



## A Case Study of Rifampicin Resistance in Newly Detected Sputum Negative Pulmonary Tuberculosis Patients by CBNAAT

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### Abstract

**Background:** Tuberculosis is the most common communicable disease causing morbidity and mortality worldwide. India is the country with the highest burden of MDR-TB. The WHO has endorsed CBNAAT in December 2010, an automated real time PCR based rapid diagnostic test, simultaneously detecting mycobacterium tuberculosis and rifampicin resistance within two hours.<sup>1,2,3</sup> WHO has recommended in 2013, the use CBNAAT in sputum AFB negative pulmonary tuberculosis, extra pulmonary tuberculosis, paediatric tuberculosis and in immune compromised patients like HIV and diabetes mellitus.<sup>4,5,6</sup> Various studies shows the prevalence of MDR/RR TB in previously treated patients.

**Aim of the Study:** This study was done to detect the rifampicin resistance in newly detected sputum AFB negative pulmonary tuberculosis patient through CBNAAT.

**Material and Methods:** This study includes 140 cases of Presumptive pulmonary tuberculosis patients who were smear negative during the period between Nov 2016 to April 2018 in all presumptive pulmonary tuberculosis patients who attended OP or got admitted in Rajah Muthiah medical college and hospital, Chidambaram. These patients were enrolled and CBNAAT was provided.

**Results:** Out of these 140 cases, MTB was detected in 54 cases (38.6%) and in rest of the 86 cases MTB not detected. Among 54 cases, 2 were rifampicin resistance and rest of them were rifampicin sensitive. These 2 rifampicin resistance was diagnosed in newly detected pulmonary tuberculosis patients. This study shows the prevalence of 3.7% MDR-TB in primary pulmonary tuberculosis.

**Conclusion:** This study shows that CBNAAT is more superior to sputum microscopy by detecting additional pulmonary tuberculosis cases and drug resistance which are missed by sputum AFB stain within few hours. We concluded that all sputum AFB negative presumptive pulmonary tuberculosis patients should undergo novel technique like CBNAAT and LPA to diagnose the undetected TB and DR-TB.

**Keywords:** Sputum AFB negative, CBNAAT, New case, Rifampicin resistance.

### Introduction

Tuberculosis is the oldest disease caused by mycobacterium tuberculosis transmitted from

infectious pulmonary tuberculosis patients through the airborne spread of droplet nuclei, most often affecting the lungs. It still remains the one of

the commonest infectious cause of death in developing countries. According to the WHO report in 2016, 10.4 million cases developed TB and 1.7 million people died from the disease including 400,000 people who are co infected with HIV. Out of 10.4million cases, 490,000 incident cases of MDR/RR – TB are reported. Out of 10.4million cases, only 6.3 million cases were detected. Remaining 4million cases were not detected. Among 10.4 million cases, 2.79 million cases were reported from India. India constitutes about 27% of global TB burden.<sup>8</sup> India ranked the top of seven globally TB burden countries followed by Indonesia, China, Philippines, Pakistan, South Africa and Nigeria. Disease caused by mycobacterium tuberculosis which is resistant to at least Isoniazid (H) and/or Rifampicin (R) with or without other first line antituberculosis drugs is defined as MDR/RR – TB. About 47% of MDR/RR-TB was in India, China and Russia. Out of 2.79million Indian cases, 147000 cases were MDR/RR –TB and 87000 were co infected with HIV. Overall mortality rate was 15.6% in India in 2016.<sup>7</sup>

Smear microscopy by Ziehl Neelsen staining which is the cornerstone for the diagnosis of tuberculosis is very rapid, cheap and easy which requires at least 10,000 bacilli per ml of sputum to make the diagnosis of tuberculosis. It has a specificity of 99% and sensitivity of only 20 – 80%.<sup>9</sup>Major disadvantage of smear microscopy is that it fails to detect the rifampicin resistance. Gold standard technique is sputum culture which takes about 6 – 8weeks for growth and has sensitivity of 80 -85% and specificity of 98%.where liquid culture (MGIT) require 4-21days for growth of at least 10-100 tuberculosis bacilli/ml. Drug susceptibility takes 15-28days.<sup>10</sup>MDR TB and XDR TB should be confirmed by culture. Major drawback of culture is time lag for initiation of appropriate treatment. Drug resistance is the major challenge to end TB. Emergence of MDR –TB is not due to single factor.<sup>11</sup> Majority of active pulmonary tuberculosis patients manifest as sputum AFB negative.

Mainstay of Tuberculosis management is early diagnosis and appropriate treatment thereby reducing the chance of transmission. To overcome all these drawbacks, the WHO has endorsed a newer rapid diagnostics technique like Line probe assay (manual) and automated technique like CBNAAT detecting DR - TB within two days and two hours respectively. CBNAAT (Cartridge based nucleic acid amplification test) which was recommended as add on test for sputum smear negative specimens and first line diagnostic test in MDR/RR – TB and HIV associated TB.

GENE XPERT (MTB/RIF - assay) recommended in 2010 as an automated hemi nested real time PCR and reverse transcriptase PCR based diagnostic tests detects both mycobacterium tuberculosis complex DNA and rifampicin resistance using 5 molecular beacons(A-E)within 2 hours.<sup>2</sup> G4 version has been recommended by the WHO from 2013 designed by Cepheid, Sunnyvale, CANADA.<sup>12</sup> It helps the physician to make expert decision in undetected cases from smear microscopy in presumptive pulmonary tuberculosis patients and to interrupt the transmission in drug resistant strains and start the appropriate treatment without any time lag on the same visit. It helps to avoid injudicious use of ATT in the better interest of patients. CBNAAT assay is performed by sample processing, DNA extraction, nucleic acid amplification and detects the target sequences and mutation in *rpo* gene which codes for rifampicin resistance.<sup>1,13,14</sup> CBNAAT has sensitivity of 99% and >80% in smear positive and smear negative cases respectively. It also has sensitivity of 95.1% and specificity 98.4% in detecting rifampicin resistance.<sup>15</sup>

### Material and Methods

This study was conducted at Rajah Muthiah Medical College and Hospital, Chidambaram, during the period between Nov 2016 to April 2018 in all presumptive pulmonary tuberculosis patients who attended OP or got admitted in our hospital. Inclusion criteria include patients above

14yrs of age with sputum smear Negative, Patients co infected with HIV, Microbiologically confirmed retreatment patients (recurrent, treatment after loss of follow up and failure cases). Exclusion criteria includes smear positive patients, extra pulmonary tuberculosis patients and patients currently on ATT drugs. Patient's demographic data was noted. Detailed history, clinical examination, routine investigation, chest X ray, sputum smears AFB staining was done. 140 Sputum negative patients were evaluated in this study and sputum sample has been sent for CBNAAT. This study was done to evaluate the role of CBNAAT in diagnosing pulmonary tuberculosis and rifampicin resistance in sputum smear negative patients. The statistical analysis was done with Pearson Chi Square test.

Sputum sample should be collected in falcon tube are mixed with sample reagent (sodium hydroxide and isopropanol) in the ratio of 2:1. Sample is mixed well and incubated for 15mins at room temperature. The treated sample is transferred into the single use plastic cartridge which is preloaded with liquid buffers and lyophilized reagent beads

required for sample processing. The CBNAAT assay is fully automated with d''38 cycles threshold. It detects mycobacterium tuberculosis when at least two of the five microbe probes show positive signals and also states rifampicin resistance when  $\Delta CT > 3.5$  cycles.<sup>1,16,17</sup> Results of CBNAAT obtained as: MTB not detected, MTB detected rifampicin resistance detected, MTB detected Rifampicin resistance not detected, invalid and indeterminate.<sup>4,19</sup>



Gene Xpert



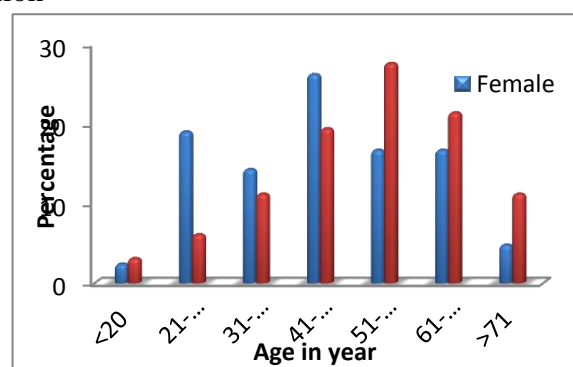
Cartridge

## Observations and Results

**Table 1:** Age vs Sex Distribution

Age group in yrs	Female	Male	Total	Percentage
<20	1	3	4	2.9%
21-30	8	6	14	10.0%
31-40	6	11	17	12.7%
41-50	11	19	30	21.4%
51-60	7	27	34	24.3%
61-70	7	21	28	20.0%
>71	2	11	13	9.3%
Total	42	98	140	100%

**Graph 1:** Age vs Sex Distribution



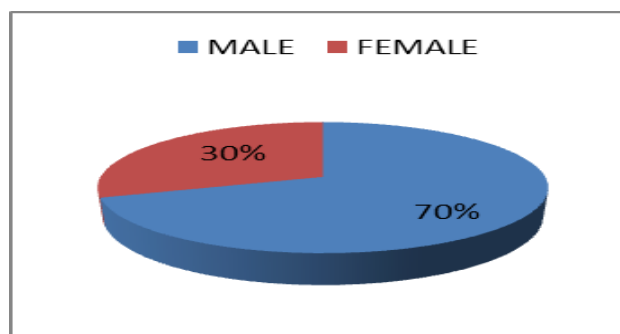
34 patients are in 51 to 60 yrs followed by 30 were in 41- 50yrs and 28 were in 61-70yrs of age group are most commonly involved age groups in this study .Only 4 patients are below 20 yrs of age

group which is least commonly involved. Other age groups like 31-40yrs, 21-30 yrs and above 71yrs are moderately involved in the frequency of 17, 14 and 13 respectively.

**Table 2:** Sex Distribution

Sex	Total	Percentage
Male	98	70%
Female	42	30%
Total	140	100%

**Graph 2:** Sex Distribution



In our study, out of 140 smear negative pulmonary tuberculosis patients, 98 patients were males and remaining 42 were female which correlates with

Mohanty et al and Ishwarappagol et al of male predominance.

**Table 3:** Clinical Features of Smear Negative Presumptive Pulmonary Tuberculosis

Symptoms	No. of Cases	%
Cough with expectoration	133	95%
Fever	90	64.2%
Breathlessness	99	70.7%
Chest pain	44	31.4%
Hemoptysis	28	20%
Wheeze	18	12.9%

Majority of patients (133) presented with cough with expectoration predominantly followed by 99 (70.7%) of them presented with breathlessness

and 90 patients (64.2%) presented with fever in this study which is similar to France Le Palud et al study (2014).

**Table 4:** Diabetic status

	Total	%
Diabetic	46	32.9%
Non diabetic	94	67.1%
Total	140	100%

Only 46 patients (32.9%) were diabetic in this study population of 140 which similar to pradeep Ishwarappagol et al study (28.6%). Rest of the

study population about 94 (67.1%) were non diabetic.

**Table 5:** Risk factor distribution in smear negative presumptive pulmonary tuberculosis patients

Risk Factor	No. of Cases	Percentage%
Smoking	80	57.1%
Alcohol	53	37.9%
Contact history	37	26.4%
Past h/o PTB	38	27.1%

In this study, 80 out of 140 patients were smokers, being the major risk factor (57.9%) followed by 53 patients were alcoholics. 38 Patients had Past

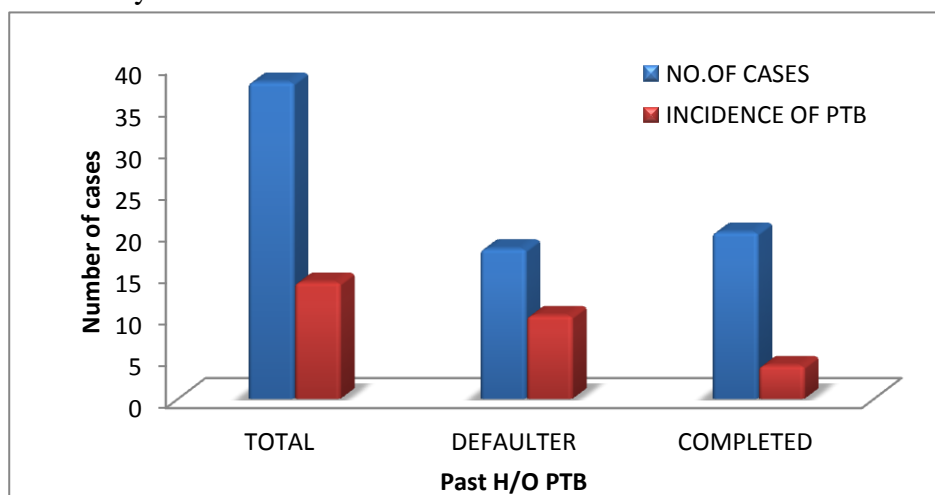
H/O PTB and 37 had Contact history constitutes about 27.1% and 26.4% respectively.

**Table 6:** Treatment History of Past PTB

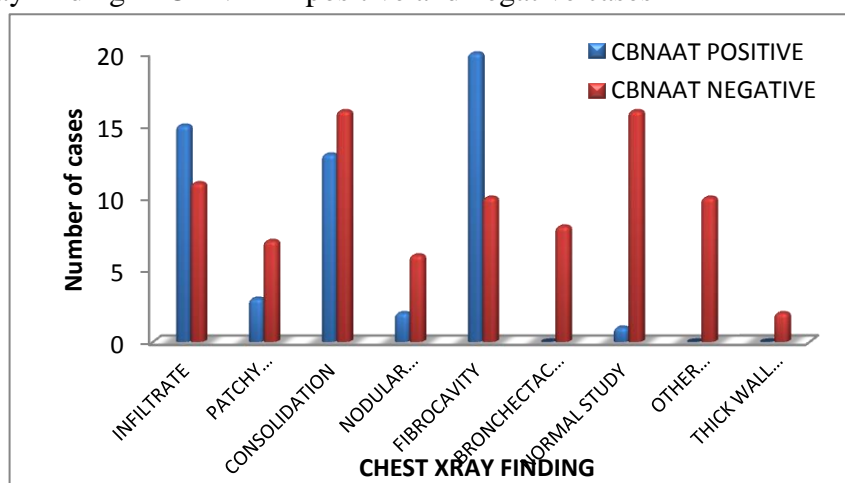
Past H/O PTB	No. of cases	Percentage%	Incidence of PTB
Treatment after loss to follow up	18	47.4%	10
Completed	20	52.6%	4
Total	38	100%	14

Out of 38 patients with past h/o PTB, 18(47.6%) were Treatment after loss to follow up and remaining 20 patients completed the treatment.

Among these 18, 10 of them developed reactivation and among 20 who completed their retreatment had recurrence.

**Graph 3:** Treatment History of Past PTB**Table 7:** Chest X-ray Finding

Chest xray finding	No. Of cases	Percentage	CBNAAT Positive	CBNAAT Negative
Infiltrate	26	18.6%	15	11
Patchy infiltrate	10	7.1%	3	7
Consolidation	29	20.7%	13	16
Nodular opacity	8	5.7%	2	6
Fibrocavity	30	21.4%	20	10
Bronchectatic changes	8	5.7%	0	8
Normal study	17	12.1%	1	16
Other abnormalities	10	7.1%	0	10
Thick wall cavity	2	1.4%	0	2
Total	140	100%	54	86

**Graph 4:** Chest X-ray finding in CBNAAT positive and negative cases

In this study, 36 patients had radiological finding of Infiltrate including patchy infiltrate is the most common radiological finding reported followed by 30 had fibro cavity lesion and 29 patients had consolidation. In CBNAAT positive cases, fibro

cavity and infiltrate being predominate, whereas consolidation in CBNAAT negative cases which is same line with Poojan shretha et al study with infiltrate predominate in CBNAAT positive cases.

**Table 8:** Chest X Ray Involvement

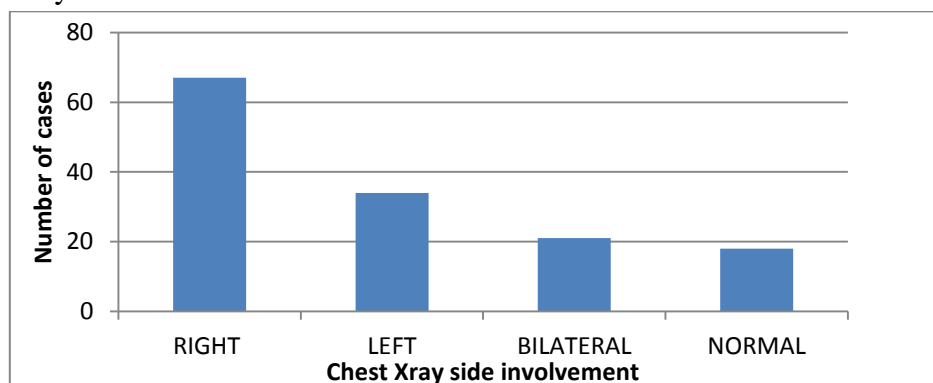
Xray involvement	No. Of cases	Percentage
UZ	58	41.4%
MZ	5	3.6%
LZ	31	22.1%
UZ+MZ	2	1.4%
MZ+LZ	11	7.9%
All zone	16	11.4%
Normal	17	12.1%
Total	140	100%

76 patients of 140 cases presented with upper zone involvement predominately followed by lower

zone involvement and less frequently middle zone are involved.

**Table 9:** Chest X-ray Side Involvement

Chest x-ray side involvement	No.of cases
Right	67
Left	34
Bilateral	22
Normal	17

**Graph 5:** Chest X-ray Side Involvement



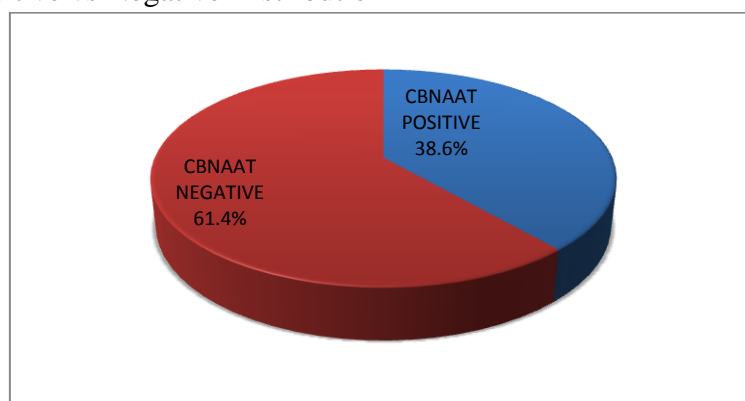
Right sided radiological finding are most commonly involved in this study. About 67 cases reported to have right sided involvement. 17 of them reported to have normal chest X ray. Rest left sided and bilateral involvement have more or else equal distribution in 34 and 22 cases respectively.

**Table 10:** CBNAAT Positive vs Negative Distribution

CBNAAT Positive	54	38.6%
CBNAAT Negative	86	61.4%
Total	140	100%

Out of 140 cases, 98 cases were reported to be CBNAAT positive and rest of the 86 patients were reported as CBNAAT negative. Among these 86 cases, 2 of them were started on ATT based on their Xray finding. These 2 patients who reported as CBNAAT negative may due to paucibacillary, inadequate sputum sample and small lung lesion. 38.6% of CBNAAT positivity correlates with other Pradeep Ishwarappagol et al study of 36.3% and 37.5% of Sowjanya et al.<sup>17, 18</sup>

**Graph 6:** CBNAAT Positive vs Negative Distribution



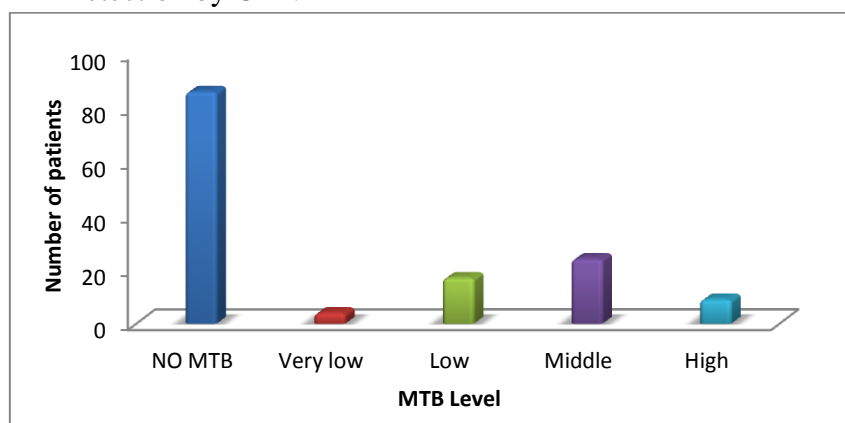
**Table 11:** Level of MTB Detection by CBNAAT

	Total	Percentage
No MTB	86	61.4
Very low	4	2.9
Low	17	12.1
Medium	24	17.1
High	9	6.4
Total	140	100.0

Out of 54 CBNAAT positive cases, majority of cases detected as medium level of MTB in 24

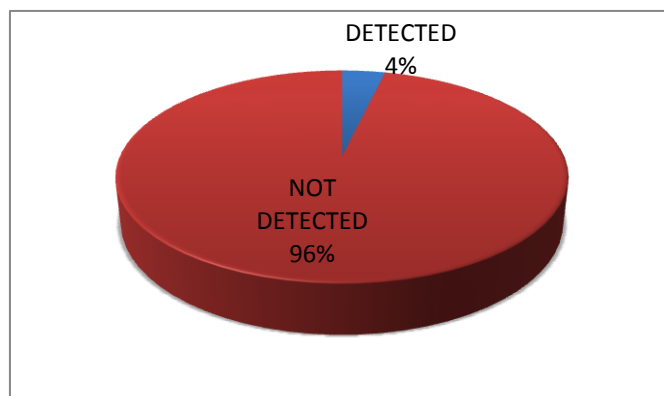
cases, low level in 17 cases, 9 of them as High level and very low level in only 4 of the cases.

**Graph 7:** Level of MTB Detection by CBNAAT



**Table 12:** Rifampicin Resistance Detection

Rifampicin resistance	No.of cases	Percentage
Detected	2	3.7%
Not detected	52	96.3%
	54	100%

**Graph 8:** Rifampicin Resistnace Distribution

Rifampicin resistance was detected in 2 out of 54 smear negative MTB detected cases which is similar to Sowjanya et al (3.6%)

### Discussion

In present study, out of 140 cases, 98 (70%) were males and rest 42 (14%) were females. Male predominance was in same line with similar with Mohanty et al (23/71) and Ishwarappagol et al (28/80) studies. In this study population, 51- 60yrs age group was commonly affected. Cough with expectoration followed by breathlessness being the predominant features in this study as same as France Le Palud et al (2014) and Mishra et al study.<sup>20,21</sup> 32.9% of study population were diabetics; out of them, 17 were detected to PTB. Among these diabetic PTB patients, 10 (59.1%) have lower zone involvement as same as Pradeep Ishwarappagol et al study.<sup>18</sup> Only 5(3.6%) of study population were HIV reactive. Rest of 135(96.4%) were HIV Non-reactive. Out of 5 PLHIV, only 2 of them were reported as MTB detected.

Smoking and alcohol being a major risk factor contributes about 57.1% (80) and 37.9% (53) for causing pulmonary tuberculosis in this study population respectively. Among study population, 37 (26.4%) have positive contact history. 27.1% (38) of study population have past history of

pulmonary tuberculosis. Out of these, 18 were defaulter and rest of the 20 completed the treatment. 35% (49) of study population are in low BMI group. Infiltrates (36) are the most common radiological lesion in this study similar to poojan shrestha et al<sup>22</sup> (2015) followed by fibro cavity and consolidation. In CBNAAT positive cases, fibro cavity and infiltrate reported as predominant finding whereas in CBNAAT negative, consolidation is the more frequently reported correlating with Poojan Shretha et al.

This study consists of 140 smear negative presumptive pulmonary tuberculosis cases. CBNAAT was conducted. Out of these 140 cases, MTB was detected in 54 cases (38.6%) and in rest of the 86 cases MTB not detected. Among 54 cases, 2 were rifampicin resistance and rest of them were rifampicin sensitive. Among 86 MTB not detected cases, 2.3% (2/86) of them were started on ATT drugs based on the clinical features and chest X-ray finding. The sensitivity and specificity in smear negative sputum sample correlates with other studies from 57% – 75% and 97 – 100% respectively.<sup>23 -26</sup> In this study MTB was detected at medium level in 24 cases (17.1%) followed by low level in 17(12.1%) of them.

Rifampicin resistance has been reported in 2 out of 54 CBNAAT positive cases, which is similar to Sharma et al study. These 2 rifampicin resistance



was diagnosed in newly detected pulmonary tuberculosis patients. Interestingly both of them have lower zone involvement. Drug surveillance was performed in many countries indicating the prevalence of MDR TB in 2-3% of newly diagnosed pulmonary tuberculosis patient and 17% of retreated cases. This study shows the prevalence of 3.7% MDR-TB in new case which correlates with state-wide study done at Gujarat by ramachandranetal.<sup>27</sup>



**Chest x-ray of rifampicin resistance in new smear negative cbnaat positive pulmonary tuberculosis**

### Conclusion

In this study, we concluded that CBNAAT is more superior to sputum microscopy by detecting additional pulmonary tuberculosis cases which are missed by sputum AFB stain. CBNAAT's additional benefit of detecting rifampicin resistance gives confidence to many clinicians by starting appropriate treatment on the same day and reducing the risk of transmission of MDR TB. Various studies prove that MDR TB is common

among retreated cases. But this study proves the possibilities of MDR TB in new smear negative CBNAAT positive pulmonary tuberculosis. We concluded that all sputum AFB negative presumptive pulmonary tuberculosis patients should undergo novel technique like CBNAAT and LPA to diagnose the undetected TB and DR – TB. CBNAAT negative does not exclude the diagnosis of tuberculosis.

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