Rifampicin Monoresistance in Treatment Naïve Pulmonary Tuberculosis Patients

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Abstract

Background: Multi-drug resistant TB has become an area of growing concern nowadays in treatment naïve pulmonary TB patients, despite the global efforts in controlling TB. It has emerged as a posing threat to human survival and major challenge for the NTEP (National Tuberculosis Elimination Programme) in achieving the global target of ending TB in 2035. Rifampicin monoresistance will contribute towards amplification of resistance, eventually leading to emergence of MDR, if they are not managed properly.

Objectives: The present study aims to detect Rifampicin monoresistance in treatment naïve or newly diagnosed pulmonary Tuberculosis patients using GeneXpert MTB/RIF assay and 1% proportion method. The study is also aimed at evaluating the diagnostic efficacy of both the tests in detecting the same.

Materials and methods: Rifampicin monoresistance (RMR) was diagnosed by molecular/ genotypic testing (GeneXpert MTB/RIF assay) and conventional phenotypic DST (drug sensitivity testing) method (1% proportion method) among 162 sputum/ BAL samples obtained from treatment naïve or newly diagnosed pulmonary Tuberculosis patients.

Results: Out of the 162 samples subjected to GeneXpert MTB/RIF (CBNAAT) assay, *Mycobacterium tuberculosis* was detected in 108 (67%) samples and rifampicin resistance (rpoB gene) was detected in 8 (5 %) samples. Whereas phenotypic DST by 1% proportion method detected Rifampicin resistance in 13 (8%) cases.

Conclusion: The study reveals that there is a discordance between molecular/ genotypic test, GeneXpert MTB/RIF assay and conventional phenotypic DST method,1% proportion method in detecting rifampicin resistance in newly diagnosed/ treatment naïve pulmonary Tuberculosis patients. Phenotypic method detected more isolates with rifampin resistance compared to molecular method.

Key words: *Rifampicin monoresistance, Gene Xpert MTB/RIF assay, 1% proportion method*

Introduction

Tuberculosis (TB), a curable, preventable, communicable disease is a major cause of ill health and one of the top 10 causes of death worldwide. It is a disease of poverty, and economic distress, vulnerability, marginalization, stigma and discrimination. It is one of the leading causes of death due to a single infectious agent. Globally, an estimated 10.0 million people fell ill with TB in 2019. There were an estimated 1.2 million TB deaths among HIV-negative people and an additional 208 000 deaths among HIV-positive people. Men (aged ≥15 years) accounted for 56% of the people who developed TB in 2019; women accounted for 32% and children (aged < 15 years) for 12%. Among all,8.2% were people living with HIV[1].

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India, highest TB burden country in the World, recorded a high notification of 24 lakh cases in the year 2019. Of the 24 lakh TB cases, 90% were incident TB cases (new and relapse/ recurrent). A total of 66,359 Multi drug resistant / rifampicin resistant (MDR/RR) TB cases were notified and 56,500 (85%) of them put on treatment by the Revised National Tuberculosis Control Programme (RNTCP), recently renamed as National Tuberculosis Elimination Programme (NTEP) by the Central TB division, Government of India[2].

Drug resistant TB has been frequently encountered from the time anti-tuberculosis drugs were introduced for treatment of TB. Drug resistance is of two types- primary and acquired. Primary drug resistance is defined as the drug resistance in a patient who has not received any anti-tuberculosis treatment. Acquired resistance is defined as the drug resistance developed in a patient who has received prior chemotherapy. MDR or multi-drug resistant Tuberculosis is defined as the disease due to *Mycobacterium tuberculosis* strains that are resistant to Rifampicin and Isoniazid with or without resistance to other drug [3].

For the purpose of drug resistance surveillance (DRS) in TB, WHO and IUATLD have revised the definitions of primary drug resistance and acquired drug resistance as 'resistance among new cases' and 'resistance in previously treated patients'. **'**Resistance among new cases' is defined as the 'presence of resistant strains of *Mycobacterium tuberculosis* in a patient who, in response to direct questioning, denies having had any prior antituberculosis treatment (for more than one month), and in countries where adequate documentation is available, for whom there is no evidence of such history'[4].

Even though, the TB incidence rate is falling, it is not as fast enough to reach the global targets of a 20% reduction between 2015 and 2020. Worldwide in 2019, close to a half million people developed Rifampicin-resistant TB (RR-TB), of which 78 % had MDR-TB. Globally, 3.3% new TB cases and 17.7% of previously treated cases had MDR-TB/RR-TB in the year 2019. Moreover, the economic impact of Covid 19 pandemic has threatened to worsen the recent progress in reducing the global burden of TB disease. Additionally,43 countries, including 13 high TB burden countries are using their GeneXpert machines for Covid 19 testing instead of diagnostic testing for TB [1]. Global TB report 2018, reported a rise in DR-TB cases with 558000 estimated incident cases of MDR plus RR-TB and more than 230000 deaths in 2017 [5].

According to India TB report 2019, 27 lakh new TB cases were reported in India, which accounted for a quarter of the global TB burden. Majority of the TB burden was among the working age group.89% of TB cases came from the age group of 15-69 years. About two-third of the TB cases were males. TB strains with MDR/RR-TB are more difficult to treat than DS-TB and is considered as one of the major challenges to progress towards the country's target to end TB by 2025 [6].

The molecular basis of multiple drug resistance in *Mycobacterium tuberculosis* (MTB) is mainly due to the following reasons-mutations in genes coding for drug target proteins, complex cell wall, drug efflux pumps and multi-functional proteins [7& 8]. Intrinsic drug resistance in MTB is due to a combination of highly impermeable mycolic acid containing cell wall and an active drug efflux mechanism[9& 10]. Acquired drug resistance occur by spontaneous mutations in specific target genes rendering the bacteria resistant to a given drug[11] . Rifampicin is a lipophilic ansamycin introduced in 1972, active against growing and non-growing bacilli. The mode of action of rifampicin is by binding to the beta subunit of RNA polymerase inhibiting the initiation and elongation of transcription. Mutations in the rifampicin resistance determining regions or 'hot spot region' of 81 base pair spanning codons,507-533 of the rpo B gene are mostly responsible for rifampicin resistance in 96% of MTB isolates. Some reports have noted the occurrence of mutations outside of the hotspot region of rpo B gene.[7 &8.]

Resistance to rifampicin leads to selection of mutants that are already resistant to other components of short-course treatment. Therefore, Rifampicin resistance (RR) is often regarded as an excellent surrogate marker for MDR-TB[12]. Resistance to rifampicin, one of the most effective and frontline anti-TB antibiotics that form the backbone of the short-course treatment, would necessitate the use of more toxic and expensive drugs which are administered for a long period, prolonging the hospital stay and increasing the mortality rate of the

patient. Hence, there is a need for continuous and / or periodic survey of rifampicin monoresistance apart from TB control programme which could provide information on the type of chemotherapy to be used for the treatment of patients and also serve as a useful parameter in the evaluation of current and past chemotherapy programmes.

Objectives of The Study

1. To detect Rifampicin monoresistance in treatment naïve or newly diagnosed pulmonary Tuberculosis patients using molecular testing method, GeneXpert MTB/RIF assay and phenotypic DST method,1% proportion.

2. To evaluate the diagnostic efficacy of both the testing methods in detecting Rifampicin resistance in treatment naïve Pulmonary TB patients.

Materials and Methods

Study design and sample collection

This prospective, observational study was conducted on treatment naïve / newly diagnosed presumptive pulmonary TB patients visiting TB and Chest department and General Medicine departments of Vydehi Institute of Medical Sciences and Research Centre during the period between September 2018-September 2020.A total of 162 samples were collected from clinically suspected new Pulmonary TB patients for ZN microscopy, Gene Xpert MTB/RIF assay, LJ culture and DST (1% proportion method). The study was approved by Vydehi Institutional Ethics Committee and informed consent was taken from each patient.

Inclusion criteria

a) Patients of all age groups, both male and female, presumptive for pulmonary tuberculosis attending the TB and Chest and General Medicine Departments of Vydehi Institute of Medical Sciences and Research centre were included

b) Patients those who have taken anti-tubercular drugs for less than 28 days were included.

Exclusion criteria

a) Presumptive patients of extra-pulmonary tuberculosis were excluded from the study.

b) Patients those who have taken anti-tubercular drugs for more than 28 days were excluded from the study.

Sample Collection

One overnight and one spot sputum sample were collected from each patient, presumptively diagnosed with pulmonary tuberculosis after taking their informed consent.

Techniques Employed

Ziehl-Neelsen Staining

Ziehl-Neelsen smear for detection of AFB was conducted from each sample and reported as per RNTCP guidelines [13].

GeneXpert MTB/RIF assay

All those samples showing positive by ZN staining were subjected to Gene Xpert-MTB/RIF assay. Such samples were immediately processed after decontaminating and diluting. The CB-NAAT system detects DNA sequences, specific for *M. tuberculosis* and rifampicin resistance by PCR. The Xpert purifies and concentrates *M. tuberculosis* bacilli from clinical samples and extracts the genomic material by sonification and subsequently amplifies the genomic DNA by PCR The process identifies clinically relevant, rifampicin resistance inducing mutations in the RNA Polymerase beta (rpoB) gene in the *M. tuberculosis* genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes[14].

Bacteriological methods

a) **Sample processing:** The samples were processed by modified Petroff's method of decontamination using 4% NaOH. The pellet obtained was washed with sterile distilled water twice before inoculation for culture [13]

b) **Culture:** All the samples were cultured on to LJ (Lowenstein Jensen) slopes in a Biological safety cabinet class 2, type 2A. The cultures were incubated at 37 and observed for growth every week up to a maximum of eight weeks.[13]

Identification of growth: All the isolates grown were identified as *M.tuberculosis* by their slow growth rate, colony morphology, smear microscopy from cultures and SD Bioline TB Ag MPT64 Rapid test [15].

c) Drug susceptibility testing: All culture positive samples with sufficient growth for the preparation of inoculum were subjected to DST using the 1% proportion method on Lowenstein Jensen (LJ) medium containing Rifampicin (40 µg /ml) antibiotic. The slants were incubated at 37 . The results were read on 28th day and finalized on the 42nd day as per the RNTCP protocol.[13]

Results

A total of 162 samples were collected from newly diagnosed, pulmonary Tuberculosis patients enrolled in the study. Of these, 149 (92%) were sputum samples and 13 (8%) were BAL samples (Table 1). Among them, 115 (71%) patients were male while the remaining 47 (29%) were female. The sex ratio (man/woman) was of 2.4. Most of the samples (47%) were from patients aged 20-40 years. None of the patients were previously treated with first or second-line drugs. Of the 162 samples subjected to GeneXpert MTB/RIF (CBNAAT) assay *Mycobacterium tuberculosis* was detected in 108 (67%) samples and rifampicin resistance (rpoB gene) was detected in 8 (5 %) samples, and MTB was not detected in 54 (33%) samples (Table 2). Among the 162 samples subjected to solid culture on LJ media, 81(50%) samples were culture positive. Of these 81-culture positive and GeneXpert positive isolates, subjected to phenotypic DST by 1% proportion method, 13 (8%) showed phenotypic resistance to rifampicin (40 µg /ml), antibiotic (Table 3).

Table 1: Distribution of samples from new/treatment naïve pulmonary TB patients (n=162)

Sputum samples	BAL samples	Total (n=162)
149 (92%)	13(8%)	162

Table 2: GeneXpert MTB/RIF findings (n=162)

	Rifampicin resistance detected	Rifampicin resistance not detected	Total
MTB detected	8	100	108(67%)
MTB not detected	0	54	54(33%)
Total	8 (5%)	154(95%)	162

Table 3: Rifampicin susceptibility testing by Xpert MTB/RIF and phenotypic DST (1% proportion method) (n=162)

RpoB gene detection by GeneXpert (n=162)	Rifampicin resistance detection by 1% proportion method
8 (5%)	13(8%)

Photo 1: Cepheid's GeneXpert MTB/RIF assay machine attached with computer monitor

Photo 2: Growth of Mycobacterium tuberculosis on LJ media

Photo 3: SD Bioline TB Ag MPT64 Rapid test showing positive reactions.

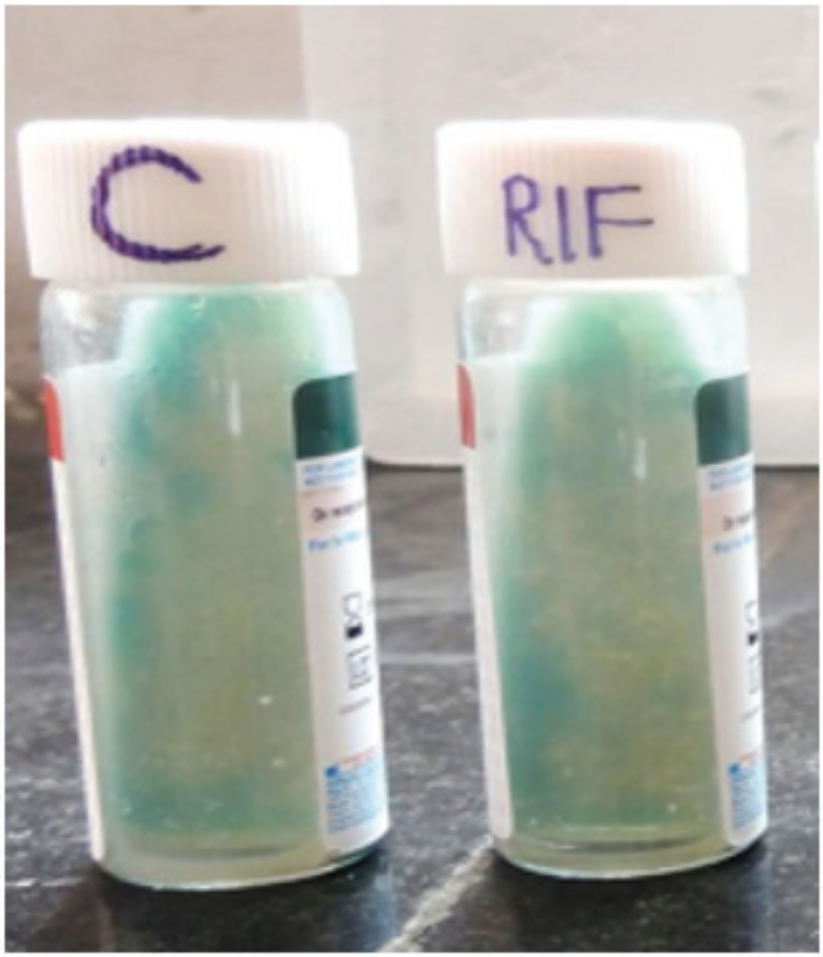

Photo 4: MTB isolate showing phenotypic resistance to Rifampicin by 1% proportion method

Discussion

MDR-TB is a manmade phenomenon. The use of inadequate regimens, poor adherence to treatment, inappropriate DOTS therapy leads to increase in the emergence of drug resistant strains in the community. Undiagnosed and improperly treated patients harbouring resistant strains of TB acts as a source of on-going transmission of resistant strains. India, being a resource-limited country; there are constraints on the availability and affordability of DST facilities. Hence, much of the anti-TB drug resistance is diagnosed presumptively based on the lack of response to treatment or relapse of symptoms in patients. Timely diagnosis of MDR-TB and treatment is important in new cases.

Detection of MDR/RR-TB requires bacteriological confirmation of TB and testing for drug resistance using rapid molecular tests, culture methods or sequencing technologies. Rapid diagnosis of TB significantly

decreases the lag time in initiation of treatment, thereby reducing transmission rates [16]. The Xpert assay (Cepheid, Sunnyvale, CA, USA), a cartridge-based, automated hemi-nested real time PCR system uses five overlapping molecular beacon probes (A-E) targeting the RRDR of rpoB gene to detect MTBC along with the mutations conferring RR [17]. The results for both, presence of MTB and rifampicin resistance, are available in less than 2 hours, in stark comparison to the turnaround time of conventional drug-sensitivity testing of 8–10 weeks[18].

In our study, GeneXpert MTB/RIF assay detected MTB in 108(67%) samples, out of 162 samples and rifampicin resistance (rpoB gene) was detected in 8 (5%) samples**,** which was in comparison with the study done by Subhasis Mukherjee et al.[19] , where Rifampicin resistance was detected in 5(2.2%) out of 111 positives with 100% sensitivity and specificity. In a study done by R Dewan et al.[20] the Rifampicin resistance was found in 10 patients out of 40 by CBNAAT.

In our study, GeneXpert was compared with 1% proportion method in detecting Rifampicin resistance. Out of 162 clinical samples subjected to both CBNAAT & 1% proportion method, resistance to Rifampicin was detected by CBNAAT in only 8 isolates, whereas, 13 isolates showed resistance to Rifampicin by the 1% proportion method. In the study by Guenaoui et al (2016),[21]all Rifampicin resistant strains detected by GeneXpert MTB/RIF were phenotypically confirmed as MDR strains. Sensitivity, specificity, positive predictive value and negative predictive value of Xpert MTB/RIF to detect Rifampicin resistance in comparison to conventional phenotypic drug susceptibility technique were found equal to the rates of 100%, 100%, 100% and 100%, respectively.

In the study conducted by Sharma et al [18], results for molecular/genotypic DST for rifampicin obtained by Xpert MTB/RIF were compared with the phenotypic DST done on solid media. Of the 430 Xpert and culture positive samples, 8 samples were indeterminate for RIF resistance by Xpert MTB/RIF assay and were excluded from the analysis. However, when these 8 samples were put for DST on solid media, 2 were RIF resistant and 6 were RIF sensitive. Out of the remaining 422 samples, 110 were resistant for rifampin by phenotypic DST of which 104 were resistant by Xpert MTB/RIF and six were sensitive for rifampin. Three hundred and twelve samples were sensitive for RIF by DST, of which 305 were sensitive and seven were RIF resistant by Xpert MTB/RIF. There were 13 discrepant samples, six where only Xpert was resistant for RIF and seven where only culture was resistant for RIF.

Similar findings were observed in the study conducted by Atashi et al [22]. They found 8 inconsistent results between the two methods, of which proportional method detected rifampicin-resistant TB in 2 cases whereas GeneXpert were unable to do so. Their findings demonstrated that GeneXpert can detect a large number of rpoB mutations, but not all mutations that cause rifampicin resistance. They suggested that the results of GeneXpert should be confirmed by the standard proportional method also. They added that GeneXpert and proportional method complement but do not replace each other.

A study by Zetola et al, (2014)[23] observed false negative results for rifampicin resistance by GeneXpert in pulmonary TB patients. When the Xpert assay results for rifampicin resistance were compared against phenotypic DST results, 5 results were discordant. One sample showed resistance to rifampin in the Xpert results but not on phenotypic DST (false-positive Xpert assay result for rifampin resistance). Four samples had genotypic/phenotypic discordant DST results showing resistance to rifampin but negative Xpert assay results for such resistance (false-negative Xpert assay result for rifampin resistance).

In our study, there were 5 inconsistent results between GeneXpert and 1% proportion method. Hence, GeneXpert rpo B gene detection and phenotypic DST by 1% proportion method needs further evaluation by studying more samples

Conclusion

The study reveals that there is a discordance between molecular/ genotypic test, GeneXpert MTB/RIF assay and conventional phenotypic DST method,1% proportion method in detecting rifampicin resistance in newly diagnosed/ treatment naïve pulmonary Tuberculosis patients. Phenotypic method detected more isolates with rifampin resistance compared to molecular method.

Conflict of Interest: None

Ethical Clearance: Done

Source of Funding: Nil

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