AN AUDIT OF SPUTUM SMEAR MICROSCOPY, GENE XPERT AND DST IN A TERTIARY CARE HOSPITAL IN PATIENTS OF SUSPECTED MDR/XDR-TB

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ABSTRACT:

Objective: To audit the results of sputum smear microscopy & gene expert and DST in NMU, Hospital Multan.
Patients and Methods: **Methodology;** The gene expert MTB-Rif assay is available in the department of Pulmonology NMU Hospital Multan from November 2011. Since November 2011 about 13000 tests have been performed, sputum smear microscopy have already being 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 expert, DST, Data.

INTRODUCTION:

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.

TB 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

Just under one third of mankind suffers from latent tuberculosis infections and is at risk for reactivation [1]. There were estimated 8.7 million new cases of tuberculosis in 2011 along with 1.7 million mortalities all over the world[1]. In 2016, 10.4 million people developed TB whereas mortality rate was 1.7 million and more than 95%of TB related deaths occured 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 phillipines and Pakistan[12,13]. Of these 8.7 million new cases of TB 30,000 hapless patients suffered from the MDR-TB caused by organisms resistant to at least Rifampicin and Isoniazid. The poorer the country, the more the burden of MDR-TB[1] and is on the rise.

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[1, 2], Whereas XDR-TB, a subset of MDR-TB with added resistance to all floroquinolones plus one of the any three injectables anti TB drugs viz Kanamycin, Amikacin and Capreomycin[1, 2, 3] is also on the rise.

Traditionally, sputum smear microscopy is employed to detect MTB in sputum samples. It is cheap with high specify 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, nasocomial outbreaks abound. Lack of rapid and accurate diagnoses and case detection are major obstacles to TB control[1, 3].

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].
L J 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[4].
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[4].

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[4] Bacterial lysis, nuclear acid extraction and amplification , and amplification detection are carried out in a fully automated manner[5].

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[6]. Data yielded by this assay shows greater than 95 % detection of all TB patients [7], 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 in non-respiratory specimen comprising stool, urine, and tissue and CSF samples were calculated to be 77.3% and 98.2 % respectively."[8]

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 mints) [11].

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.

PATIENTS AND METHODS

We collected the data since the availability of gene Xpert MTB-Rif in department of Pulmonology NMU i.e 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. 2 sputum samples were processed for microscopy and 1 sample for gene xpert.

VENUE OF STUDY

MDR TB clinic attached with department of Pulmonology Nishtar medical university Multan Punjab Pakistan

STUDY DESIGN; It is a descriptive study in which data was collected from MDRTB clinic and analyzed

SAMPLING: Non probability consecutive sampling was done

DATA ANALYSIS: Descriptive statistics were applied, frequencies and percentages were calculated using SPSS 17.
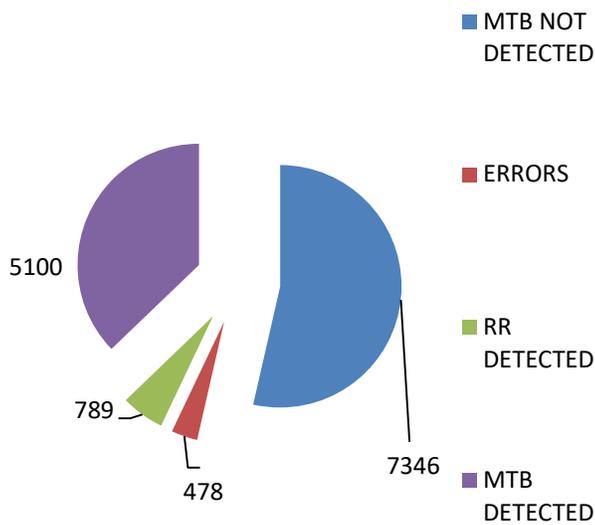
RESULTS

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 repeat testing of Xpert Mtb-Rif were performed due to technical errors. Xpert were found negative in **7346** of total testings.

Sputum smear microscopy detected about **4502** cases of Tuberculosis while smear microscopy were unsuccessful in **8422** patients.

SM +ve	SM -ve	ERRORS	RR	MTB ND	MTB DD	TOTAL	YEARS
16	22	0	9	20	18	38	2011
206	377	40	100	302	241	583	2012
303	339	33	75	265	344	642	2013
626	1006	25	183	924	683	1632	2014
882	1306	22	171	1161	1005	2188	2015
1110	2108	182	100	1787	1249	3218	2016
1212	2814	169	126	2501	1356	4026	2017
147	450	7	25	386	204	597	Till Feb 2018
450	**8422**	**478**	**789**	**7346**	**5100**	**12924**	**TOTAL**

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Xpert Mtb/ Rif interpretation

- MTB NOT DETECTED
- ERRORS
- RR DETECTED
- MTB DETECTED

5100
789
478
7346

RESULTS (in terms of percentages)

- MTB NOT DETECTED
- ERRORS
- RR detected
- MTB DETECTED

37%
6.%
3%
54%

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 hinderance 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 millions cases which are co – infected with HIV all over the world, each year, have access to proper case detection or DST. [14] 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. [15-16]

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% [9]. 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[10].

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. [17-18]

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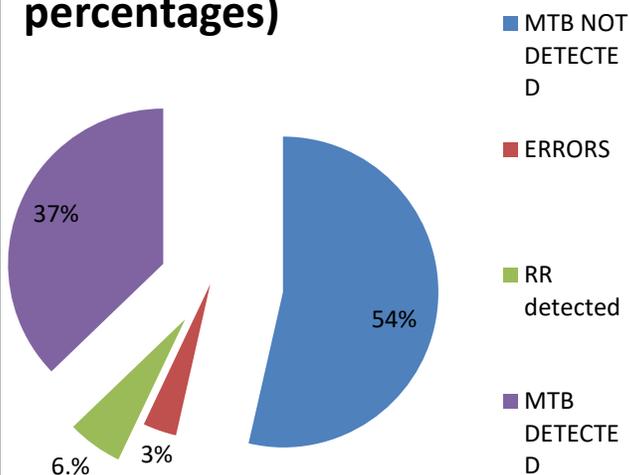
different modalities available to diagnose tuberculosis in Pulmonology department of Nishtar, we performed an audit of data gathered from MDR-TB clinic situated in OPD of chest ward .
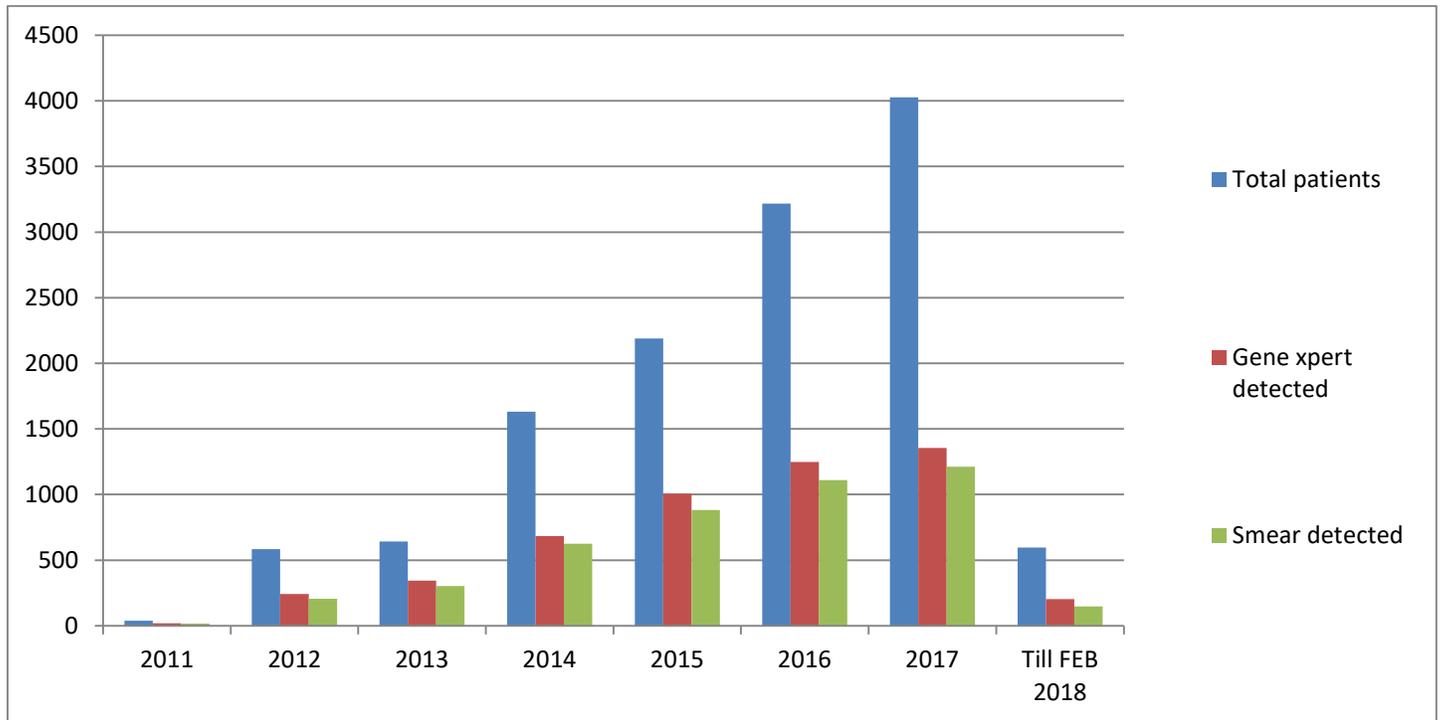

Table 4.
Comparison of Gene xpert and Sputum Smear Microscopy in detection of MTB.

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, amongest them Gene Xpert were able to detect Mtb in 5100 while about 7346 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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