

Prevalence of Rifampicin-resistant *Mycobacterium tuberculosis* by CBNAAT in a Tertiary Care Hospital of West Bengal, India

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Abstract

Background: Tuberculosis (TB) remains one of the commonest infectious diseases in India, where control is difficult due to the emergence of multi-drug-resistant TB (MDR-TB). The rifampicin resistance (RR) is an important surrogate marker of MDR-TB. **Aims and Objectives:** The aim of this article is to determine the prevalence of RR-TB among all symptomatic presumptive TB patients by cartridge-based nucleic acid amplification test (CBNAAT) in our institution. **Materials and Methods:** A retrospective cross-sectional study was conducted from January 2018 to December 2020 in the Directly Observed Treatment (DOT) center of a tertiary care hospital. All presumptive cases of pulmonary TB patients coming to the DOT center were subjected to CBNAAT by GeneXpert *Mycobacterium tuberculosis* rifampicin (MTB/RIF) (Cepheid). Data were collected from a data-extraction sheet from registration books and analyzed using SPSS version 20. Fisher's exact test was applied to examine the association between categorical variables and *P*-value less than 0.05 was considered significant. **Results:** A total of 6171 presumptive TB patients were included in the study. The overall prevalence of TB was 1590 (25.77%) in all age groups. The prevalence of RR-TB among all confirmed TB cases was 10.75% (171/1590) with the majority being males (130 cases). The prevalence of RR cases among new and previously treated was 3.1% and 36.34%, respectively, which were statistically significant (*P* < 0.05). **Conclusion:** We found that RR-TB is still much prevalent in our area, and it was significantly higher in previously treated cases. The CBNAAT is a good molecular assay method for rapid detection of TB and also for the detection of RR-TB.

Keywords: CBNAAT, MDR-TB, rifampicin resistance, tuberculosis

INTRODUCTION

Tuberculosis (TB) is one of the oldest known diseases that affect humans and remains one of the commonest infectious diseases in India till date.^[1,2] Diagnosis, treatment, and control of TB become difficult with the emergence of multi-drug resistance TB (MDR-TB), with rifampicin resistance (RR) being an important surrogate marker.^[2,3] According to the World Health Organization (WHO) Global TB report, the estimated burden of TB in 2019 was about 10 million population worldwide and two-thirds of it were contributed by eight countries with the highest proportion of cases being reported from India (26%), followed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.^[4]

The incidence of MDR-TB has been increasing all over the world, both among newly diagnosed TB cases and previously treated cases.^[3] The strongest risk factor for the development of MDR-TB is a previous treatment followed by spontaneous mutations or transmission of resistant strains among newly detected TB patients.^[5-7]

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Globally, 465,000 people fell ill with RR-TB in 2019, of which 206,030 people were identified as MDR-TB, which is a 10% increase from 2018. The treatment success rate for MDR/RR-TB is 57% globally, which is very low.^[4]

WHO along with the Revised National Tuberculosis Control Programme (RNTCP), India has affirmed Gene Expert MTB/RIF assay for the rapid detection of TB with RR in every district hospital as well as tertiary care centers.^[2] It has been shown that detection of RR is very important as it can be used as a surrogate marker for MDR-TB in about 90% of cases.^[8] For this reason, WHO recommends that RR-TB patients should be treated similar to those with MDR-TB.^[9]

Cartridge-based nucleic acid amplification test (CBNAAT), a novel diagnostic test, is an automated, semi-quantitative real-time polymerase chain reaction (PCR) assay that detects MTB with RR simultaneously within 2 h.^[10] This diagnostic tool is more sensitive than smear microscopy, even conferring the RIF resistance mutations directly from the sputum and other body fluids.^[2] Although MTB culture in Lowenstein–Jensen (LJ) media remains the gold standard technique for determining the infection, it takes several weeks to generate thus resulting in a delay in treatment initiation. Also, RR cannot be detected in culture.^[11] Moreover, CBNAAT by GeneXpert MTB/RIF is a cost-effective molecular assay unlike others such as latest-generation liquid culture diagnostics and molecular line probe assays which are costly and need biosafety measures and specialized staff. Therefore, CBNAAT, which is highly specific, having five unique molecular probes to target the *rpoB* gene of MTB associated with RR, is an esteemed diagnostic tool.^[12]

Considering the above facts, and that there is no such study in this part of our country where our institution is situated, we had undertaken this study with the aim to detect the prevalence of RR-TB among all presumptive pulmonary TB patients referred to the Directly Observed Treatment (DOT) center for CBNAAT at our institute.

MATERIALS AND METHODS

Study design, area, and period

This was a retrospective, observational, record-based study conducted from January 2018 to December 2020 in a tertiary healthcare institution situated in the state of West Bengal, India. The study population was all sputum smear-positive symptomatic patients and all presumptive pulmonary TB patients who visited the DOT center for CBNAAT during the study period.

Definition of presumptive tuberculosis^[13]

Presumptive pulmonary TB is defined as patients with any symptoms and signs suggestive of TB including cough >2 weeks, fever >2 weeks, significant weight loss, hemoptysis, and any abnormality in chest radiograph.

Inclusion and exclusion criteria

Inclusion criteria

The patients whose clinico-demographic data and CBNAT reports along with RR reports available were included in the study regardless of age.

Exclusion criteria

Human immunodeficiency virus (HIV)-positive cases, diabetic patients, rheumatic patients, and cancer patients were excluded from the study.

Laboratory investigation and data collection

From presumptive pulmonary TB cases, samples of sputum, bronchoalveolar lavage, and bronchial aspirations were obtained. Sputum smears were made from the samples and stained with Ziehl–Nielsen (Z–N) stain and examined under a microscope to detect acid-fast bacilli (AFB). Samples were processed as per standard operating procedures led by GeneXpert MTB/RIF guidelines. In case of presumptive pulmonary TB, 2–4 mL of sputum sample was collected. The samples were mixed with the buffered solution in the ratio 1:2. Then the whole solution was made to stand for 15–20 min with intermittent shaking. The mixer solution was then transferred to the GeneXpert cartridge in the GeneXpert MTB/RIF system. Then the tests were run on CBNAAT machine as per procedures laid by the manufacturer.

Data regarding demographic variables, TB-positive, and RR-TB cases were extracted from records.

Statistical analysis

Data were entered in EPI Info version 7 and then exported to SPSS version 20 for analysis. Proportions and crude odds ratio (COR) were calculated. Fisher's exact test was applied to examine the association between categorical variables and *P*-value less than 0.05 was considered significant.

Ethical consideration

The study was approved by the institutional Ethics Committee before commencing the study vide memo no. IEC/2021/02/003 dated February 12, 2021. Individual patient consent was not taken as it was a retrospective record-based study.

RESULTS

A total of 6171 presumptive pulmonary TB cases were included in our study. Out of these, 1590 (25.77%) patients were diagnosed as TB in GeneXpert MTB/RIF assay in all age groups, of which 1292 (81.26%) were males and 298 (18.74%) were females [Table 1]. The youngest RR-TB patient was 13 years old female, and the oldest RR-TB

patient was 67 years old male. Majority of the cases belonged to the 21–60 years age group.

The overall prevalence of RR-TB was 2.77% (171/6171) among presumptive TB patients and 10.75% (171) in all TB-diagnosed patients [Table 2]. Though the prevalence of RR-TB was more among males, the association is not statistically significant ($P = 0.0772$) [Table 2]. Among various age groups, the maximum number of RR-TB patients was in 21–30 years (48); however, COR in all age groups was <1 , signifying that age had lower odds [Table 3]. Previously treated cases had 17.82 times the odds of RR-TB when compared with new cases, and this difference was statistically significant also [Table 4].

DISCUSSION

Although there is a considerable advancement in the diagnosis and treatment, TB continues to be prevalent in many developing countries including India. TB is one of the top 10 causes of mortality across the world. The rate of decline of annual incidence and mortality rate is slow, i.e., 2% and 3%, respectively.^[14] Various factors are responsible for hindrance against TB control such as lack of early diagnosis, inappropriate treatment and a delay in treatment initiation, poor socioeconomic status, lack of awareness among people, etc.

Table 1: Sociodemographic and clinical characteristics of tuberculosis patients ($n = 1590$)

Variables	Total TB patients	Percentage (%)
Sex		
Male	1292	81.26
Female	298	18.74
Age group (in years)		
0–10	0	0
11–20	113	7.11
21–30	356	22.39
31–40	391	24.59
41–50	322	20.25
51–60	217	13.65
61–70	148	9.31
>70	43	2.70
History of TB treatment		
Previously Rx cases	366	23.02
New cases	1224	76.98

In our study, the prevalence of *Mycobacterium tuberculosis* was found to be 25.77% among the presumptive pulmonary TB patients. Previous studies from India have reported the prevalence of CBNAAT-confirmed TB cases among those suspected in the range of 16.36% and 32.9%, which is comparable to our study.^[15–17] Studies from outside of India, mainly from African subcontinent, have reported a prevalence of TB between 15.11% and 37.7%.^[18–20] The difference in variation of prevalence of TB cases may be related to various factors such as geographic variation, method of diagnosis, TB control practices, sociodemographic factors, laboratory method used, awareness among people, etc.

We found much higher positivity among males when compared with females (81.26% vs. 18.74%), which is in with the global picture. The prevalence of TB among males is found to be higher than females both in India and abroad. This reflects that males are affected more than females by TB.^[21] The reason for high positivity among men could be because of the fact that they seek health care more often than women, whereas women tend to self-medicate.^[22]

The Government of India is committed to eliminate TB from India by the year 2025 as stated by the National Strategic Plan (NSP) 2017–2025.^[23] Under RNTCP, every presumptive pulmonary TB patient should undergo sputum microscopy test for AFB. Nowadays, with the use of GeneXpert MTB/RIF assay by CBNAAT, the turnover time for TB diagnosis is reduced with the added advantage of testing of RR.^[10] The test detects mutation in the *rpoB* gene and is able to differentiate between conserved wild-type sequence and mutations in the core region that is associated with RR.^[16] So, for early and rapid diagnosis of TB cases along with detection of RR, the CBNAAT method is used under RNTCP.^[24] RR is also associated with resistance to other antitubercular drugs, and so RR can be successfully utilized as a surrogate marker for MDR-TB.^[2,3,25]

One of the important factors that hurdle the control of TB is the existence of RR-TB. RR-TB is less likely to respond to first-line antitubercular drugs and therefore their early detection is of utmost important for treatment and prevention of the further spread of drug-resistant TB. Drug-resistant TB may develop when there is inadequate treatment of active cases of pulmonary TB and spread of TB from drug-resistant cases or rarely by spontaneous mutation. There may

Table 2: Prevalence of rifampicin-resistant TB in TB patients according to sex distribution

Gender	RR-TB (%)	Not RR-TB (%)	COR (95% CI)	P-value
Male	130 (8.18)	1162 (73.08)	Reference	
Female	41 (2.58)	257 (16.16)	1.43 (0.98–2.08)	0.0772
Total	171 (10.75)	1419 (89.25)		

TB = tuberculosis, RR-TB = rifampicin-resistant tuberculosis, COR = crude odds ratio

Table 3: Prevalence of rifampicin-resistant TB in TB patients according to age (n = 1590)

Age group in years	RR-TB, N (%)	Non RR-TB, N (%)	COR (95% CI)
11–20	24 (21.24)	89 (78.76)	Reference
21–30	48 (13.48)	308 (86.52)	0.58 (0.34–1.00)
31–40	31 (7.93)	360 (92.07)	0.32 (0.18–0.57)
41–50	30 (9.32)	292 (90.68)	0.38 (0.21–0.69)
51–60	14 (6.45)	203 (93.55)	0.26 (0.13–0.52)
61–70	24 (16.22)	124 (83.78)	0.72 (0.38–1.34)
>70	0 (0)	43 (100)	—

TB = tuberculosis, RR-TB = rifampicin-resistant tuberculosis, COR = crude odds ratio, CI = confidence interval

Table 4: Prevalence of rifampicin-resistant TB in TB patients according to treatment history (n = 1590)

TB treatment history	RR-TB, N (%)	Non RR-TB, N (%)	COR (95% CI)	P-value
Previous Rx cases	133 (36.34)	233 (63.66)	17.82 (12.1–26.23)	0.00001
New cases	38 (3.10)	1186 (96.90)	Reference	

TB = tuberculosis, RR-TB = rifampicin-resistant tuberculosis, COR = crude odds ratio, CI = confidence interval

be multiple reasons for inadequate therapy which include irregular medication by patients, adverse effects leading to stoppage of drugs, inappropriate prescribing practices such as insufficient duration of treatment, selection of poor drugs, etc.^[26,27] Our study has found less RR prevalence (10.75%) when compared with previous studies (14.6–26.1%), which may be due to different patient selection criteria, geographic variation, and variation in sample size (smaller the sample size, more likely higher resistance rate).^[14,18,20,28] Many of the patients acquire drug resistance from the environment in which the prevalence of drug resistance cases is high.^[28] Thus, estimation of RR-TB is also important from the epidemiological point of view as it gives an estimate of the prevalence of MDR-TB in community.

The prevalence of RR-TB was found to be higher in the 11–20 years age group (21.24%). We have not found any definite cause of higher prevalence in this age group. In the present study, RR-TB was significantly high among patients previously treated with antitubercular drugs. This finding corroborates well with previous studies.^[14,18,20] This may be due to various factors such as non-adherence to treatment and incomplete and inadequate treatment. However, a good number of naive patients also developed resistance to rifampicin as found in our study. This finding suggests that there is presence of active person-to-person transmission and the existence of undiagnosed new RR-TB cases in our area. Again, the high percentage of RR-TB cases among previously treated cases indicates that there must be some sort of poor performance, especially in patient follow-up and compliance of TB control program.

Limitations of our study

There are a few limitations of our study. First, we have done a retrospective study in which we are not able to

collect data from the participants, which are associated with a high prevalence of TB, like average monthly income, history of cigarette smoking, level of education, etc. As these variables are usually associated with high prevalence of TB, we are not able to analyze these factors.

Secondly, we collected data for presumptive pulmonary TB only as data of extrapulmonary TB were not available. Had both pulmonary and extrapulmonary cases been included, the exact picture of the burden of TB and prevalence of RR could have been estimated.

CONCLUSION

The current study has revealed that TB is quite prevalent among presumptive TB patients, which indicates that cases are reporting early and awareness about TB is high. The RR-TB is also much prevalent among confirmed TB cases, and it is significantly associated with the previous history of anti-TB therapy. Early case detection and appropriate case management is a much-needed element to minimize the spread of drug resistance in the community. The study provides a rough estimate of drug-resistant TB in this area and may help the stakeholders to plan and manage drug-resistant TB in this geographic area.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Moule MG, Cirillo JD. *Mycobacterium tuberculosis* dissemination plays a critical role in pathogenesis. Front Cell Infect Microbiol 2020;10:65.

2. Sujatha R, Deepak S, Nashra A, Pratima. Detection of rifampicin resistance by CBNAAT in sputum samples at a tertiary centre in Kanpur. *Rama Univ J Med Sci* 2019;5:1-7.
3. WHO/IUATLD Global Anti-Tuberculosis Drug Resistance in the World. Fourth Global Report (WHO/HTM/TB/2008.394). Geneva: World Health Organization; 2008.
4. WHO Global Tuberculosis Report 2020. Geneva: World Health Organization; 2020. Available from: https://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf. [Last accessed on June 10, 2021].
5. Sharma SK, Mohan A. Multidrug-resistant tuberculosis: A menace that threatens to destabilize tuberculosis control. *Chest* 2006;130:261-72.
6. Espinal MA, Laserson K, Camacho M, Fusheng Z, Kim SJ, Tlali RE, *et al.* Determinants of drug-resistant tuberculosis: Analysis of 11 countries. *Int J Tuberc Lung Dis* 2001;5:887-93.
7. Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis in India. *Indian J Med Res* 2004;120:377-86.
8. O'Riordan P, Schwab U, Logan S, Cooke G, Wilkinson RJ, Davidson RN, *et al.* Rapid molecular detection of rifampicin resistance facilitates early diagnosis and treatment of multi-drug resistant tuberculosis: Case-control study. *PLoS One* 2008;3:e3173.
9. World Health Organization. WHO Treatment Guidelines for Drug-Resistant Tuberculosis, 2016 Update. WHO/HTM/TB/2016.04 [Internet]. Geneva: World Health Organization; 2016. Available from: <http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/resources/en/>. [Last accessed on 2021 Nov 20].
10. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, *et al.* Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J Clin Microbiol* 2010;48:2495-501.
11. Das PK, Ganguly SB, Mandal B, Khan A. Prevalence of rifampicin-resistant pediatric tuberculosis by cartridge-based nucleic acid amplification test at the intermediate reference laboratory under revised National Tuberculosis Control Program, India: A multi-dimensional approach. *Biomed Biotechnol Res J* 2018;2:300-5.
12. Agrawal N, Patel P, Modi K, Amin V, Kapadiya D, Patel GC, *et al.* Role of CB-NAAT in diagnosis of mycobacterial tuberculosis and rifampicin resistance among key population under programmatic condition in Gujarat, India. *Healthline J* 2020;11:59-63.
13. Revised National TB Control Programme (RNTCP): Technical and Operational Guidelines for Tuberculosis Control in India. New Delhi: Government of India; 2016.
14. World Health Organization. Global Tuberculosis Report 2017. Geneva, Switzerland: WHO; 2017. WHO/HTM/TB/2017.23.
15. Gautam PB, Mishra A, Kumar S. Prevalence of rifampicin resistant *Mycobacterium tuberculosis* and associated factors among presumptive tuberculosis patients in eastern Uttar Pradesh: A cross sectional study. *Int J Community Med Public Health* 2018;5:2271-6.
16. Shunmugam M, Pratheeban J, Karthiga G. A study on the evaluation of CBNAAT—A novel diagnostic tool for rapid and specific diagnosis of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens in a tertiary care hospital of South India. *J Clin Sci Med Res* 2019;7:665-8.
17. Raj A, Baliga S, Shenoy MS, Dhanashree B, Mithra PP, Nambiar SK, *et al.* Validity of a CB-NAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India. *J Clin Tuberc Other Mycobact Dis* 2020;21:100198.
18. Arega B, Menbere F, Getachew Y. Prevalence of rifampicin resistant *Mycobacterium tuberculosis* among presumptive tuberculosis patients in selected governmental hospitals in Addis Ababa, Ethiopia. *BMC Infect Dis* 2019;19:307.
19. Alemu A, Tesfaye E, Yaregal Z, Amare M, Dagne Z, Addise D, *et al.* Status of Xpert MTB/RIF assay implementation in Ethiopia. *J Infect Dis Ther* 2017;5(suppl):3.
20. Adejumo OA, Olusola-Faleye B, Adepoju V, Bowale A, Adesola S, Falana A, *et al.* Prevalence of rifampicin resistant tuberculosis and associated factors among presumptive tuberculosis patients in a secondary referral hospital in Lagos Nigeria. *Afr Health Sci* 2018;18:472-8.
21. Neyrolles O, Quintana-Murci L. Sexual inequality in tuberculosis. *Plos Med* 2009;6:e1000199.
22. Hoa PN, Thorson AEK, Long NH, Diwan VK. Knowledge of tuberculosis and associated health-seeking behaviour among rural Vietnamese adults with a cough for at least three weeks. *Scand J Public Health* 2003;31(Suppl. 62):59-65.
23. Khaparde SD. The National Strategic Plan for tuberculosis step toward ending tuberculosis by 2025. *J Mahatma Gandhi Inst Med Sci* 2019;24:17-18.
24. Patel M, Bhavsar K, Rami K, Vala A, Shringarpure K. Role of CBNAAT in diagnosis of new pulmonary TB cases under RNTCP: A retrospective analysis from Ahmedabad, India. *Indian J Immunol Respir Med* 2019;4:221-23.
25. Andarge DB, Anticho TL, Jara GM, Ali MM. Prevalence of *Mycobacterium tuberculosis* infection and rifampicin resistance among presumptive tuberculosis cases visiting tuberculosis clinic of Adare General Hospital, Southern Ethiopia. *Sage Open Med* 2021;9:20503121211045541.
26. World Health Organization. The Global MDR-TB and XDR-TB Response Plan 2007–2008. 2007. Available from: https://www.who.int/tb/publications/2007/global_response_plan.pdf. [Last accessed on March 23, 2021].
27. Onyedum CC, Alobu I, Ukwaja KN. Prevalence of drug-resistant tuberculosis in Nigeria: A systematic review and meta-analysis. *PLoS One* 2017;12:e0180996.
28. Ikuabe PO, Ebuonyi ID. Prevalence of rifampicin resistance by automated GeneXpert rifampicin assay in patients with pulmonary tuberculosis in Yenagoa, Nigeria. *Pan Afr Med J* 2018;29:204.