Section: Microbiology

Original Research Article

DIAGNOSTIC IMPLICATIONS IN THE INTERPRETATION OF RIF RES/INDETERMINATE IN CB-NAAT WHILE TESTING SPUTUM SAMPLES WITH LOW BACTERIAL LOAD

K. Lakshmi[1], R.Vijayapriya[2], M.Bhuvaneswari[3]

[1]Associate Professor, Department of Microbiology, KAPV Government Medical College, Tamilnadu, India.
[2]Assistant Professor, Department of Microbiology, KAPV government medical college, Tamilnadu, India.
[3]Associate Professor, Department of Microbiology, Government Thiruvarur Medical College, Tamilnadu, India.

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Corresponding Author:
Dr. K. Lakshmi,
Email: lakmekands@yahoo.com.

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Abstract

Background: The development of the Xpert MTB/RIF assay was a significant step forward in improving the diagnosis of TB and detecting rifampicin resistance globally. This study aimed to study the diagnostic implications in sputum samples with low mycobacterial load. **Material & Methods:** This was a study conducted at KAPV Government Medical College, Trichy, where we tested clinically suspected PTB patients whose samples were sent to the laboratory for CBNAAT to identify Mycobacterium TB/RIF (MTB/RIF) testing from January 2023 to December 2023. **Results:** Out of the 5,807 sputum samples tested, 4514 tested negative (77.73%). Low and very low TB was detected in 213(3.68%) and 194 cases (3.34%). While considering samples with low mycobacterial load alone, 60 cases showed indeterminate results, and 12 showed Rif Resistance. 53 out of the 60 indeterminate samples were re-tested; 22 (41.51%) were MTB negative, whilst 31 samples (58.49%) were positive. All 12 cases were re-tested; 10 (83.33%) were positive, while 2(16.67%) samples tested negative. No indeterminate results were seen in high and medium TB-detected cases, which form about 63.5% of the positive cases. When the test was repeated for indeterminate Rif-detected cases, 41.5% were Tb negative. **Conclusion:** Though our diagnostic algorithm directs us to repeat the test for all indeterminate cases, the Rif resistance cases amongst the low mycobacterial load cases are not re-tested but directly sent for MDR treatment. Hence, we suggest that it might be better to re-test all samples with very low MTB detected showing Rif resistance before initiation of MDR treatment.

INTRODUCTION

Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) is a rapid molecular diagnostic test. This test is used to diagnose Tuberculosis (TB) and detect rifampicin resistance in patients diagnosed with TB. Results are obtained in 2 hours, which helps in early detection and initiation of appropriate treatment. The CB-NAAT system detects DNA sequences specific for Mycobacterium tuberculosis complex and rifampicin resistance by Real-Time Polymerase Chain Reaction (PCR). It concentrates Mycobacterium tuberculosis bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication, and amplifies the genomic DNA by PCR. In real-time, the process identifies clinically relevant rifampicin resistance-inducing mutations in the Mycobacterium tuberculosis genome's RNA polymerase beta (rpoB) gene using fluorescent probes called molecular beacons. The limit of Detection of CBNAAT is (LOD ~ 130 CFU/ml).[4]

The single-use cartridge contains reagents for DNA extraction, PCR amplification, internal controls and five partially overlapping fluorescent probes, A, B, C, D and E, targeting the 81 bp Rifampin Resistance Determining Region (RRDR) of MTB rpoB gene. The test provides semi-quantitative MTB detection based on the probes' Cycle Threshold (Ct) – several PCR cycles are required to amplify MTB DNA to a detectable level. MTB detection result is reported as High (Ct<16), Medium (Ct 16–22), Low (Ct 22–28), or Very Low (Ct28).[5] The latest, 4th version of CBNAAT software interprets samples with >4 cycles difference in Ct values between any two probes as resistance to RIF. Because the assay terminates after 39 cycles, a sample might be reported indeterminate for RIF resistance if the first

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probe's Ct is >34.5 cycles and the last probe's CT is >38 cycles.[6]

MATERIALS AND METHODS

This cross-sectional study was conducted on 5,807 pulmonary samples in the Department of Microbiology, KAPV Government Medical College, Trichy, from January 2023 to December 2023.

Inclusion Criteria

All pulmonary samples with Rif indeterminate and resistance were included in the study.

Exclusion Criteria

All extra-pulmonary samples, samples in which TB was not detected, and samples in which TB was detected and Rif sensitive were excluded.

All samples that showed Low (Ct 22–28) and Very Low - Ct values (<28) in MTB detection were selected. Such cases were selected with very low mycobacterial load and Rif indeterminate and Rif Resistant cases. As per the Diagnostic algorithm for pulmonary TB, A Rif indeterminate will get an additional CBNAAT for a valid result. Samples with both results consistently negative were subjected to LPA or MGIT as appropriate.

Data Analysis

Data entry and analysis were done with WHONET software. The assay used version 4 cartridges according to the manufacturers' recommendations.

RESULTS

A total of 5,807 sputum samples were tested during our study period, of which 4,514 were negative, accounting for 77.73%. TB was detected high in 351 samples (6.04%) and detected medium in 358 samples (6.16%). Our concern was about the low mycobacterial load samples, which include the low MTB detected samples 213 (3.68%) and very low MTB detected samples 194(3.34%). [Table 1]

There were no indeterminate samples amongst the high and medium TB detected samples as the mycobacterial load was high. Among the low TB detected samples (213), we had 11 indeterminate samples, constituting 18.33%. Among the very low TB detected samples (194), we had 49 indeterminate samples, constituting 81.67%. [Table 2]

Among the low TB detected samples (213), we had 4 Rif resistance samples, constituting 1.88%. Among the very low TB detected samples (194), we had 8 Rif resistance samples, constituting 4.12%. [Table 3]

53 out of the 60 Rif indeterminate samples were re-tested; 22 were TB negative (41.51%), and TB was detected in 31 samples (58.49%). All 12 samples were re-tested, and two samples turned out to be TB-negative (16.67%), and ten samples turned out to be TB-positive (83.33%). [Table 4]

Table 1: Distribution of pulmonary samples

Pulmonary samples	Number	Percentage
TB not detected	4514	77.73
TB detected high	351	6.04
TB detected medium	358	6.16
TB detected low	213	3.68
TB detected very low	194	3.34
Error	113	1.95
Invalid	64	1.1
Total	5807	100

Table 2: Rifampicin indeterminate amongst TB-detected samples

Rifampicin indeterminate amongst TB-detected samples	Number	Percentage
Tb detected high	0	0
Tb detected medium	0	0
Tb detected low	11	18.33
Tb detected very low	49	81.67
Total	60	100

Table 3: Rifampicin resistance amongst low mycobacterial load

Rifampicin resistance amongst low mycobacterial load	Number	Percentage
Tb detected low	4	1.88
Tb detected very low	8	4.12

Table 4: Result of re-test of Rif indeterminate/resistant samples

Result of re-test of Rif indeterminate/ resistant samples		
	TB detected	TB not detected
Rif indeterminate	31(58.49%)	22(41.51%)
Rif resistant	10(83.33%)	2(16.67%)

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DISCUSSION

In the present study, TB was detected in 77.73% of samples, similar to the study conducted by Sajjad Ahmad et al. in 2019. We also noted that Rif's indeterminate results were found predominantly in the low and very low TB-detected samples and not in the high and medium TB-detected samples with high mycobacterial load, similar to the study by Ajbani et al. in 2019. The basis for the detection of RIF resistance is the difference between the first (early Ct) and the last (late Ct) MTB-specific beacon (ΔCt). Since the assay terminates after 38 cycles, the assay was deemed indeterminate for RIF resistance if the first probe Ct is >34.5 cycles and the last probe has a Ct of >38 cycles, as confirmed by Asish K Prakash et al. in 2018.

WHO recommends an additional CBNAAT for" RIF indeterminate result" to get a valid result. If the second result is also indeterminate, then an additional specimen should be sent for line tuberculosis probe assay (LPA) or liquid culture and drug susceptibility testing (DST).[10] In the present study, we had an indeterminate Rif result in 1.03% of samples, which correlates well with the study done by Devrari et al. in 2023, which had a rate of 1.8% indeterminate results. In our study, we could not get a repeat sample from 7 out of the 60 patients with indeterminate Rif results. We collected a repeat sample, which took 5-10 days, and did a re-test. We had 41.51% of samples that showed a negative TB result, which could be probably due to the anti-tuberculous treatment already initiated for these patients. Similarly, a re-test was done from low mycobacterial load samples, which showed Rif resistance; 16.67% of samples tested negative for TB.

CONCLUSION

There is a diagnostic implication when interpreting TB results, which shows TB detected as low and very low, especially when such cases show Rif as indeterminate or resistant. WHO recommends a repeat test in case of Rif indeterminate, but acquiring repeat samples for re-testing is challenging. Our study shows that the same recommendations can be followed for Rif-resistant cases from low- and very low-TB-detected samples.

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