International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Drug Resistance Pattern and Changing Diagnostic Trends of Multidrug-Resistant Tuberculosis at Tertiary Care Centre in Western Rajasthan: A 5-Year Experience

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Abstract: ***Purpose: Multidrug-resistant tuberculosis (MDR-TB) is a serious obstacle to successful TB control.India has a high burden of drug-resistant tuberculosis (TB). Microscopy is the most available diagnostic technique but drug resistance cannot be diagnosed by microscopy. As Culture method is the gold standard important in diagnosis and drug resistance testing, but it has the prolonged turnaround time, high costs and unavailability. The Gene Xpert method for XpertMTB/Rif assay, allows rapid diagnosis of tuberculosis/multiple drug resistance Mycobacterium tuberculosis (MTB/MDRTB). The aim of thisstudy is to evaluate the day by day increase role of the XpertMTB/Rifassay method in the diagnosis of MTB and drug resistance -tuberculosis (DR-MTB) after its installation inwestern Rajasthan. The objectives of this study were to determine the first-line drug resistance patterns and its changing diagnostic trends in western Rajasthan in the 5 years. In view of this, we reviewed our data over last five years. Materials and Methods: This five year observational, retrospective, hospital base, study was carried out in tertiary center of western Rajasthan, Kamla Nehru chest hospital, Dr. S.N. medical college Jodhpur Rajasthan, India. During the period of March 2012 to December 2016, 1225 cases of multidrug resistant tuberculosis.Results:In our study we observed progressive increase in percentage of CBNAAT based diagnosis of MDR-TB in our study population as 9% in 2012, 23% in 2013, 29% in 2014, 39% in 2015 and 46.96% in 2016. 16.9% patients were rifampicin mono-resistant and 53.6% patients were both isoniazid and rifampicin resistant based on LPA. 29.5% patients on retreatment group were put on treatment on the basis of gene expert.Conclusion: This study recommends that Xpert MTB/RIF should be incorporated in the diagnostic algorithm of pulmonary tuberculosis, which has been dealt in the latest tuberculosis guideline.***

Keywords: DR TB (Drug Resistant Tuberculosis), INH (Isoniazide), LPA (Line Probe Assay),

1. Introduction

The emergence of resistance to anti-tubercular drugs in general is a significant public health problem that threatens tuberculosis care and control worldwide. Inspite of hard efforts taken to control tuberculosis(TB),[1] this disease continues to be one of the major publichealth problems worldwide, particularly in developingcountries.[2]Globally, an estimated 3.3% of new patients and20% of previously treated patients have MDR-TB.[3]Multi-drug-resistant tuberculosis (MDR-TB) is defined as tuberculosis that is resistant to at least Isoniazid (INH) and Rifampicin (RMP), the two most powerful first-line treatment anti-TB drugs. The advent of HIV/AIDS contributed to this substantially.

In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide. Globally, an estimated 3.3% of new patients and 20% of previously treated patients have MDR-TB.[4]There are an estimated half million (480,000) new cases of multi drug resistance tuberculosis around the world every year. TB creates more orphans than any other infectious disease.[3]

MDR-TB results from infection with already drug-resistant bacilli or may develop during a patient's treatment, mostly due to administration of improper regimens in chemotherapy of drug-susceptible TB patients and failure to ensure patient compliance. The cause of drug resistant tuberculosis is loss of Sensitivity to drugs due to genetic mutation.[4]

Almost half of the global MDR-TB cases were reported to be from China and India.[5]In order to formulate a national treatmentpolicy reliable and periodic updates on the prevalence ofdrug resistance for the entire country is needed, which wouldserve as an indicator of the transmission of drug resistantorganisms as well as the efficacy of the National Tuberculosis Programme (NTP).[6]

Sputum smear microscopy is the only accessible technique inmost facilities in the developing world. The technique isinexpensive requiring no complicated equipment and candiagnose the most infectious patients with relatively highspecific results that always prompt treatment without furtherconfirmations [7].Culture is the gold standard method and drug sensitivitytesting (DST) can be done but it is costly and thereforeunavailable in most sites. There is also the risk of contaminationand prolonged turnaround time due to the slow growth rate ofthe TB bacilli [8].

The gene Xpert machine (XpertMTB/RIF Assay method) hasthe potential to revolutionize the diagnosis of TB based on itsspeed, sensitivity and specificity [9]. It is a cartridge-basedautomated diagnostic test that can, in less than 2 h,simultaneously detects *Mycobacterium tuberculosis* organismsas well as rifampicin resistance by using three

specific primersand five unique molecular probes which ensure a high degree ofspecificity. The use of sealed and disposable cartridgesapparently overcomes the problem of cross contamination.

There is minimal bio-hazard so that bio-safety cabinets are notcompulsorily needed. The Xpert MDR/Rif assay method which isa nucleic acid amplification test (NAAT) based on the principle ofpolymerase chain reaction (PCR) has opened a new era ofwidespread molecular diagnosis of TB. Its hands-on operation iseasy and requires minimal technical expertise [10].

In Western Rajasthan, MDR-TB patients are frequently admitted at the kamla Nehru chest hospital, of Dr. S.N. medical college, Jodhpur, Rajasthan for initiation of category 4. This study presents our five year experience with MDR-TB patients in terms of changing diagnostic trend over time and drug susceptibility patterns.

2. Material and methods

This is five years retrospective, hospital data based, observational study (March2012-Desember 2016) was carried out in tertiary care center of western Rajasthan, Kamla Nehru chest hospital, Dr. S.N. medical college, Jodhpur, Rajasthan. Approval was received from ethical committee of Dr. S.N. medical college ethical committee. Informed consent was taken. To determine the drug resistance pattern and increase role of gene xpert in diagnosis of MDR TB cases in western Rajasthan admitted in kamla Nehru chest hospital, Jodhpur, to facilitate early diagnosis and treatment. Also to find out the number of those diagnosed cases who were successfully initiated the treatment in MDR TB Centre of western Rajasthan.

Data was entered using Microsoft Excel 2007 and analyzed using Software SPSS 24th Version. Statistical analysis was done by using Chi square test and P Value <0 .05 as significant.

Inclusion Criteria

1) Rifampicin mono-resistant cases confirmed by molecular DST (Line Probe Assay).
2) All multidrug (isoniazid and rifampicin) resistant tuberculosis cases.
3) All rifampicin resistant (gene expert based) retreatment case.

Exclusion Criteria

1) Extra pulmonary MDR-TB cases
2) Resistant Tuberculosis other than Multi drug resistant tuberculosis
3) Transferred in patients

Total 1225 MDR-TB cases were admitted in tertiary care center, Jodhpur for initiation of category 4, according to the above criteria. Two diagnostic technologies were used: Line Probe Assay (LPA) and Cartridge-Based Nucleic Acid Amplification Testing (CBNAAT) method. After confirmation of MDR-TB, patient is referred to the drug resistant tuberculosis center (DR-TB center) forinitiation of regimen for MDR-TB.

All sputum microscopy positive samples were subjected to Line Probe Assay (LPA) by Genotype MTBDR plus kit, where as microscopy negative samples were subjected to culture by LJ media. Culture was observed for 8 weeks and weekly reading was done. If culture became positive then they were subjected to LPA to know their drug sensitivity by Genotype MTBDR plus kit. Then resistance to INH or Rifampicin or both could be determined by comparing the strip given along with the diagnostic kit.

The whole procedure was divided into three parts: 1) DNA extraction; 2) PCR amplification and; 3) Hybridization. We have used MTBDR plus kit for the present study and followed the instructions given by the manufacturer of the kit while performing each of the above mentioned procedures. [7]

In gene xpert two (2.0) ml aliquots of each diluted specimen were dispensed into the cartridge port and loaded accordingly into the Xpert machine for automated processing which included ultrasonic lysis of the mycobacterium to release DNA, washing and filtration, amplification and quantification of the DNA by real time PCR as well as detection of mutations on the rpoB gene using molecular beacons, with display of result within 2 h.

3. Results

In present study, 1225 MDR-TB cases were admitted in our center from six districts of western Rajasthan, over five years march 2012 to December 2016. Age of the patients was ranging from 9 to 75 years and male-female ratio was 4:1. Study population consists of predominantly male 78.6 %(963/1225).(table no. 1)

The study population comprised of 1225 patients, 963 male (78.64%) and 262 females (21.4%). Of these 24 (1.8%) were HIV positive. Further categorization of the patients based on thereasons for doing the test showed that most of patients wererelapse 465/1225 (37.8%) followed by defaulters 386/1225(31.5%) and 105/1225(8.5%) had previous history of second line drugs exposure cases as shown inTable 1.

207/1225(16.9%) patients were rifampicin mono-resistant and 657/1225(53.6%) patients were both isoniazid and rifampicin resistant based on LPA. 361/1225(29.5%) patients on retreatment group were put on treatment on the basis of gene expert.(Table no. 2).

In our study We observed progressive increase in percentage of CBNAAT based diagnosis of MDR-TB in our study population as 9% in 2012, 23% in 2013, 29% in 2014, 39% in 2015 and 46.96% in 2016(table no. 3)(figure).

Table 1: The patient's gender, HIV status and reason for testing
Represented in percentage

Patients characteristics	No of Patients
Total no. of patients	1225
No. of male	963
No. of female	262
Reasons of test	
Defaulter	386/1225(31.5%)

Relapse	465/1225 (37.8%)
Tb contact	16/1225(1.3%)
Hivtb	9/1225(0.7%)
Treatment failure	300/1225(24.4%)

Table 2: Distribution of patients according to methods of diagnosis andResistance pattern

Resistance pattern	Number (%)
Rifampicin mono-resistant (LPA)	207(16.9)
Multi-drug resistant (LPA)	657(53.6)
Rifampicin resistant retreatment (Gene expert based)	361(29.50)
Total	1225(100)

Table 3: Year Wise Resistance Pattern

Resistance Pattern/Year	2012	2013	2014	2015	2016
LPA	176	188	214	159	140
CBNAAT	19	56	88	101	124
Total	195	244	302	260	264*

*data collected up to Dec 2016

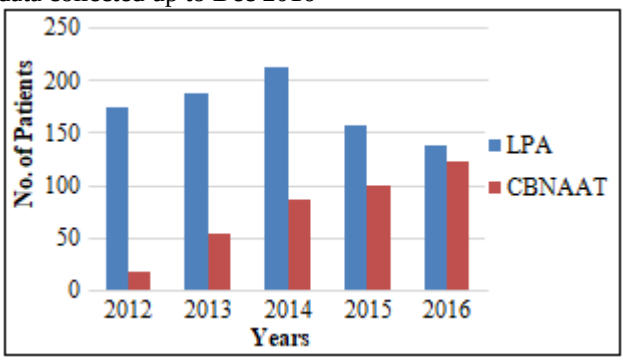

Figure 1: Diagnostic trends over time of resistance patterns among DR-TB patients diagnosed in western Rajasthan, 2012–2016

4. Discussion

To our knowledge, this is the first study of multidrug-resistant tuberculosis (MDR-TB) diagnostic trendsover an extended period of time (2012-2016) in western part of Rajasthan.The prevalence of Multi drug resistance tuberculosis is on a rise since decades globally.

This study was conducted to evaluate the increase role of CBNAATin diagnosing pulmonary tuberculosis anddetection of rifampicin resistance among these patients. Toaddress these challenges, there is a critical need in suchsettings for rapid, effective screening for TB and detectionof drug resistance for early initiation of appropriatetreatment. The WHO policy guidance on the use of CBNAAT wasissued in December 2010. The recommendations werethat it should be used as the initial diagnostic test inindividuals at risk of having MDR-TB[11]

Rapid detection of TB/MDRTB is critical for improving patientscare and decreasing TB transmission. The second line drugs fortreatment of MDRTB are more expensive, not commonlyavailable, have more adverse side effects and with extendedtreatment duration [12].

The MTB/RIF assay method is a good and reliable proxy forMDRTB and enables earlier initiation of treatment and bettertreatment outcome which are essential in decreasing MDRTBtransmission [13].

Out of the 1225 study participants, 963/1225(78.6%) patients were male, 262 (21.4%) patients were female and mean age was about 36.33 years. Similar finding was found in other studies [14-19] (30.32-37.08 years). DrNirmalya Manna et al[14] from Kolkata in 2014 found that 70.7% were male and 29.2% female,SunitaTripathy et al[19]from north Bihar in 2013 found that 79.4% were male patients and 20.6% were female.

864/1225(70.5%) patients were LPA based diagnosed and 361/1225(29.5%) patients were put on treatment on the basis of gene expert. 207/1225(16.9%) patients were rifampicin mono-resistant and 657/1225(53.6%) patients were both isoniazid and rifampicin resistant based on LPA. These findings were similar to DrNirmalya Manna et al [16] a study in Kolkata, where among total 53.7% patients were found to be resistant to Rifampicin only and 46.3% to both Rifampicin and Isoniazid. SunitaTripathy et al[19] study in north bihar showed 65.4% LPA and 25.6% CBNAAT based diagnosis, In DrNirmalya Manna et al [16] study showed 65.8% LPA based and 24.7% CBNAAT based and SaralaMenon et al[17] study in Mumbai showed 74.4% LPA based and 25.6% patients were put on treatment on the basis of gene expert.

Trends of diagnosis of MDR-TB from 2012 to 2016. In the present study, a continuous increasing trend of increase role of gene xpert for diagnosis of MDR-TB was observed from 2012 to2016. A total cases of 19 (9%), 56 (23%), 88(29%), 101(29%), and 124 (46.96%) were identified as gene xpert based MDR-TB cases in 2012, 2013, 2014, 2015and 2016, respectively [Figure 1]. Overall gene xpert based MDR-TB gradually increased from 2012 to 2016 in bothnew and previously treated cases. Possible reasons include increasing awareness amongst clinicians of the possibility of MDR-TB and the availability CBNAAT facility on district and sub district level.

Previously LPA and CBANAAT sample were sent to Ajmer for their drug resistance testing, but now LPA machine is established in Dr. S.N. Medical College. Therefore all the cases of multidrug resistant TB are diagnosed and treated here. Since CBNAAT is available in all the districts of western part of Rajasthan so that number of cases will probably increase in future.

RNTCP adopted CBNAAT in India in April 2012. In thegovernment set up, CBNAAT was launched in 2012 as apilot project in Maharashtra by the State tuberculosisdepartment. By the end of 2012, under EXPANDx-TBproject, 12 CBNAAT labs were established all over Indiaacross different states(20).WHO recommends CBNAAT fordiagnosis of pulmonary tuberculosis and detection ofrifampicin resistance, especially in PLHIV and re-treatmentcases who are at risk of MDR-TB(21).

5. Conclusion

CBNAAT detects pulmonary TB with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours. It also detects rifampicin resistance with high specificity and can be used for screening for MDR-TB so

that early therapy can be started, thus decreasing the incidence of MDR-TB.

This study recommends that Xpert MTB/RIF should be incorporated in the diagnostic algorithm of pulmonary tuberculosis, which has been dealt in the latest tuberculosis guideline.

References

[1] Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ,Reniero A, et al. Global trends in resistance to antituberculosisdrugs. World Health Organization-International Unionagainst Tuberculosis and Lung Disease Working Group onAnti-Tuberculosis Drug Resistance Surveillance. N Engl JMed 2001;344:1294-303

[2] Gupta R, Espinal MA, Raviglione MC. Tuberculosis as amajor global health problem in the 21st century: A WHOperspective. SeminRespirCrit Care Med 2004;25:245-53.

[3] World Health Organization. Global tuberculosis report, 2015.WHO/HTM/TB/2015.08. Geneva, Switzerland: WHO, 2015.

[4] David HL. Probability distribution of drug-resistant mutants in unselected [7] populations of Mycobacterium tuberculosis. ApplMicrobiol. 1970;20:810-14.

[5] World Health Organization. Multidrug and ExtensivelyDrug-Resistant TB (M/XDR-TB). 2010 Global Report onSurveillance and Response. Geneva, Switzerland: WorldHealth Organization; 2010 (WHO/HTM/TB/2010.3). [Lastaccessed 2011 Aug 13].

[6] Paramasivan CN, Venkataraman P. Drug resistance in tuberculosis inIndia. Indian J Med Res 2004; 120:377‑86.

[7] Mark DP, Giorgio R, Alimuddin Z (2006) Progress towardsimproved tuberculosis diagnostics for developing countries.Lancet 367: 942-943.

[8] Cuevas LE, Vassin MA, Al-Sonboli, Lawson N, BAhader J (2011) Amulti-country non-inferioriy cluster randomize trial of front–loaded smear microscopy for the diagnosis of pulmonarytuberculosis. PLOS Med 8: l000403.

[9] Carlton AA (2011) Gene Xpert: Agame changer for tuberculosiscontrol? PLOS Medicine.

[10] World Health Organisation (2012) WHO Nigeria supportsintroduction of Xpert MTB/RIF technology for diagnosis of MDRTBin Nigeria.

[11] WHO Policy statement: automated real-time nucleic acidamplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: XpertMTB/RIF system 2011. Available at: http://www.who.int/tb/laboratory/en/

[12] Otu A, Umoh V, Habib A, Ameh S, Lawson L, et al. (2013) Drugresistance among pulmonary tuberculosis patients in Calabar, Nigeria. Pulmonary Medicine.

[13] Papaventsis D, Ioannidis P, Evangelos V (2014) Tuberculosisdiagnosis update: the new gene xpert MTB /RIF assay. Hellenic centre for disease control and prevention. E-bulletin.

[14] DrNirmalya Manna et al Kolkata, India Drug resistance pattern, related socio- demographic factors and preventive practices among MDR-TB patients: An experience from a tertiary care setting (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 9 Ver. VI (Sep. 2014), PP 16-21 www.iosrjournals.org

[15] Gnenyabhatt et al an epidemiologica study of multi drug resistant tuberculosis cases registered under rntcp of ahmedabad city. Indian j tuberc 2012: 59: 18-27

[16] Julia V. Ershova et al in 2012 Epidemiology of Primary Multidrug-Resistant Tuberculosis, Vladimir Region, Russia Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 11, November 2015

[17] SaralaMenon et al Drug resistance profiles of Mycobacterium tuberculosis isolates to first line anti-tuberculous drugs: A five years study Lung India • Vol 29 • Issue 3 • Jul - Sep 2012

[18] Poulomi Mukherjee et al R. G. Kar Medical College, Kolkata Sociodemographic and clinical profile of multi drug resistant tuberculosis patients: a study at drug resistant tuberculosis centers of Kolkata (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279 0861. Volume 14, Issue 8 Ver. IV (Aug. 2015), PP 52-58 www.iosrjournals.org

[19] SunitaTripathy et al Prevalence of Multidrug Resistant Pulmonary Tuberculosis in North Bihar Journal of Clinical and Diagnostic Research. 2015 Nov, Vol-9(11): LC09-LC12

[20] Theron G, Peter J, Zyl-Smit RV et al. Evaluation of the Xpert MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in a HighHIV Prevalence Setting. Am J RespirCrit Care Med 2011; 184 (1):132-40.

[21] Chauhan LS. Drug resistant TB-RNTCP response. Indian J Tuberc2008; 55 (1): 5-8.