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Study of gene patterns for Rifampicin in LPA, which were discordant in CBNAAT in the state of Telangana, India

Abstract

Introduction: Among the first line drugs used for treatment of Drug Sensitive Tuberculosis, Rifampicin plays a crucial role. Hence it is important to know the resistance of Rifampicin using molecular technologies like CBNAAT and line probe Assay. However, a diagnostic dilemma occurs when there is discordance in result of rifampicin resistance at periphery and in higher laboratories like Intermediate Reference Laboratory (IRL).

Aims and objectives: To study various patterns of Gene for rifampicin that is demonstrated in probes of Line Probe Assao for First line –Line Probe Assay, which showed discordance with CBNAAT at periphery.

Methodology: A retrospective observational study was conducted from 1st January 2021 to 31st December 2021 for all those samples which showed rifampicin discordance.

Result: A total of 203 results were found discordant in Rifampicin. The resolution for rifampicin discordance is resolved using the NAAT facility at (IRL). Most of the resolution occurred in concurrence with result of Rifampicin in field. Eight of them gave inconclusive results (MTB not detected).

Conclusion: It is very important to understand the gene patterns in LPA for providing appropriate regimen to the patients.

Keywords: Rifampicin, discordance, gene patterns, NAAT, LPA

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Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis is disease of major concern as India accounts to 27% of Global burden. Diagnosis of drug resistant TB and their management remained challenge to National TB program because of poorer outcomes. The important first line drugs Isoniazid, Rifampicin, Pyrazinamide and Ethambutol remained powerful weapons to combat TB disease when used in correct doses and duration.

National drug resistant surveys in 2016 mentioned that Rifampicin resistance is observed in 2-3 % in new cases and 11-12% in retreatment cases, overall amounting to 6% of all TB Cases. The tests to detect Rifampicin status have been increasing at district level and sub district level. The molecular tests like CBNAAT (Cartridge Based Nucleic Acid Amplification Test) and FLLPA (First Line –Line Probe Assay). First line Probe Assay gives information of resistance about two drugs i.e. Isoniazid and Rifampicin. The genes that are detect for Isoniazid are *Kat G* and *Inh A* whereas *rpoB* is the gene detected for Rifampicin.

Rifampicin was discovered in 1965, marketed in Italy in 1968, and approved in the United States in 1971. Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase. Crystal structure data and biochemical data suggest that rifampicin binds to the pocket of the RNA polymerase β subunit within the DNA/RNA channel, but away from the active site. The inhibitor prevents RNA synthesis by physically blocking elongation, and thus preventing synthesis of host bacterial proteins. By this "steric-occlusion" mechanism, rifampicin blocks synthesis of the second or third phosphodiester bond between the nucleotides in the RNA backbone, preventing elongation of the 5' end of the RNA transcript past more than 2 or 3 nucleotides.

Rifampicin is the drug used in combination for treatment of Mycobacterium tuberculosis, Mycobacterium leprae, A typical mycobacterium, Zoonotic infections like Brucella species.

Resistance to rifampicin arises from mutations that alter residues of the rifampicin binding site on RNA polymerase, resulting in decreased affinity for rifampicin. Resistance mutations map to the *rpoB* gene, encoding the beta subunit of RNA polymerase. An alternative mechanism of resistance is through Arr-catalyzed ADP-ribosylation of rifampicin. With the assistance of the enzyme Arr produced by the pathogen *Mycobacterium smegmatis,* ADP-ribose is added to rifampicin at one of its ansa chain hydroxy groups, thereby inactivating the drug.

Rational of study

Rifampicin resistance is surrogate marker for Multi drug resistance (MDR). The resistance for rifampicin prompts program managers to tailor the suitable treatment regimen.

It is very important to know the status of rifampicin in diagnosis and management of DRTB. The status of rifampicin determines the MDR status. CBNAAT /Tru NAAT are molecular tests which will not only detect TB infection but also determine the resistance to Rifampicin. The CBNAAT (cartridge based Nucleic acid amplification test) at district level and sub district level are made available as expansion of diagnostic services for DRTB.

As part of diagnostic DRTB (Drug resistant Tuberculosis) algorithm 2021 in National TB Elimination Program, India, and all the samples which detected Rif resistance should undergo both first line and second line Probe Assay along with liquid Culture. The First line LPA not only reconfirms Rif's resistance but also gives information about

genes related to Isoniazid (Inh A & Kat G). Similarly, the samples where Gene Xpert showed no resistance to rifampicin, are subjected to FLLPA. At times there is an observation that the discordance in the both molecular tests leads management of such TB patient in cross roads.

Research question

1) What are reasons for rifampicin discordance?
2) What are suitable solution to resolve to design appropriate DRTB regimen?

Aim of the study

To study various patterns of Gene that are demonstrated in probes of Line Probe Assay for First line –Line Probe Assay which were discordant from the results in CBNAAT.

Objectives

1) To understand the patterns of rifampicin resistance detected and rifampicin resistance inferred which showed rifampicin resistance detected in CBNAAT at district and sub district level.
2) To resolve discordance by offering CBNAAT at Culture and drug susceptibility laboratory (C&DST) which prompts the appropriate decision on Rifampicin Status.

Methodology

All the consecutive samples will be taken from January 1st 2021 to December 31th 2021, which showed discordance in Rifampicin.

Study period: January 2021- December 2021

Place of Study: State TB training and demonstration Center, Hyderabad.

Samples size: All consecutive samples which showed rifampicin discordance during the study period.

Type of Study: retrospective Observational study

Inclusion criterion

1) All samples which are Rif resistant at district level and rif resistance not detected in FLLPA at C&DST laboratory
2) All samples which were Rif's resistance not detected and Rif Resistance detected in FLLPA at C&DST laboratory.

Exclusion criterion

There was no exclusion criterion for this study.

All samples which showed discordance in rifampicin resistance are recorded. The samples showing discordance are resolved by performing Gene Xpert again at C&DST laboratory. Two out of three molecular test performed is taken as the final result of Rifampicin.

The entire data was collected in Excel sheet and will be updated according.

Training was being given to assigned person on updating of the data. Appropriate statistics was applied along with demographics.

Results

A total of 40 TruNAAT and 38 CBNAAT were available in public sector in the state of Telangana in the year 2021. First line LPA was offered in Public sector at Intermediate Reference laboratory and is collocated at State TB training and Demonstration Center (STDC), Hyderabad, Telangana, India.

A total of 203 were found to be rifampicin discordant when tested in NAAT at districts and LPA at Reference laboratory in the year 2021.

The more number of samples were shown in districts of Hyderabad, Sangareddy, Rangareddy and Nalgonda.

Kumurambheem Asifabad was only district which did not show any discordance in Rifampicin when tested in CBNAAT and TruNAAT.

Only one discordant result was seen in Mahabubnagar, Nagarkurnool, Rajanna Siricilla and Yadadri bhongir districts.

All the districts had TruNAAT facility at the time of study. Only one district of Narayanpet did not had CBNAAT facility at the time of study.

Having NAAT facility in the districts was very important in conducting this study as this had impact and the diagnosis of Rifampicin resistance. District wise distribution is shown in Table 1.

Table 1 District wise distribution of discordance in rifampicin

S. no	Name of District	Number of discordant results	S. no	Name of District	Number of discordant results
1	Adilabad	3	19	Nalgonda	10
2	Badradri Kothagudem	6	20	Narayanpet	2
3	Hyderabad	37	21	Nirmal	4
4	Jagityal	6	22	Nizamabad	2
5	Janagaon	4	23	Peddapally	5
6	Jayasankarbhupalpally	7	24	Rajanna Siricilla	1
7	Jogulamba Gadwal	5	25	Rangareddy	18
8	Kamareddy	2	26	Sangareddy	14
9	Karimnagar	11	27	Siddipet	5
10	Khammam	12	28	Suryapet	6
11	Kumurambheem Asifabad		29	Vikarabad	6
12	Mahabubabad	2	30	Wanaparthy	5
13	Mahabubnagar	1	31	Warangal urban	4
14	Mancherial	6	32	Warangal rural	4
15	Medak	1	33	Yadadri Bhongir	1
16	Medchal Malkajgiri	7		Total	203
17	Mulugu	5			
18	Nagarkurnool	1			

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As a policy and protocol of CDST laboratories, all samples (irrespective of Rif's status) reaching CDST laboratories would be subjected to smear microscopy. All those which read smear negative would be inoculated in liquid culture for reading in Line probe Assay (LPA).All those which are positive will be directed to LPA

Smear results of those 203 samples which showed discordance were recorded (Table 2).

The second sample according to algorithm are directed to CDST laboratory (Table 3).

Type of samples received (Table 4).

In FLLPA tests all the Samples read PCR, TUB band Rpo B gene and following gene types were studied (Tables 5–7).

Table 2 Smear microscopy results in IRL

Smear results at IRL	Total in number
Neg	27
Scanty	3
Smear 1+	100
Smear 2+	37
Smear 3+	36
Total	203

Table 3 Samples received for various TB tests

Sample received for tests	Total in number
CBNAAT	2
FLLPA	114
SLLPA	87
Total	203

Table 4 Types of samples received for discordance

Type of Sample	Total in number
Sputum	198
BAL	1
Pus	2
Abscess from Lymph node	1
Biopsy from shoulder	1
Total	203

Table 5 Gene read in FLLPA

Gene	Present	Absent
WT1	202	1
WT2	199	4
WT3	189	14
WT4	194	9
WT5	197	6
WT6	191	12
WT7	195	8
WT8	152	51
MUT1	6	197
MUT2A	18	185
MUT 2B	21	182
MUT3C	35	178

Table 6 Results of discordance for Rifampicin resistance cases

Number of samples which were rifampicin resistance detected in NAAT in district	Number of samples which were Rifampicin resistance not detected in FLLPA	Discordant results		
		Resistance	Resistance not detected	MTb not detected
88	88	34	53	1

Table 7 Results of discordance for Rifampicin resistance not detected cases

Number of samples which were rifampicin resistance not detected in NAAT in district	Number of samples which were Rifampicin resistance detected in FLLPA	Discordant results		
		Resistance	Resistance not detected	MTb not detected
115	115	53	55	7

Discussion

Rifampicin, as the most effective first-line antituberculosis drug, also develops resistance due to the mutation on *Mycobacterium tuberculosis* (Mtb) RNA polymerase. Multidrug-resistant tuberculosis (MDR-TB) is defined as disease due to *Mycobacterium tuberculosis* that is resistant to isoniazid (H) and rifampicin (R) with or without resistance to other drugs. Rifampicin-resistant TB (RR-TB) defined as resistance to rifampicin detected using genotypic or phenotypic methods with or without resistance to other first-line anti-TB drugs. MDR-TB/RR-TB has been an area of growing concern to human health worldwide and posing a threat to the control of TB. The Global TB Report 2016 estimated that of 3.9% newly diagnosed and 21% of previously treated TB cases had MDR-TB. It has been estimated that 580,000 cases of TB resistant to at least rifampicin (RR-TB) globally in 2015, of whom, 480,000 were having resistant to both rifampicin and isoniazid (MDR-TB), and 250,000 deaths occurred due to MDR-TB/RR-TB in 2015 globally. Out of estimated 580,000 MDR-TB/RR-TB cases, only 132,120 (23%) were detected, and even fewer 124,990 (20%) started treatment, and only 52% of them were treated successfully.[1]

Rifampicin resistance status was made more available after decentralizing the TB services in form of TruNAAT and CBNAAT (Cartridge Based Nucleic Acid Amplification test) at block level. According Programmatic management of Drug resistance TB (PMDT) guidelines 2019 and 2021, The samples which detected rifampicin resistance in the field would go through the cascade of

FLLPA (First line -Line Probe Assay), SLLPA (Second line-line probe Assay) and LC-DST (liquid Culture Drug susceptibility testing).At times the discordance in the result of rifampicin is encountered which is amounting to 1.7%(i.e. 203 out of 11839 samples tested in FLLPA).

As per PMDT guidelines 2019 and 2021, discordance in RR results between NAAT & FL-LPA to be resolved with a repeat NAAT at C&DST lab and microbiologists will provide the final decision.[2]

A total of 203 results showed discordance in CBNAAT and LPA. Among the geographical distribution, Hyderabad holds 37 (majority of cases), amounting to more than 15% of total cases.

This could be attributed to reasons like more number of NAAT machines doing more number of tests. All the 37 tests which showed rifampicin discordance were resolved.

In our study the samples which detected Rifampicin resistance in field NAAT, showing sensitive in FLLPA were 88. Out of 88, when discordance was resolved, 34 out of 88 showed resistance, 53 out of 88 showed resistance not detected. Only one case showed MTB not detected.

The samples which detected Rifampicin Sensitive in field NAAT, showing resistance in FLLPA were 115.Out of 115, when discordance was resolved, 53 out of 115 showed resistance, 55 out of 115 showed resistance not detected. Seven case showed MTB not detected.

Whenever the result of MTB is not detected, the treating physician had to take a decision on whether to treat the patient as drug sensitive TB or Drug resistant TB. Molecular test like CBNAAT and LPA failed to demonstrate the status even after subjecting to NAAT third time.

The line probe Assay which is read manually should be understood in depth on presence of TUB band, expression of wild types and mutations. The following table helps to understand the interpretation's in LPA (Table 8).

Table 8 Interpretation of rifampicin in FLLPA

Probe	Result interpretation	Clinical interpretation
rpoB WT1 not developed	Resistance to rifampicin inferred	Rifampicin is not effective
rpoB WT2 not developed	Resistance to rifampicin inferred	
rpoB WT2 and WT3 not developed	Resistance to rifampicin inferred	
rpoB MUT1 developed	Resistance to rifampicin detected	
rpoB WT3, WT4 and MUT1 not developed	Resistance to rifampicin inferred	
rpoB WT4 and WT5 not developed	Resistance to rifampicin inferred	
rpoB WT5 and WT6 not developed	Resistance to rifampicin inferred	
rpoB MUT2A developed	Resistance to rifampicin detected	
rpoB MUT2B developed	Resistance to rifampicin detected	
rpoB WT7, MUT2A and MUT2B not developed	Resistance to rifampicin inferred	
rpoB MUT3 developed	Resistance to rifampicin detected	
rpoB WT8 and MUT3 not developed	Resistance to rifampicin inferred	

Resistance to rifampicin is predominantly (95% of cases) caused by genetic variants in the rifampicin resistance-determining region (RRDR) of the RNA polymerase β subunit (*rpoB*) gene.[3] Discordant results occurred mostly (20/22) in specimens with very low bacterial load.[4] This can be known in present study by smear results of negative, scanty and 1+ are more in number approximately 70% of cases.

Few studies have investigated the mechanism causing discordances between two molecular tests. In a recent article, Hofmann-Thiel *et al.* hypothesized that several mechanisms can cause discordances, but they did not provide evidence for or the relative importance of these mechanisms in routine conditions.[5] The most frequent cause of discordance observed was false-negative results by MTBDR*plus* in the presence of 'disputed' mutations, defined as *rpoB* mutations associated with low-level rifampicin resistance that is missed by rapid phenotypic drug susceptibility tests.[6]

Discordant results between two molecular tests (especially Xpert and MTBDR*plus*) can have important implications for clinical care and public health as they can result in additional tests requested and can pose challenges to patient management. Patients wrongly diagnosed with rifampicin-resistant TB may receive a prolonged period of unnecessarily toxic and poorly effective drugs, while those misclassified as having drug-susceptible TB after an initial diagnosis of rifampicin-resistant TB may receive ineffective first-line treatment. Our observation that three in four patients with discordant results can be resolved by a follow-up Xpert assay support the recent guidelines that recommend a confirmatory Xpert assay in patients at 'low rifampicin-resistant TB risk' and/or a MTBDR*plus* assay to assess for MDR TB following a rifampicin-resistant TB diagnosis.[7]

Conclusion

Molecular assays have become the main method for detection and confirmation of rifampicin resistance in *M. tuberculosis*, but discordances between test results have important implications for the laboratory, clinician and patient. Our findings of a high (1.7%) prevalence of discordant results between CBNAAT and LPA under routine conditions and an array of underlying causes highlight both the importance and complexity of this issue. Future research should assess the value of a repeat CBNAAT in the case of a discrepant result to guide healthcare workers in their treatment decisions.

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Conflicts of interest

The author has no conflicts of interest to declare.

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References

1. World Health Organization. Global Tuberculosis Report 2016. Geneva: World Health Organization. 2016.
2. Guidelines on Programmatic management of drug resistance Tuberculosis in India. 2021. 25 p.
3. Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. *Lancet*. 1993;341(8846):647–650.
4. Sahrin M, Rahman A, Uddin MKM, et al. Discordance in Xpert® MTB/RIF assay results among low bacterial load clinical specimens in Bangladesh. *Int J Tuberc Lung Dis*. 2018;22(9):1056–1062.
5. Hofmann Thiel S, Hoffmann H , Hillemann D, et al. How should discordance between molecular and growth-based assays for rifampicin resistance be investigated?. *Int J Tuberc Lung Dis*. 2017;21(7):721–726.
6. Miotto P, Cabibbe AM, Borroni E, et al. Role of disputed mutations in the *rpoB* gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of Mycobacterium tuberculosis. *J Clin Microbiol*. 2018;56(5):e01599–17.
7. Piubello A, Aït Khaled N, Caminero JA, et al. Field guide for the management of drug-resistant tuberculosis. 2018.

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