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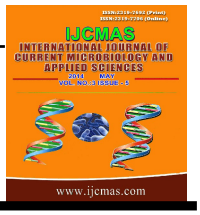

Original Research Article

Extensively Drug Resistant Tuberculosis (XDR-TB) by Phenotypic Drug Susceptibility Using BACTEC Micro MGIT Culture System – A Pilot study in Hospital Based Population in Chennai, India

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Keywords

MGIT, DST, XDR-TB; *Mycobacterium tuberculosis*

A B S T R A C T

Tuberculosis (TB) is the leading cause of death in the world and drug resistant tuberculosis has been reported since the early days of introduction of anti-tubercular chemotherapy. Extensively drug-resistant tuberculosis (XDR-TB) is a form of TB caused by *Mycobacterium tuberculosis* that are resistant to Isoniazid and Rifampicin (i.e. MDR-TB) as well as to any fluoroquinolone and to at least one of three injectable second-line drugs (amikacin, Capreomycin or Kanamycin). There is no published report exclusively on XDR-TB in local population of Chennai. The main scope of this study is to Standardize and apply phenotypic drug susceptibility testing for second line drugs on Multi drug resistant tuberculosis (MDR-TB) isolates for the detection of XDR-TB using microMGIT BACTEC culture system. A total of 322 (40.1%) *M.tuberculosis* isolated from 802 new cases of clinically suspected tuberculosis patients were included in this study. Among the 322 *M.tuberculosis* isolates 13 (4%) were MDR-TB by BACTEC micro MGIT method, 9 (2.7%) were sensitive to all the second line drugs and 4 (1.2%) were resistant to one or more second line drug of which 2 (0.6%) were XDR-TB. This is the first report on XDR-TB from hospital based population in Chennai from a non-governmental organization.

Introduction

Tuberculosis (TB) is the leading cause of death in the world and humans are the only reservoir of this organism (Collee et al., 2002). *M. tuberculosis* strain that is resistant to Isoniazid and Rifampicin, two in the treatment of the disease is called as multi drug resistant tuberculosis (MDR-TB). Drug resistant tuberculosis has been reported since the early days of introduction of anti-tubercular

chemotherapy, but recently MDR-TB is posing threat to global efforts of tuberculosis control programmes. Three most important risk factors, identified in the causation of drug resistant tuberculosis are inappropriate previous treatment with anti-tubercular drugs, high prevalence of drug resistant tuberculosis in the community and contact with patients known to have drug resistant tuberculosis [Collee et al., 2002].

MDR-TB cases threaten the effectiveness of chemotherapy for both treatment and control of TB and require the use of second-line drugs (SLDs) that are more expensive, toxic, and less effective than first-line anti-TB drugs [Zumia et al., 2001]. While assisting MDR-TB treatment programs worldwide, and ensuring the proper use of SLDs in resource limited countries the committee encountered reports of multiple cases of TB with resistance to virtually all SLDs. This led to the emergence of new terminology in relation to drug-resistant TB, i.e., extensively drug-resistant TB (XDR-TB). XDR-TB is a form of TB caused by *M. tuberculosis* that are resistant to Isoniazid and Rifampicin (i.e. MDR-TB) as well as any one of the fluoroquinolone and second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin) [Masjedi et al., 2006, Banerjee et al., 2008]. Because XDR-TB is resistant to first line and second line drugs, treatment options are seriously limited.

The emergence of XDR-TB has re-focused attention on TB as a disease of continuing significance in the developed and developing world [Pooja singla et al., 2014]. Approximately 9% of MDR-TB cases detected to have XDR-TB. Recent reports states that, 84 countries had reported at least one XDR-TB case [Pooja singla et al., 2014]. The situation has turned into a pressing demand for drug-susceptibility testing (DST) for the second line drugs in order to develop efficient regimens for appropriate treatment of individual cases [Raviglione et al., 2007]. Rapid diagnostic methods enabling accurate susceptibility testing of second-line drugs are critical for the early diagnosis of extensively drug-resistant tuberculosis so that the initiation of effective regimens can be possible [Shah et al., 2007]. Drug susceptibility testing by the conventional solid medium culture method is highly sensitive and specific but extremely slow, due to the slow growth of *M. tuberculosis*. The BACTEC MGIT culture systems for SLDs are more accurate and has a less turn around time.

The present study was focused on the standardization and application of phenotypic drug susceptibility testing for second line drugs on MDR-TB isolates for the detection of XDR-TB.

Materials and Methods

The study was carried out at L& T Microbiology Research Centre, Vision Research Foundation, Chennai after obtaining approval from Institutional Research and Ethical board (IRB).

Clinical isolates

Respiratory specimens were collected from patients attending OPD at Institute of thoracic medicine (ITM) and Stanley medical college (SMC). A total of 322 *M. tuberculosis* isolates from newly diagnosed tuberculosis patients were included in this study. The specimen wise distribution of 13 MDR isolates is mentioned in table 1.Age group of patients included in this study ranged from 22-60 years and the mean age was 34.8.

Among the 322 *M.tuberculosis* isolates, 13(4%) were MDR-TB strains and the phenotypic drug susceptibility pattern of all the MDR-TB strains are given in table 2. Further, phenotypic DST for SLD was performed for the 13 MDR-TB strains by following the procedure (kit insert) recommended by BACTEC micro MGIT culture system [BD Diagnostics BACTEC manual].

Phenotypic Drug Susceptibility Testing Method for second line anti-tuberculous drugs

Phenotypic Second Line Drug Susceptibility Testing was optimized using BACTEC Micro MGIT culture system following the instructions given by the manufacturer's kit insert [BD Diagnostics BACTEC manual]. The second line drugs used for drug susceptibility test were Ofloxacin (2.0µg/ml), Levofloxacin (2.0µg/ml), Ciprofloxacin (1.0 µg/ml), Amikacin (1.0µg/ml), Kanamycin (2.5 µg/ml), Capreomycin (2.5 µg/ml), Para-amino salicylic acid (4.0 µg/ml) and Ethionamide (5.0 µg/ml).

The antibiotic solutions were filtered sterilized (except Ethionamide) using 0.22 µm polycarbonate filters and stored in aliquots at –20°C until use.

Nine tubes were taken and labelled as Control, Ofloxacin, Levofloxacin and Ciprofloxacin, Amikacin, Kanamycin, Capreomycin, Para amino salicylic acid and Ethionamide. 500µl of positive cultures (Day 1 or Day 2 positive tube) was used directly as the inoculum for drug susceptibility testing, while for a Day 3, Day 4 or Day 5 positive tubes, 1:5 diluted (1 ml of positive broth in 4 ml of sterile saline) suspension was used for the inoculation procedures as per the instructions given by the manufacturer.Briefly 800µl of BACTEC MGIT DST supplement (OADC) was added to all the nine tubes and 100 µl of the appropriate drug solution was added to the respective labelled MGIT tubes except the control. 500 µl of 1:100 diluted culture suspensions was added to control. All the tubes were tightly recapped, mixed well and incubated at 37ºC. The tubes were read in micro MGIT reader from day 3 till control tube became positive. An isolate was considered susceptible if the drug-containing tube did not fluoresce within 2 days of positivity in the control tube and resistant if the drug-containing tube showed growth on the day of control tube positivity or within 2 days [BD Diagnostics BACTEC manual].

Results and Discussion

Of the 13 MDR-TB strains tested, 9 were sensitive to all the eight SLDs and 4 were resistant to one or more SLDs. Among 4 MDR resistant strains, 2 were XDR-TB [1 was resistant to Ciprofloxacin, Ofloxacin (flouroquinones) and Amikacin (Aminoglycoside) and the other strain was resistant to Ciprofloxacin (fluoroquinones) and one of the Aminoglycosides (Amikacin)].The 2 XDR-TB strains were confirmed by repeating the phenotypic drug susceptibility testing to all the second line drugs. The results of phenotypic drug susceptibility were showed in Table3. The present study showed that the MDR *M.tuberculosis* strains were resistant to Amikacin, Ciprofloxacin, Kanamycin, Ethionamide, Para-amino salicylic acid (15.3 %) of the SLDs and the lowest resistance was observed with Capreomycin and Ofloxacin (7.6%).

The important aspects to control tuberculosis are prompt identification of new cases and rapid implementation of

effective treatment regimens to interrupt transmission of the disease. The chance of incidence of XDR-TB is on the rise due to improper use of second line anti-tubercular drugs leading to drug resistance. Delay in the diagnosis of XDR-TB is mainly due to slow growth of the organisms which eventually reflects further identification of XDR-TB strains and therefore delays in performing drug susceptibility testing for the second line drugs. Since conventional drug susceptibility testing using liquid and solid medium takes nearly about 22 and 68 days respectively, there is a need for the rapid diagnostic drug susceptibility testing methods to identify XDR-TB and further prevent the spread and emergence of drug resistant tuberculosis which results in early initiation of the treatment.

The first outbreak of XDR-TB occurred in South Africa in 2006 studied by Gandhi et al [Gandhi et al., 2006], who reported1428 patients with suspicion of tuberculosis. Of 1428 patients analysed, 475 (33%) of 1428 patients were culture-positive for *Mycobacterium tuberculosis*. Among the confirmed culture positive cases, they found that the prevalence of MDR tuberculosis was 39% (185/475) and of XDR tuberculosis was 6% (30/475) by proportional method on Middlebrook 7H10 agar. In 2005 Zarir Udwadia from Hinduja Hospital reported first case of XDRTB in India. Of 3,904 samples included in their study, 409 samples (32.35%) were found to be MDR-TB, out of which 33 (8%) were XDRTB [Deepak et al., 2005].Jain et al [Jain et al., 2009] in 2008 reported on the High Prevalence of XDR TB from a Tertiary Care Hospital in India. Of 3904 samples (sputum, pleural fluid, CSF etc.) included in their study, 1264(32.3%) samples were culture positive for *Mycobacterium tuberculosis*. 409 samples (32.35%) were found to be MDR-TB, out of which 33 samples (8%) were XDR-TB. In 2010, Balaji et al [Balaji et al., 2010] studied the clinical and demographic risk factors associated with the isolation of XDR-TB in a tertiary hospital laboratory in South India by performing drug susceptibility testing. Datta et al [Datta et al., 2010] studied the prevalence of MDR-TB and XDR-TB in Kashmir valley of India, during the period of March 2003 to February 2007.Out of 910 cases of pulmonary tuberculosis they found 52 (5.7%) cases of MDR-TB, among which 8 (15.3%) were diagnosed as XDR-TB by BACTEC MGIT 960 method. A recent study from Hinduja hospital (2011), Udwadia et al [Zarir et al., 2011] reported four cases of "total drug resistant tuberculosis" (TDR-TB).According to this report, 12 patients have shown resistance to all the first line TB drugs (isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin) and to seven second line anti-TB drugs (ofloxacin, moxifloxacin, kanamycin, amikacin, capreomycin, para-aminosalcyclic acid and ethionamide). However, within a couple of weeks the Indian health authorities had rejected their claim, saying that all the cases were in fact extensively drug resistant, that is XDR-TB. So far, three of the TDR-TB patients died, one of them after lung surgery. One of the patients passed on the infection to her daughter. The doctors were pessimistic saying that they have little to offer those 12 patients except for drastic surgery and medication for some relief. It was also said that TDR TB had emerged because of the failure of the overall health system as these patients received erratic, unsupervised second line drugs, added individually and often in incorrect doses, by multiple private practioners (Zarir et al., 2011) .

Table.1 Clinical specimen wise distribution of 13 MDR strains

s.no	Clinical specimens (N)	Male	Female	Mean age group
1	Sputum (10)	8	2	30.9
2	Bronchial wash (2)	Nil	2	33
3	Fine needle aspirate biopsy from right axillary lymphnode (1)	1	Nil	45
Total	(13)	9	4	

Table.2 The First line drug susceptibility pattern of 13 MDR strains of *M.tuberculosis* by BACTEC MGIT system

S.no	MDR strains	Age/sex	Clinical specimens	First line DST pattern
1	26/09	45/M	Sputum	S,H,R,E,Z-resistant
2	136/10	22/M	Sputum	S,H,R,E,Z-resistant
3	103/10	28/M	Sputum	S,H,R,E,Z-resistant
4	172/10		FNAB right axillary lymphnode	S,H,R,E,Z-resistant
5	254/10	25/M	Sputum	S,H,R,E,Z-resistant
6	480/10	42/M	Bronchial wash	H,R-resistant
7	543/10	26/F	Sputum	S,H,R,E,Z-resistant
8	584/10	19/F	sputum	H,R-resistant
9	680/10	49/M	Sputum	S,H,R,Z-resistant,
10	849/11	29/M	Bronchial wash	S,H,R,E,Z-resistant
11	670/10	42/M	Sputum	S,H,R,E,Z-resistant
12	728/10	30/M	Sputum	S,H,R,E,Z-resistant
13	962/11	23/M	Sputum	H,R-resistant

S-Streptomycin,
H-isoniazid,
R-rifampicin,
E-Ethambutol,
Z-Pyrazinamide

Table.3 Phenotypic second line drug susceptibility results of 13 MDR strains for second line drugs by BACTEC culture (MGIT) system

S.No	Clinical specimens	Reference number	Age/Sex	Second line DST	
				Resistant	Sensitive
1	**Sputum**	**LTITM 136/10**	**22/M**	**CAP, OFX, CPX, ETH, PAS**	**AMK,KAN, LVX**
2	**Sputum**	**LTITM 254/10**	**25/M**	**AMK,CPX**	**ETH,PAS ,KAN, CAP,OFX,LVX**
3	Sputum	LTITM 728/10	30/F	AMK,KAN, ETH	CAP, OFX, LVX, PAS,CPX
4	Sputum	LTITM 543/10	26/F	KAN,PAS	ETH,AMX CAP,OFX,LVX ,CPX
5	Sputum	LTITM 103/10	28/M	Nil	All SLDs
6	Fine needle aspirate biopsy from right axillary lymphnode	LTITM 172/10	45/M	Nil	All SLDs
7	Sputum	LTITM 26/10	45/M	Nil	All SLDs
8	Bronchial wash	LTITM 480/10	42/F	Nil	All SLDs
9	Sputum	LTITM 584/10	19/F	Nil	All SLDs
10	Sputum	LTITM 680/10	49/M	Nil	All SLDs
11	Bronchial wash	LTITM 849/11	24/F	Nil	All SLDs
12	Sputum	LTITM 670/11	42/M	Nil	All SLDs
13	Sputum	LTITM 962/11	43/M	Nil	All SLDs

CPX –Ciprofloxacin
AMK-Amikacin
ETH- Ethionamide
CAP-Capreomycin
OFX -Ofloxacin
KAN -Kanamycin
PAS-Para amino salicylic acid
SLDs-Second line drugs

In the present study, the standardized phenotypic drug susceptibility testing for second line drugs by BACTEC micro MGIT culture system showed 2/13 MDR strains (15.3%) were XDR-TB strains.

To the best of our knowledge, this is the first report on the detection of XDR-TB from non governmental organization (private laboratory) in Chennai. The limitation of the present study is the number of MDR strains tested are minimal and the optimized technique to be applied on more number of MDR strains to get the incidence rate of XDR-TB in Chennai population.

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