

# An audit of sputum smear microscopy, Gene Xpert and DST in a Tertiary care hospital in patients of suspected MDR/XDR-TB

Muhammad Wajahat Tariq<sup>1</sup>, Azam Mushtaq<sup>2</sup>, Shahid Pervaiz<sup>3</sup>, Tahir Khan<sup>4</sup>,  
Muhammad Atiq UI Mannan<sup>1</sup>, Sarmad Ali Naqvi<sup>1</sup>

<sup>1</sup>Department of Pulmonology, Nishtar Medical University, Multan – Pakistan

<sup>2</sup>Department of pulmonology, Bahawalpur Victoria hospital, Bahawalpur – Pakistan

<sup>3</sup>Department of Pulmonology, Sahiwal Medical College and Hospital, Sahiwal – Pakistan

<sup>4</sup>Department of Pulmonology, Jinnah Hospital, Quetta – Pakistan

**Address for correspondence**  
**Azam Mushtaq**

Department of pulmonology,  
Bahawalpur Victoria hospital,  
Bahawalpur – Pakistan  
E-mail:

azammushtaq7@gmail.com

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## Author Contributions

AM MAM conceived idea, AM SP MAM drafted the study, MWT TH SAN collected data, MWT SP did statistical analysis and interpretation of data, AM MAM SP critical review manuscript, All approved final version to be published.

## Declaration of conflicting interests

The authors declare that there is no conflict of interest.

## Abstract

**Background:** The gene expert MTB-Rif assay is available in the department of Pulmonology NMU Hospital Multan from November 2011, It is working in collaboration with National TB control program (NTP). As per NTP guidelines, it is advisable to high risk groups only & not as a primary diagnostic tool. These high risk groups include retreatment cases, treatment after default, treatment failure & even in new cases who are high risk e.g DM, HIV, Long standing use of steroids, those having malignant disease, health care providers & contacts of MDR-XTR TB patients

**Objective:** Objective of the present study was to audit the results of sputum smear microscopy & gene expert and DST in NMU, Hospital Multan.

**Methodology:** It was a descriptive study in which non probability consecutive sampling method was used. It was conducted in MDR clinic of department of pulmonology. From November 2011 to February 2018 total 12924 tests have been performed, sputum smear microscopy has already been performed for years & is being continued in the presence of gene expert as well. In selected cases, where gene expert MTB is detected & Rif resistance is also present and the patient are either non affording these cases are funded by NTP for culture and DST. It is considered worthwhile to simply audit the results of sputum smear microscopy, gene expert & DST and share this data of our PMDT clinic.

**Results:** A total of 12924 gene experts were performed. MTB were detected in 5100, Rif resistance were detected in 789, sputum smear was positive in 4502 & negative in 8422 cases. The gene expert showed error in 478 cases out of them 200 were re-checked which showed expert positive in 98 cases & Rif resistance in 12 cases.

**Conclusion:** The gene expert MTB Rif-Asaay is an effective & revolutionary tool to diagnose the MDR TB patients and it minimizes the time to start MDR treatment. Our unit is being benefited very effectively from this game-changer and that's why we wanted to share our data & perspective.

**Key words:** Sputum Smear Microscopy; Gene Xpert; DST

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## Introduction

**T**B is a chronic infectious disease which is caused by a bacterium named as mycobacterium tuberculosis; it is specified by formation of caseating granulomatous tubercles from cell-mediated immunity and delayed-type hypersensitivity.

From being “the disease of antiquity” to acquiring the dubious distinction of the one of the leading causes of deaths from a single infectious disease, tuberculosis (TB) has inflicted unparalleled morbidity and mortality upon mankind.

Just under one third of mankind suffers from latent tuberculosis infections and is at risk for reactivation.<sup>1</sup>

There were estimated 8.7 million new cases of tuberculosis in 2011 along with 1.7 million mortalities all over the world.<sup>1</sup> In 2016, 10.4 million people developed TB whereas mortality rate was 1.7 million and more than 95% of TB related deaths occurred in low- and middle-income countries. Of these deaths, 90% were adults, 65% were male, 10% were HIV positive (74% in Africa) and 56% in 5 countries: India, Indonesia, China, the Philippines and Pakistan.<sup>12,13</sup> Of these 8.7 million new cases of TB 30,000 hapless patients suffered from the MDR-TB caused by organism resistant to at least Rifampicin and Isoniazid. The poorer the country, the more the burden of MDR-TB and is on the rise.<sup>1</sup>

It has been estimated that well over 60% of the MDR-TB cases were reported from China, India, The Russian federation, Pakistan and South Africa,<sup>1,2</sup> Whereas XDR-TB, a subset of MDR-TB with added resistance to all fluoroquinolones plus one of the any three injectable anti TB drugs like Kanamycin, Amikacin and Capreomycin is also on the rise.<sup>1,2,3</sup>

Traditionally, sputum smear microscopy is employed to detect MTB in sputum samples. It is cheap with high specificity but low sensitivity, thereby resulting in many false negative cases, Moreover, it cannot detect the resistance to anti tuberculosis drugs. Hence the accelerated pace of morbidity, mortality, nosocomial outbreaks abound. Lack of rapid and accurate diagnoses and case detection are major obstacles to TB control.<sup>1,3</sup>

DST of sputum samples takes almost 12 weeks to yield results which means belated diagnosis and treatment of drug susceptible as well as potentially resistant cases of tuberculosis. Culture of MTB remains the gold standard for the purpose of

diagnosis of disease and drugs sensitivity testing (DST). However, it requires dedicated facilities and staff 4. LJ cultures take 20 to 56 days for diagnosis and 4 to 6 weeks for subsequent drug sensitivity testing. 7H11 medium takes 17 to 21 days for diagnosis and further 3 to 6 weeks for drug susceptibility testing.<sup>4</sup>

Rapid liquid TB culture medium that is BACTEC, MGIT 960® (automated) Yields diagnosis in 7 days for sputum positive and up to 42 days for sputum negative results. DST takes 8 to 12 days starting from culture, but this equipment is very expensive and requires specific training for medical personnel.<sup>4</sup>

NAAT (nucleic acid amplification test) have been in use for several years. The Gene Xpert MTB/Rif assay is fully automated NAAT platform that can detect MTB and Rif resistance. It employs real time PCR to amplify mycobacterium tuberculosis-specific sequence of the rpo B gene. To determine rifampicin resistance, the rifampicin-resistance- determining region of the rpo B gene is probed with molecular beacons<sup>4</sup> Bacterial lysis, nuclear acid extraction and amplification, and amplification detection are carried out in a fully automated manner.<sup>5</sup> The recent Cochrane database system review “ shows that the expert assay as an initial diagnostic test for tuberculosis detection and rifampicin resistance detection in patients suspected of having TB , MDR-TB or HIV – associated TB is sensitive and specific<sup>6</sup>. Data yielded by this assay shows greater than 95 % detection of all TB patients <sup>7</sup>, not with-standing its high specificity and positive predictive values and low sensitivity and negative predicative value especially in smear negative and extra pulmonary disease. In a remarkable German study, it was concluded that “the combine sensitivity and specificity of the Xpert assay

Table 1. Distribution of cases according to Gene Xpert (Detected/ Not detected), Rifampicin Resistance and Smear result (Positive/ Negative)

Year	Total cases	MTB detected	MTB Not detected	Rif Resistance	Error	Smear-ve	Smear+ve
2011	38	18	20	9	0	22	16
2012	583	241	302	100	40	377	206
2013	642	344	265	75	33	339	303
2014	1632	683	924	183	25	1006	626
2015	2188	1005	1161	171	22	1306	882
2016	3218	1249	1787	100	182	2108	1110
2017	4026	1356	2501	126	169	2814	1212
Till Feb 2018	597	204	386	25	7	450	147
<b>TOTAL</b>	<b>12924</b>	<b>5100</b>	<b>7346</b>	<b>789</b>	<b>478</b>	<b>8422</b>	<b>4502</b>

Table 2. Result of Gene Xpert vs Sputum Smear Microscopy

Test Category	Gene Xpert		Sputum Smear	
Result Category	MTB Detected	MTB Not Detected	Smear+ve	Smear-ve
Cases	5100	7346	4502	8422
Percentages	39.46 %	56.83%	34.83%	65.16%

in non-respiratory specimen comprising stool, urine, and tissue and CSF samples were calculated to be 77.3% and 98.2 % respectively.<sup>8</sup>

WHO has recommended the use of expert test as the first line diagnostic test among persons who are suspected of having MDR-TB and/or HIV co-infection with TB. WHO has also noted that the expert test does not obviate the need for conventional microscopy, culture and drug sensitivity testing which is necessary to monitor treatment progress (Xpert detects both dead and live bacteria) and to detect resistance to drugs other than Rifampicin. Never the less, Xpert is more reliable than sputum smear microscopy and speedier than culture. (It provides results in about 90 mins).<sup>11</sup>

With the availability of the facilities for microscopy, Gene Xpert and drug sensitivity testing in the OPD of our department with the collaboration of NTP (Nov 2011), it was considered worthwhile to report the results of these diagnostic tools in cases of suspected MDR/XDR pulmonary TB cases, Hence a study was under taken to simply audit the results yielded by these diagnostic methods being performed in our OPD for quite some time now.

### Methodology:

A descriptive study was conducted at MDR-TB clinic attached with department of Pulmonology Nishtar medical university Multan, Pakistan. Samples were collected on Non probability consecutive sampling

method. During this study data was collected from MDRTB clinic and analyzed. For study purpose all collected data were entered into SPSS version 17 software. Descriptive statistics were applied, frequencies and percentages were calculated.

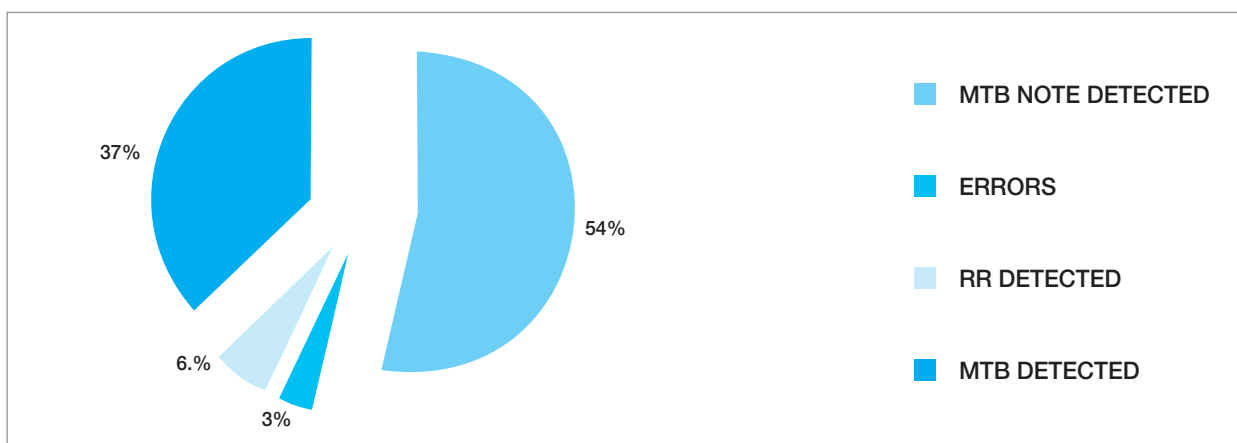
We collected the data since the availability of gene Xpert MTB-Rif in department of Pulmonology NMU from November 2011 till February 2018 of those patients having sputum smear microscopy followed by Xpert Mtb-Rif. In patients having Rif resistance positive DST were performed.

Patients were being enrolled consecutively from MDR clinic located in OPD of chest department NMU. patients having signs and symptoms suggestive of pulmonary tuberculosis either susceptible or DR-TB and were able to provide good quantity of sputum sample (1.5 ml) were included. Two sputum samples were processed for microscopy and 1 sample for gene xpert.

### Results

Total of 12924 patients were tested with Xpert Mtb-Rif in period of about more than six years from Nov. 2011 till Feb.2018 accounting 38 in 2011, 583 in 2012, 642 in 2013, 1632 in 2014, 2188 in 2015, 3218 in 2016, 4026 in 2017 and 597 till Feb. 2018.

MTB were detected in total of 5100 patients (39.46%) with Rif resistance were found in 789 patients (15.47%) using Xpert Mtb-Rif. In 478 cases (3.69%)



Graph 1: Pie chart showing results of gene xpert in percentages

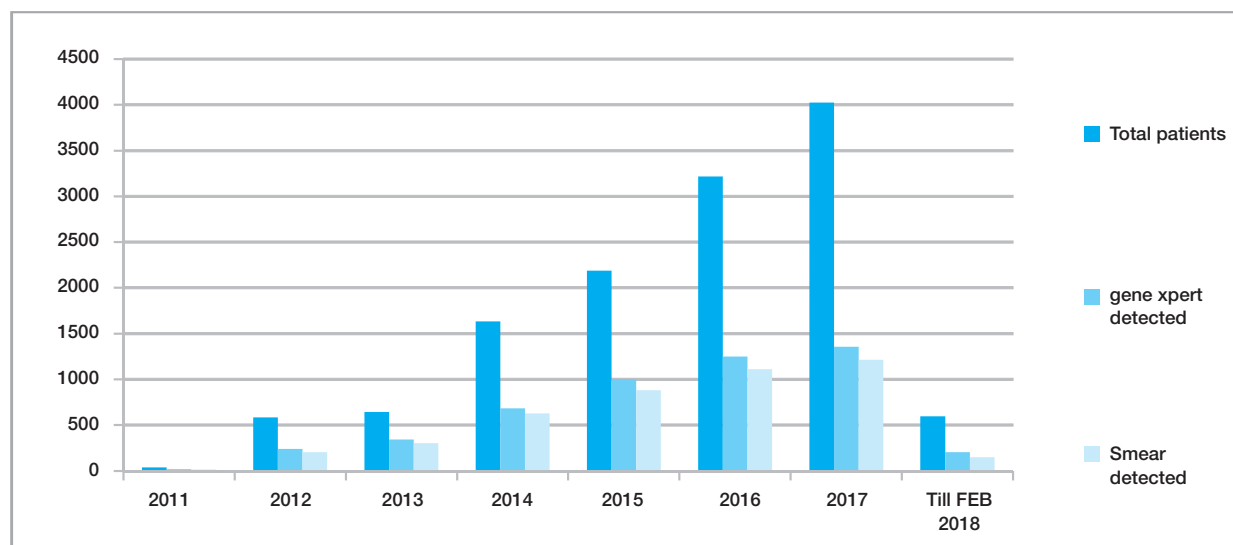


Table 2. Comparison of Gene xpert and Sputum Smear Microscopy in detection of MTB.

repeat testing of Xpert Mtb-Rif were performed due to technical errors. Gene Xpert was negative in 7346 patients (56.83%). Sputum smear microscopy detected about 4502 cases (34.83%) of Tuberculosis while smear microscopy were unsuccessful in 8422 patients (65.16%)

### Discussion

In Pakistan, burden of tuberculosis with regards to Drug susceptible TB and Drug Resistant Tuberculosis is already high, on the other side Pakistan is a resource limited country this poses hindrance in diagnosis and treatment of disease causing high toll on already burdened economy of country.

Only a limited proportion of tuberculosis patients i.e. estimated 500000 patients which have acquired MDR-TB and 1.37 million cases which are co-infected with HIV all over the world, each year, have access to proper case detection or DST.<sup>14</sup> The delay in diagnosis and consequently treatment of these patients leads to significant increase in mortality along with secondary resistance to anti tuberculous drugs and disease spread.<sup>15-16</sup>

To respond to this situation requiring simple and rapid diagnostic tool, a fully automated molecular test for tuberculosis detection and rifampicin resistance testing was developed. MTB/RIF test made possible detection of MTB in sputum samples with relatively higher sensitivity in short duration of about 2hours.

In one study it has been shown that efficiency of GeneXpert test in diagnosis of pulmonary TB was 100 % among all tuberculous patients showing sensitivity more than 97%.<sup>9</sup> Another study explored the sensitivity, specificity, positive and negative predictive values in microscopically negative

specimens as 86.3%, 93%, 79% and 95.6 % respectively. It further concluded that the Xpert assay correctly detected rifampicin resistance in all but one, which harbored mixed population.<sup>10</sup>

Xpert MTB/RIF uses heminested real-time PCR assays for the amplification of MTB specific sequence of the rpo B gene which are probed with molecular beacons for the presence of certain mutations inside rifampicin resistance determining unit.<sup>17-18</sup>

To know about the actual burden of tuberculous patients coming to chest OPD of Nishtar hospital and to find out diagnostic accuracy of different modalities available to diagnose tuberculosis in Pulmonology department of Nishtar, we performed an audit of data gathered from MDR-TB clinic situated in OPD of chest ward.

This was just an audit of the data compiled over 6 years to find out the results of two tests being carried out in our department. Data results are suggestive of superiority of Gene Xpert over AFB smear. Authors did not find any such data of audit as most of the studies compare both tests in cohort.

### Conclusion

Out of total patients tested with sputum smear microscopy, 4502 had smear positive pulmonary tuberculosis (34.83%) and 8422 have smear negative tuberculosis (65.16 %).

12924 patients were tested with Xpert/Mtb Rif, amongst them Gene Xpert were able to detect Mtb in 5100 (39.46%) while about 7346 (56.83%) remained negative.

Predictive value of gene xpert in detecting MTB was found higher than sputum smear testing , so

suggestion can be made for use of gene xpert in diagnosing MTB in New patients as well as retreatment cases.

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