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Genexpert[®] MTB/RIF for Rapid Identification of Clinically Suspected MDR-TB Cases

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Abstract

Introduction: An alarming increase in global incidence of drug-resistant *Mycobacterium tuberculosis* infection has created a critical need for methods that can rapidly detect it. Multidrug-resistant tuberculosis is defined as tuberculosis caused by *M. tuberculosis* that are resistant to at least isoniazid and rifampicin (RIF). The process to detect *M. tuberculosis* is time consuming. GeneXpert[®] MTB/RIF detects it within two hours.

Aim and Objectives: The study was undertaken to detect *M. tuberculosis* and simultaneously RIF resistance in clinically suspected MDR cases of pulmonary TB by using GeneXpert[®] MTB/RIF. Compare MTB detection with sputum smear status and geographical mapping of clinically suspected MDR-TB cases and confirmed by GeneXpert[®] MTB/RIF.

Material & Methods: Sputum samples from 100 clinically suspected MDR-TB cases were subjected to sputum smear microscopy followed by GeneXpert[®] MTB/RIF.

Results: Amongst 100 clinically suspected MDR-TB cases, 81 (81%) were smear-positive and 19 (19%) smear-negative. *M. tuberculosis* was detected amongst 69 (85.19%) smear-positive and 6 (35.29%) smear-negative cases. Amongst 75 MTB detected cases, 58 (77.33%) were RIF sensitive, 12 (16%) resistant and 5 (6.67%) indeterminate.

Discussion: In 100 clinically suspected MDR-TB cases, only 12 (16%) showed RIF resistance. The MDR-TB appears to be low in our area against clinically suspected MDR cases. This may be due to irregular, inadequate and intermittent treatment leading to clinical failure of primary line anti-TB drugs. Emphasis on proper counselling is needed while starting primary line anti-TB drugs.

Conclusion: GeneXpert[®] MTB/RIF assay may prove to be useful in rapid diagnosis and initial management of clinically suspected MDR-TB cases.

Key words: *M. tuberculosis*, geneXpert, multidrug resistance (MDR)

Introduction

Tuberculosis (TB) remains a major public health problem, accounting for more than 9.4 million incident cases and 1.3 million deaths every year, worldwide.^[1] The TB burden in India is still staggering. Every year, 1.8 million persons develop the disease, of which about 800,000 are infectious; and, until recently, 370,000 died annually -1,000 every day. In India today, two deaths occur every three minutes from TB.^[2] The emergence and spread of multidrug (MDR) and extensively (XDR) drug-resistant *Mycobacterium tuberculosis* complex (MTBC) strains poses significant challenges to disease control.^[1] In spite of aggressive TB control measures, multidrug resistant (MDR) tuberculosis continues to harbor in the community. Continuous monitoring of drug resistance patterns is necessary to establish the efficacy of public health interventions.

In early 2012, four cases of extremely drug resistant (XXDR) TB had been diagnosed in Mumbai, as they were resistant to twelve TB drugs. Although the number of known cases of XXDR TB in India is small, they are geographically dispersed.^[3] Therefore, early and rapid detection of mycobacteria and its drug resistance is required. GeneXpert[®] MTB/RIF is a machine which simultaneously detects *M. tuberculosis* and tests for drug resistance in less than two hours. Single GeneXpert MTB/RIF assay could assist in discontinuing isolation for patients with suspected tuberculosis. These results are tremendously helpful in further establishing the clinical utility of nucleic acid amplification tests, which are already considered as standard practice

in the United States to aid in the initial diagnosis of patients with suspected tuberculosis⁴

The study was undertaken to detect *M. tuberculosis* in clinically suspected MDR cases of pulmonary TB and to detect rifampicin (RIF) resistance by using GeneXpert[®] MTB/RIF, to compare MTB detection with sputum smear status and geographical mapping of clinically suspected MDR-TB cases and MDR-TB confirmed by GeneXpert[®] MTB/RIF.

Material & Methods

A prospective study was conducted in a tertiary care hospital during 2013 to 2014. As per Revised National Tuberculosis Control Programme (RNTCP) guidelines,^[4,5] two sputum samples from each of 100 clinically suspected MDR-TB cases were collected from patients attending peripheral health centre (PHC), designated microscopy centre (DMC) and district TB centre (DTC) in Dhule district. Sputum smear microscopy was performed at DMC level whereas GeneXpert[®] MTB/RIF assay was carried out at our institute.

Early morning, deep coughed sputum samples from 100 clinically suspected MDR-TB cases were collected in wide mouth sterile containers.^[4,5]

Inclusion criteria used for MDR suspect consist of treatment failure, retreatment cases sputum positive at 4 month, contact of known MDR-TB case, sputum positive retreatment case at diagnosis, any follow up sputum positive, sputum negative retreatment case at diagnosis and HIV TB case. The exclusion criteria used is cases of pediatric age group.

The sputum samples were subjected to GeneXpert[®] MTB/RIF (Cepheid, France) for detection of *M. tuberculosis* and further RIF resistance in them.

GeneXpert[®] MTB/RIF assay is a cartridge based nucleic acid amplification technique which includes semi-quantitative, nested real-time PCR *in-vitro* diagnostic test for the detection of MTBC DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative. RIF-resistance associated mutations of the *rpoB* gene in samples from patients at risk for RIF resistance. As per manufacturer's instructions,

Two volumes (2 ml) of sample reagent added to one volume (1 ml) of each fresh sputum sample directly in the collection container. The lid was replaced and shaken vigorously 10-20 times. It was incubated at room temperature. After ten minutes of incubation, the specimen was shaken vigorously 10-20 times. It was incubated for five minutes again. Sample should be perfectly fluid before being processed, with no visible clumps of sputum. If still viscous, wait for 5-10 minutes further before processing it in the cartridge.

At least 2 ml of processed sample was taken with the plastic transfer pipette from the collection container to the single-use, disposable, self-contained GeneXpert cartridge. Then it was subjected to GeneXpert[®] MTB/RIF to create a test. After scanning the cartridge barcode, loading was done on blinking module. The results were visualized on the attached computer and interpreted by using software (GeneXpert

MTB/RIF Dx). The GeneXpert MTB/RIF test was repeated on second sputum sample, if it has shown indeterminate susceptibility to RIF.

Geographical mapping of clinically suspected MDR-TB cases and MDR-TB cases confirmed by GeneXpert[®] MTB/RIF in Dhule and Nandurbar district was done.

Results

In 100 clinically suspected MDR-TB cases, in 75 cases MTB was detected by GeneXpert[®] MTB/RIF. However, RIF resistance was detected in 12 cases by GeneXpert[®] MTB/RIF.

The male cases were 80% whereas female cases were 20%. The majority 34 cases belonged to age group 21-30 years followed by 28 cases of age group 31-40 years, 15 cases of 41-50 years, 10 cases of 51-60 years, 8 cases of more than 60 years and 5 cases of age group 14-20 years. The male: female ratio 4:1.

As per MDR suspect criteria, sputum positive retreatment case at diagnosis was the most common (49%) inclusion criteria followed by sputum negative retreatment case at diagnosis (15%). Amongst 100 clinically suspected MDR-TB cases, the distribution of remaining cases was as follows; any follow up sputum positive case (13%), retreatment cases sputum positive at 4 month (10%), HIV-TB case (7%) and failure to primary line drugs (5%). There was only one case of follow up.

Amongst 100 clinically suspected MDR-TB cases, 81% were sputum smear-positive whereas 19% were sputum smear-negative. *M. tuberculosis* was detected amongst 69 (85.19%) smear-positive and

6 (31.58%) smear-negative cases. In 12 cases, MTB was detected by sputum smear microscopy but not by GeneXpert[®] MTB/RIF (Table-1). Amongst 75 MTB cases detected by GeneXpert[®] MTB/RIF, 58 (77.33%) were RIF sensitive, 5 (6.67%) indeterminate and 12 (16%) resistant. (Figure-1)

The demographic distribution of suspected MDR-TB cases showed 91% cases in Dhule district and 9% in Nandurbar district. Dhule district showed maximum (65%) were from Dhule city followed by Sindkheda (9%), Shirpur (8%), Sakri (6%) and

Dondaicha (3%). The MDR-TB confirmed cases were majority 6 cases (71.43%) of from Dhule city and . (Figure 2). Out of 100 clinically suspected MDR-TB cases, in Dhule district, eight cases from Dhule city and one case each from Sindkheda and Dondaicha. In Nandurbar district the suspected MDR-TB cases (6%) were concentrated around Nandurbar city whereas few cases were spotted around Shahada (2%) and Dhadgaon (1%). Only one case each from Nandurbar city and Shahada was confirmed as MDR-TB by GeneXpert[®] MTB/RIF (Figure-3).

Table 1. Comparison of MTB detection by GeneXpert[®] MTB/RIF and smear status

MTB detection by GeneXpert	Smear status		Total
	Positive	Negative	
Positive	69	6	75
Negative	12	13	25
Total	81	19	100

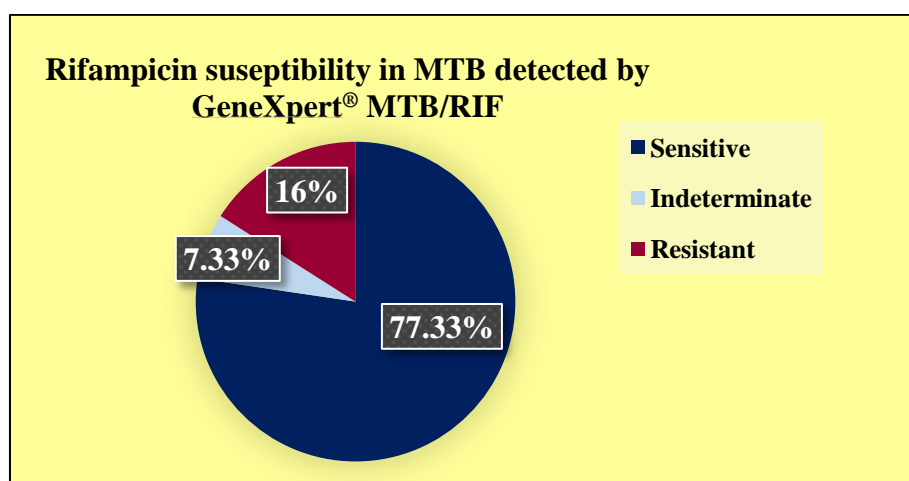


Figure-1: Rifampicin susceptibility amongst MTB detected by GeneXpert[®] MTB/RIF (n = 75)

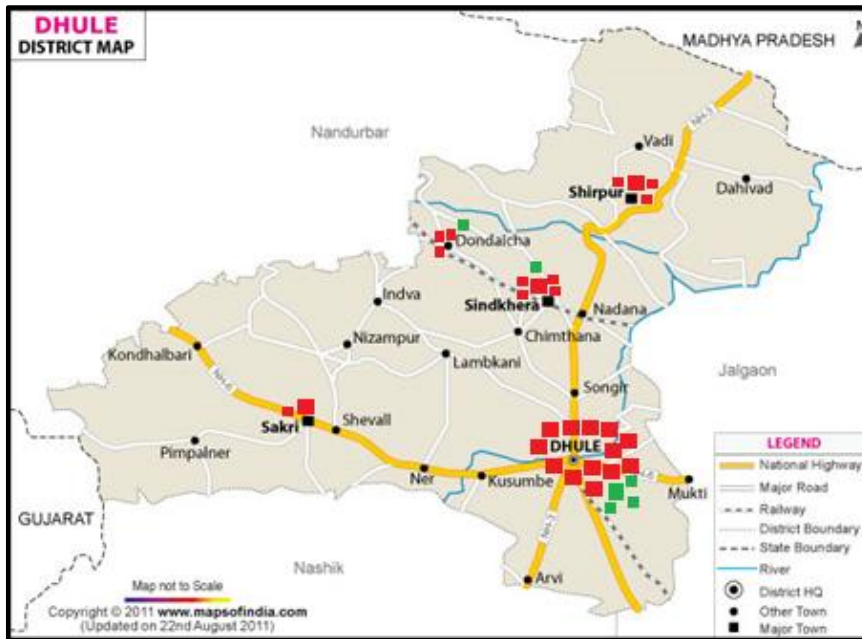


Figure-2: Distribution of clinically suspected MDR-TB cases & MDR-TB cases confirmed by GeneXpert[®] MTB/RIF in Dhule district

- - 5 Clinically suspected MDR-TB cases
- - 1 Clinically suspected MDR-TB cases
- - 5 MDR-TB cases confirmed by GeneXpert[®] MTB/RIF
- - 1 MDR-TB cases confirmed by GeneXpert[®] MTB/RIF



Figure-3: Distribution of clinically suspected MDR-TB cases & MDR-TB cases confirmed by GeneXpert[®] MTB/RIF in Nandurbar district

- - 5 MDR-TB suspected cases
- - 1 MDR-TB suspected cases
- - 1 MDR-TB cases confirmed by GeneXpert[®] MTB/RIF

Discussion

An alarming increase in the global incidence of drug-resistant *M. tuberculosis* infection has created a critical need for methods that can rapidly detect *M. tuberculosis* and identify drug-resistant cases. The failure to quickly and effectively recognize and treat patients with drug-resistant TB, particularly MDR-TB and XDR-TB, leads to increased mortality, nosocomial outbreaks and resistance to additional anti-tuberculosis drugs. However, MDR and XDR TB can be effectively treated if properly identified. The conventional methods are time consuming, tedious and technically robust whereas current nucleic acid amplification methods to detect *M. tuberculosis* are complex, labor-intensive, and technically challenging.^[7]

GeneXpert[®] MTB/RIF assay, an integrated hands-free sputum-processing and real-time polymerase chain reaction system with rapid on-demand, near-patient technology, to simultaneously detect *M. tuberculosis* and rifampicin resistance within two hours.^[7,8] However, other new methods like line probe assay (LPA) and Mycobacterium growth indicator tube (MGIT) are also available which can detect both *M. tuberculosis* and its drug resistance. But GeneXpert[®] MTB/RIF is the one which detects it simultaneously and rapidly within two hours. Whereas LPA and MGIT take five days to report it as positive and MGIT take 42 days to report it as negative.^[9] GeneXpert facilitated identification of tuberculosis in HIV patients in whom the diagnosis had been delayed.¹⁰

In present study, mostly 34% cases belonged to age group 21-30 years and 80% of patients were male. This might have occurred because chances of exposure to tubercle bacilli are more in young men due to their outdoor work pattern. Non-reporting of female patients might be due to lack of awareness about their health issues and negligence. As per MDR suspect criteria, sputum positive retreatment case at diagnosis was the most common (49%) inclusion criteria followed by sputum negative retreatment case at diagnosis (15%). There was only one case of follow up.

The 81% cases were sputum smear-positive whereas 19% were sputum smear-negative tuberculosis. *M. tuberculosis* was detected amongst 69 (85.19%) smear-positive and 6 (31.58%) smear-negative cases. The GeneXpert[®] MTB/RIF test has 88% sensitivity and 98% specificity when compared to culture as per the recent Cochrane review.^[11] (Table 1) Data from many countries, including India, clearly show substantially better performance of the GeneXpert[®] MTB/RIF test over conventional smear microscopy.^[11]

MTB detected was detected in 75 cases and 58 (77.33%) were RIF sensitive, 5 (6.67%) indeterminate and 12 (16%) resistant (Fig. 1). A recent Cochrane review has shown that the GeneXpert[®] MTB/RIF test detect RIF resistance with sensitivity of 94% and specificity of 98%.^[11] Out of five RIF indeterminate cases, in three cases the GeneXpert[®] MTB/RIF assay was repeated on the second sputum sample separately and two cases showed RIF sensitivity with MTB concentration low to medium. Therefore, results

depend on the quality of sputum. The second sputum sample was not received in remaining two cases with indeterminate RIF susceptibility.

Multidrug-resistant tuberculosis (MDR-TB) is defined as tuberculosis caused by strains of *M. tuberculosis* that are resistant to at least isoniazid and rifampicin (RIF).^[12] Mono-resistance to RIF is rare; however, 90% of RIF resistant isolates also exhibit resistance to isoniazid. Therefore, the detection of RIF resistance may serve as a surrogate marker for MDR *M. tuberculosis*.^[1] Mutations in the 81-bp RIF resistance-determining region of the *rpoB* gene, occur in 95 to 98% of all RIF-resistant strains and are almost invariably absent in RIF-susceptible strains.^[7] GeneXpert[®] MTB/RIF detects RIF-resistance associated mutations of the *rpoB* gene in samples from patients at risk for RIF resistance; therefore indirectly detects MDR-TB. We observed only 12 (16%) showed RIF resistance.

Geographical mapping of clinically suspected MDR-TB cases (91) and MDR-TB confirmed cases (10) in Dhule district whereas clinically suspected MDR-TB cases (09) and MDR-TB confirmed cases (02) from Nandurbar district.

The confirmed MDR-TB cases (16%) appear to be low in our area and it is much less than clinically suspected MDR cases. This may be due to patient from this tribal region might have taken irregular, inadequate and intermittent treatment leading to clinical failure of primary line anti-TB drugs; hence considered as MDR. Emphasis on proper counseling is needed while starting primary line anti-TB drugs. However the observed trends in TB drug-resistance patterns in Mumbai highlight

the need for individualized drug regimens, designed on the basis of DST results involving first- and second-line anti-TB drugs and treatment history of the patient. A drug-resistant TB case-finding strategy based on molecular techniques that identify only rifampicin resistance will lead to initiation of suboptimal treatment regimens for a significant number of patients, which may in turn contribute to amplification of resistance and transmission of strains with increasingly advanced resistance within the community.¹³

Conclusion

Amongst 100 clinically suspected MDR-TB cases, in 75 cases MTB was detected by GeneXpert[®] MTB/RIF assay. However, in 25 cases MTB was not detected though clinically labeled as MDR-TB. GeneXpert[®] MTB/RIF indirectly detects MDR-TB. To conclude, GeneXpert[®] MTB/RIF assay may prove to be useful in the rapid diagnosis and initial management of suspected TB cases.

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