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Research Article

PHENOTYPIC DRUG RESISTANCE CONFIRMATION OF ADDITIONAL SECOND LINE DRUGS IN EXTRA-PULMONARY MULTI DRUG RESISTANT TB PATIENT

Srashty Sharma[1,2]., Mahmud Hanif*[1]., Kamal Kishore Chopra[1]., Ashwani Khanna[3] and Sunil Kumar Chauhan[2]

[1]Intermediate Reference Laboratory, New Delhi Tuberculosis Centre, New Delhi
[2]Department of Microbiology, Maharaja Vinayak Global University, Jaipur
[3]State Tuberculosis Office, Gulabi Bagh, New Delhi

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A B S T R A C T

Introduction: Rapid phenotypic Drug Susceptibility testing (DST) on liquid media BACTEC MGIT 960 system had been applied for additional newer Second line drugs currently being used in Indian TB programme known as extended second line drugs on confirmed multi drug resistant (MDR TB) patients with extra pulmonary (EP)TB.

Material & Methods: A total of 334 different types of EP specimens were collected from MDR-TB suspects. M. tuberculosis was recovered from such EP specimens using rapid liquid culture. All Mycobacterium tuberculosis complex (MTBC) positive isolates were subjected to line probe assay (LPA) for confirmation of MDR. Confirmed MDR isolates were subjected to MGIT DST for extended second line drugs.

Result: Total culture positivity was found 17 %. Out of these 58 MTBC positive culture isolates, 15% were MDR. Second line DST was performed for Kanamycin, Capreomycin, Levofloxacin, Moxifloxacin low, Moxifloxacin high, Linezolid, and Clofazimine. Different combinations of drug resistance were observed among these isolates.

Conclusion: Significant frequency of extensively drug resistant (XDR) and pre XDR strains in the cohort of EPTB MDR cases shows that there is a certain probability of more unreported XDR cases which can be left untreated otherwise.

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INTRODUCTION

Extra-pulmonary tuberculosis (EPTB) describesthe various conditions caused by *Mycobacterium tuberculosis* infection of organs or tissues outside the lungs. There are many forms of EPTB, affecting every organ system in the body. Some forms, such as TB meningitis and TB pericarditis, are life-threatening, while others such as pleural TB and spinal TB can cause significant ill health and lasting disability. The burden of EPTB is high ranging from 15-20 per cent of all TB cases in HIV-negative patients, while in HIV-positive people, it accounts for 40-50 percent of new TB cases [1]. The estimated incidence of TB in India was 2.1 million cases in 2013, 16 percent of which were new EPTB cases, equating to 336,000 people with EPTB [1]. The prevalence of MDR may lie between 1-69% of total EPTB cases, whereas, the proportion of resistant cases to any one anti-tuberculosis drug is about 10-75 % [2]. Recently, the rare possibility for the presence of XDR strain in extrapulmonary site has also been documented in India [3].

Corresponding author:* **Mahmud Hanif
Intermediate Reference Laboratory, New Delhi Tuberculosis Centre, New Delhi

India is one of the country with highest number of MDR (multi drug resistant) and XDR (extensively drug resistant) tuberculosis. The increase in the incidence of multidrug-resistant tuberculosis (MDR TB) and the emergence of extensively drug resistant tuberculosis present tremendous challenges to the global efforts to combat tuberculosis [4]. The increase in MDR and XDR TB rates prompt effective diagnostic methods so that appropriate treatment can be given rapidly to infected patients[5].

Rapid drug susceptibility testing play an important role in the early detection and control of MDR and XDR [6]. It is well known that broth-based DST methods provide significantly rapid and reliable results. Various drug susceptibility testing (DST) methods that use solid media, including the agar proportion method (AP) and other methods, have the drawback of prolonged turnaround times (TATs). The World Health Organization and the U.S. Centers for Disease Control and Prevention have recommended the use of liquid culture systems for the diagnosis of tuberculosis and DST to improve TATs [7,8]. As a rapid phenotypic DST base on liquid media BACTEC MGIT 960 system had been applied for the DST to

standard Second line drugs and newer drugs currently being used known as extended second line drugs.

The aim of this study is to rapidly screen drug resistance for additional second line drugs in confirmed MDR TB patients with extrapulmonary TB. This study helps in screening out the drug resistance to second line drugs newly introduced in RNTCP programme in India.

MATERIAL AND METHODS

This study was carried out at New Delhi Tuberculosis Centre, Intermediate Reference Laboratory from June 2016 to October 2017. The laboratory is certified by Central TB Division, Ministry of Health and Family Welfare, Govt. of India for conducting liquid culture and DST for first & second line drugs and Line probe assay.

A total 334 different type of Extra-pulmonary specimens were collected from TB suspects which include Ascitic fluid 12(3.5%), abscess 11(3.2%), biopsy 130 (39%), CSF 45 (13.4%), Knee Aspirates 15 (4.4%), Lymph node Aspirates 33 (9.8%), Pleural Fluid 41(12.2%), Pus 28(8.3%) and Synovial Fluid 19(5.6%). All specimens were decontaminated by N-acetyl L-cysteine (NALC-NAOH) method (Kent & Kubica) [9]. The Mycobacterium Growth Indicator Tube (MGIT) with BACTEC MGIT 960(Becton Dickinson, Singapore) used for recovery of Mycobacterium from these clinical specimens (MGIT Manufacturer instruction). Positive culture isolated from MGIT liquid culture was identified for Mycobacterium tuberculosis complex (MTBC) by SD Bioline MPT64kit, as per the manufacturer instruction [10]. All MTBC positive isolate from MGIT liquid culture were subjected to line probe assay (LPA) for detection of drug sensitivity for two drugs Rifampicin and Isoniazid. This assay was performed using MTBDR Plus ver-2 test kit (as per manufacturers [Hain Life Sciences, Germany] instructions) [11].

Phenotypic DST for second line drugs

The MGIT 960 instrument (Becton Dickinson Diagnostic Systems, Sparks, MD) was used for the DST. The protocol for DST in MGIT 960 was strictly followed as recommended by Manufacturer's instructions [12]. Antimicrobial agents-Kanamycin, Capriomycin, Levofloxacin, Moxifloxacin low, Moxifloxacin high, Linzolid, Clofazimin were used in this study. These drugs used with different critical concentration as Kanamycin 2.0 µg/ml, capreomycin 2.0 µg/ml, Levofloxacin 1.0 µg/ml, Moxifloxacin (low) 0.5 µg/ml, Moxifloxacin (high) 2.0 µg/ml, Linezolid 1.0 µg/ml and Clofazimine 1.0 µg/ml. For Quality control, One quality control (QC) known well characterized *M. tuberculosis* H37Rv was also tested with each batch of DST.

Interpretation of results*:* Interpretation of results was done as per manufacturer instructions. [12].

RESULT

Total number of 334 extra pulmonary specimens was collected from 334 suspected EPTB patients during the study period. Of these total number of males was 183(55%) and females were 151(45%). Average age of the study population was 33 years ranging from 4 years to 82 years.

Out of 334 extrapulmonary specimens 58(17%) were positive MTBC, 225(67.3%) were found to be negative for MTB, 50(15%) were found to be contaminated and 1(0.2%) was found Non tuberculosis mycobacteria (NTM). Out of these 58 MTBC positive culture isolates, 9 (15%) were found to be MDR (resistant to both Rifampicin and Isoniazid), 46(79%) were sensitive to both drugs, 3(5%) were found to be mono isoniazid resistant. MDR-TB ranged from 8 % in CSF, which is high among all specimen followed by 7% in Pus, 3% in Lymph node and 1% in Biopsy isolates. All MDR strains were subjected to second line DST for extended second line drugs. Out of these 2(22%) were found resistant to kanamycin. 1(11%) was resistant to capreomycin 3(33%) were resistant to levofloxacin. 2(22%) were resistant to moxifloxacin low 1(11%) was resistant to moxifloxacin high. no one resistant to linezolid. 1(11%) was resistant to Clofazimine [Table 1].

Table 1 Result of DST in extended second line drugs used

	Susceptible	Resistant
Kanamycin (2.0)	7	2
Capreomycin (2.0)	8	1
Levofloxacin (1.0)	6	3
Moxifloxacin low (0.5%)	7	2
Moxifloxacin High (2.0%)	8	1
Linezolid (1.0)	9	0
Clofazimine (1.0)	8	1

DISCUSSION

MGIT 960 is the recommended as the Gold standard method for isolation and recovery of Mycobacterium tuberculosis from extrapulmonary specimens especially from smear negative specimens. Our study reports a higher recovery of MTBC strains 58 (17%) out of the total 334 patients included. Similar data were reported by (15.4%) by salma smaoui *et.al*. [13], (13.6%) by Reena Rveendran *et.al*. [14], (12%) by Bunger et. al. [15], (10.6%) reported by Richa kumara *et.al*. [16], (9.2%) reported by Chakraborty *et.al*. [17], Butt *et.al*. reported higher positivity rate (25.2%) in their study [18]. These differences may be due to the failure of early recognition and under-estimation of EPTB in developing countries because of lack of facilities to diagnose EPTB in these settings.

To detect MDR cases among the culture positive EPTB cases, LPA was conducted and 9 (15.5%) were confirmed as MDR. A higher percentage of MDR is reported in our study and supported by other studies like12.5% MDR in EPTB in Nepal [19] and 10% in Delhi [20]. For screening of XDR among EPTB cases, confirmed MDR samples were further tested for detection of drug resistance in extended second line drugs which shows different combinations of resistance to second line drugs. Among 58 cultures positive MDR suspects, several and disparate patterns of drug resistance is observed among extended second line drugs. XDR and pre-XDR strains were also identified and confirmed in few specimens as documented in our result. Also mono resistant was documented confirming the possibility of inherent drug resistant forms of Mycobacterium tuberculosis among targeted population of extrapulmonary TB. Due to limited availability of data on studies involving extrapulmonary cases, the results obtained in our study is fulfilling the importance of the objectives of the study. The percentage of XDR and preXDR cases reported in our study is high due to the better recovery of positive cultures and the screening of drug resistance in extended second line drugs. Resistance of levofloxacin and moxifloxacin has been reported earlier as 54.3% (Levofloxacin resistance) from Peru [21] and 28% from Taiwan [22] and is supported by our study as well. But strain showing resistance to both Moxifloxacin

lower and higher concentration simultaneously and sensitive to levofloxacin is rarely seen and same is reported in our study.

Moreover, we have conducted drug sensitivity testing for extended second line drugs and pre-XDR and phenotypically confirmed XDR strains were also recovered, patterns with different combinations of drug resistance is also reported.

CONCLUSION

Significant frequency of XDR and pre XDR strains in the cohort of EPTB MDR cases shows that there is a certain probability of more unreported XDR cases which can be left untreated otherwise. During national drug resistance surveys such data of extended second line drugs is very important to rule out the status of increasing drug resistance in population with EPTB. As evidenced by our study more such studies are further anticipated countrywide in every reference laboratory and to make a national database with respect to treatment outcome for the better management of MDR TB patients.

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