

A PRELIMINARY STUDY OF PREVALENCE OF MDR – TB IN DIFFERENT REGIONS OF WESTERN RAJASTHAN, THROUGH LINE PROBE ASSAY (LPA) : A CLINICO – BACTERIOLOGICAL CROSS – SECTIONAL STUDY.

¹Prabhat Kiran Khatri, ²Neetu Bhora, ²Laxmi Rathore, ²Suneel Tyagi, ⁵Gopal Purohit

Abstract

infections. Diagnosis of multi drug resistance strains of ten district of western Rajasthan were performed by LPA. Sputum samples from various districts from June 2014 to June 2015 were tested using LPA technology and those with LPA positive were segregated. With respect to segregation, the prevalence of multi drug resistance in respective regions was analyzed. Further, areas were categorized as those with high and low prevalence rate. Region found to have highest prevalence rates of multi drug resistance is “Pali” district and that with lowest is “Bikaner”.

INTRODUCTION

Tuberculosis (TB) is one of the deadliest infectious disease. It is transmitted through the coughing, droplets of the infected person¹. Disease account for high global burden with 9.0 million incidents per year and 1.5 million deaths according to WHO report 2014². One of the most critical phase of TB infection is development of the multi drug resistance (MDR) within the bacilli genome. It is major threat to the world with wide control of tuberculosis. More specifically multi drug resistance explained as resistance to isoniazid and rifampicin, with or without resistance to any other anti-tuberculosis drugs. Resistance to any anti mycobacterial drugs is the consequences of naturally occurring, spontaneous mutations that encode either the target of the drug or the enzymes that are involved in the drug activation.

1 Senior Professor & Head, 2 Senior Demonstrator Department of Microbiology 3 HOD, TB Chest Department, KNCH, Dr. S.N. Medical College, Jodhpur
Corresponding address: Dr. Prabhat Kiran Khatri
drpkkhatri@yahoo.co.in

Multi drug resistance develops by the subsequent acquisition and the selection of mutant at the different loci usually because of the inappropriate treatment of the patient. Inappropriate treatment may lead to the disease progression which will increase the bacterial load and the risk of the naturally occurring mutations. Isoniazid is a prodrug that requires activation of isoniazid susceptible mycobacteria species. Activation of it results in a number of highly reactive compounds that are capable of managing the mycobacterial cell wall. Isoniazid resistant clinical isolates lose their catalytic peroxidase activity. Also one of the main reasons for the treatment failure and fatal clinical outcome in TB patient is resistant to Rifampicin. Rifampicin displays the significant early bactericidal effect on metabolically active mycobacterium tuberculosis and the excellent late sterilization action on the semi dormant organism undergoing short burst of metabolic activity. Rifampicin resistance occurs more often in strains that are also resistant to Isoniazid, thus Rifampicin resistance can be used as a surrogate marker for multi drug resistance TB.

Therefore, the most important criteria for the treatment of tuberculosis also becomes the categorisation of the patient into multi drug resistance and Non-MDR.

Present study involves categorisation of MDR3 suspects testing through Line Probe Assay (LPA) into multi drug resistance and non-multi drug resistance, subsequently screening of the regions to those high multi drug resistance load and low multi drug resistance load. The results of the study could be a substantial tool

- a) knowing the high load multi drug resistance region
- b) isolating the masses from the co-habitants
- c) restricting the migrants of these masses to other regions

as they may serve as a host surviving multi drug resistance bacilli to the non-infected peoples also, which subsequent may increase the “case of new system positive with MDR”.

MATERIALS AND METHODS

Collection of Samples

As the laboratory is accredited for providing Diagnostic services through LPA, we receive multi drug resistant suspected samples from nine district of Western Rajasthan. As per RNTCP protocol⁴ they are sent to the laboratory via courier in the triple layered packing maintaining cold chain. Samples were received at laboratory within one day from the collection at various DTC's and TU's. Then samples are taken for the processing.

Processing of Samples

All the Diagnostic samples received are then subjected to a microscopy using standard Ziehl-Neelsen staining method.

All the positive samples are then subjected to the LPA which was done in the following steps:

- 1) Decontamination and digestion of the samples using NALC-NaOH method as defined in the RNTCP guidelines. The

process was carried out in the biosafety cabinet class A2.

- 2) DNA extraction using renewal Genolyse multi drug resistance kit from HAIN Life sciences, provided by the FIND India.
- 3) Preparation of master mix reagents in the cleanest room with the PCR hood.
- 4) Amplification of the extracted DNA through thermocycler.
- 5) Reverse hybridization using MTBDR genotype kit of the in HAIN Life sciences.

Once the results were obtained we then segregated the samples district wise. For each district then samples were segregated as LPA positive and negative. Further, LPA positive samples were segregated into the sensitive and resistant isolates.

RESULTS AND DISCUSSION

As the laboratory performing LPA services to nine District of Western Rajasthan, therefore in all the samples received at laboratory for one year from June 2014 to June 2015 were analysed.

Of the total 1859 were LPA positive samples at the laboratory 471 LPA positive from district Jodhpur, 205 LPA positive from Barmer district, 152 positives from Jalore district, 62 were positive from Jaisalmer district, 284 were LPA positive from Pali district, 217 were LPA positive from Sirohi district, 84 were positive from Bikaner district, 204 were LPA positive from Hanumangarh district, 180 were LPA positive from Ganganagar district. (Table: 1)

Further to analyse the multi drug resistance load in these areas, all positive samples from each district were then segregated into the sensitive and MDR. For Jodhpur district, out of 471 LPA positive samples, 397 were sensitive and 74 were multi drug resistance isolates. In Barmer district out of 205 LPA positive samples, 167 were sensitive and 38 were MDR isolates, in Jalore out of 152 LPA positive samples, 121 were sensitive and 31 were multi drug resistance isolate; in

Jaisalmer out of 62 LPA positive samples, 56 were sensitive and 6 were MDR. In Pali out of 284 LPA positive samples 224 were sensitive and 60 were multi drug resistance isolates; in Sirohi out of 218 LPA positive samples, 173 samples were sensitive and 44 MDR; in Bikaner out of 84 LPA positive samples 80 were sensitive and 4 were MDR; in Ganganagar out of 180 LPA samples 155 were sensitive and 25 were multi drug resistance isolates and in Hanumangarh out of 204 LPA samples, 181 were sensitive and 23 were MDR.

This analysis gives a clear picture of percentage load multi drug resistance patient in these districts. Figure 1

Table 1: District wise distribution of MDR Tuberculosis

District	LPA TB Detected	First Line Sensitive	MDR TB Cases
Jodhpur	471	397	74
Barmer	205	167	38
Jalore	152	121	31
Jaisalmer	62	56	6
Pali	284	224	60
Sirohi	217	173	44
Bikaner	84	80	4
Ganganagar	180	155	25
Hanumanghar	204	181	23
Total	1859	1554	305

Jodhpur having 15.71% of multi drug resistance cases, Jalore head 20.39% of multi drug resistance cases, Jaisalmer had 9.6% of multi drug resistance cases, Sirohi had 20.27% of multi drug resistance cases, Barmer with 18.5% of multi drug resistance cases, Pali 20.12 % of multi drug resistance cases, Bikaner with 4.7 % of multi drug resistance cases, Hanumangarh with 11.2% multi drug resistance cases and Ganganagar with 13.8% multi drug resistance cases.

Our study reflected that the Pali district had highest percentage of multi drug resistance

TB load in Bikaner had least. This analysis may provide a useful tool, through which the RNTCP program can manage the patients, and their treatment may be fast tracked. Most importantly, the immigration of the multi drug resistance masses may be checked. Movement of these multi drug resistance masses if not checked, then may serve as host which would transmit the co-habitants directly with the multi drug resistance tuberculosis infections. Because of all those patients with multidrug resistant tuberculosis infections if encounters a healthy person, the cough droplets would be carrying mutated (multi drug resistance) bacilli, a healthy person would be transmitted the infection already to its resistant stage, these infected co-habitants, now when screened would be given the first line drugs, which may have no effect on them, also these “new infected” masses would further serve as host transmitting MDR-TB infections to another healthy masses.

Figure 1: MDR load in Rajasthan districts, catered by Lab



Jodhpur 15.7%, Barmer – 18.5%, Jalore 20.39 %, Jaisalmer 9.6%, Pali 21.2%, Sirohi – 20.27%, Bikaner 4.7%, Ganganagar 13.8%, Hanumangarh 11.2%

Tuberculosis associated with multidrug resistance has emerged as Global problem for many decades. Present study gives the prevalence pattern of multi drug resistance TB through LPA in western regions of Rajasthan state, reflecting that Pali district has highest prevalence of multi drug

resistance TB load and Bikaner the least. The observation may be used as an important base of monitoring these regions for

- a) Regular and correct treatment
- b) Restriction of the immigrants of infected masses to nearby areas
- c) Screening of co-habitant for multi drug resistance TB diagnosis. Further, the movement of these multi drug resistance masses if not checked, then these may serve host which would transmit the co-habitants directly with the multi drug resistance tuberculosis infections. And also this "new infected" masses unscreened, would further serve as host transmitting multi drug resistance TB infections to another healthy masses.

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