figure 5: Distribution of rifampicin resistance among TrueNat-positive samples.

DISCUSSION

Tuberculosis is still a big problem for public health, especially in countries that are developing, such as India. Finding disease early and knowing if it is becoming resistant to drugs is very important for controlling it well. In this study, 13.53% of respiratory samples tested using the TrueNat assay showed the presence of *Mycobacterium tuberculosis*.

In this study, the ZN smear test found just 7 positive cases, but the TrueNat test found 18 positive cases. This result shows that molecular techniques are better at detecting the disease compared to the traditional smear microscopy method. Similar results were also found in [9], where they noticed that TrueNat was able to find more tuberculosis cases that were not detected by smear microscopy because the number of bacteria was too low.

Out of the 18 samples that tested positive with TrueNat, 11 of them were negative when checked with the smear test but still came back positive with TrueNat. This shows how important nucleic acid amplification tests are for finding cases of tuberculosis that have very few bacteria. Smear microscopy needs more bacteria to show a positive result, but molecular tests can find fewer bacteria and are more accurate. Similar results were also mentioned in [7], where they found that

molecular tests worked better in detecting pulmonary tuberculosis in cases where the smear test came back negative.

In this study, 3 out of the 18 cases that tested positive with TrueNat showed resistance to rifampicin, which is about 16.67%. Rifampicin resistance is a key sign that shows a person has tuberculosis that is resistant to multiple drugs. Early detection of rifampicin resistance is important to start the right treatment on time and stop the spread of drug-resistant strains. [6,3] suggest using quick molecular tests like TrueNat and GeneXpert to find rifampicin resistance early.

The test was very specific and had a positive predictive value of 100%, showing that the positive results are very reliable. However, the sensitivity was not as high, which means that some actual positive cases might not be found using standard methods alone. These results show that using molecular tests like TrueNat can help quickly and accurately diagnose tuberculosis and rifampicin resistance.[2] also found that traditional methods are not very good at detecting the disease, especially when there are few bacteria present, but faster molecular tests help find cases earlier.

The study shows that TrueNat is a quick, accurate, and dependable method for identifying *Mycobacterium tuberculosis* and rifampicin resistance in samples from the lungs. It can help doctors detect tuberculosis earlier and manage the disease better in large, specialized hospitals.

CONCLUSION

This study shows that TrueNat is a fast and dependable method for testing respiratory samples to find *Mycobacterium tuberculosis* and check for resistance to rifampicin. TrueNat found more tuberculosis cases than the usual ZN smear test, including some that the regular test missed, showing it is better at detecting the disease. The study also found cases where the bacteria were resistant to rifampicin, highlighting the need to catch drug resistance early so that the right treatment can start on time. TrueNat is good at identifying tuberculosis and rifampicin resistance at the same time, works quickly, and is very accurate. This makes it helpful for early diagnosis and better treatment of tuberculosis in big hospitals and places with few resources.

Ethics Statement The study was approved by Institutional Human Ethics Committee of Santosh Medical University.

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