

A Pilot Study on the Detection of Multidrug Resistant Tuberculosis in Hospital Based Population of Chennai, India

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Abstract

Background: Tuberculosis is curable, but one of the biggest challenges for TB control has been its cure. Multidrug resistant tuberculosis (MDR-TB) is defined as Mycobacterium tuberculosis (M. tuberculosis) complex isolates with in vitro resistance against isoniazid and rifampicin, with or without resistance to additional first-line anti-TB drugs.

Objective: To identify the MDR-TB by BACTEC MicroMGIT culture system among newly detected cases of tuberculosis in local population of Chennai, India.

Materials and methods: 961 clinical specimens including 801 new cases and 160 control cases were collected during the period December 2009 to September 2011. Phenotypic drug susceptibility testing for Streptomycin, Isoniazid, Rifampicin, Ethambutol and Pyrazinamide was performed by BACTEC MicroMGIT mycobacterial culture system for the 322 M. tuberculosis isolates from the new cases.

Results and Conclusion: Among the 322 M. tuberculosis isolates, 194 (60.2%) were sensitive to all the five first line drugs, 13 (4.03%) were MDR (resistant to H and R with or without resistance to other first line drugs), 62 (19.25%) were polyresistant (resistant to more than two drugs) and 53 (16.45%) were mono-resistant. This is the first pilot study conducted by a private Research Institution in Chennai, India.

Keywords: mMGIT culture; DST-M.tb; MDR-TB; Rifampicin; Isoniazid

Introduction

Tuberculosis (TB) is the leading cause of death from a curable infectious disease. Globally more than 1.3 million people die of the disease every year. Nearly one third of the world's population is infected with tubercle bacilli; approximately 10% of them have a life time risk of developing the disease [1]. The incidence rate is highest among young adults, and most cases are due to recent infection or reinfection. As transmission falls, the caseload shifts to older adults, and a higher proportion of cases are attributable to the reactivation of latent infection. The emergence of drug resistant TB, and particularly MDR-TB, has become a significant public health problem in a number of countries and an obstacle to effective TB control.

India has more new TB cases annually than any other country. In 2008, out of the estimated global annual incidence of 9.4 million TB cases, 1.98 million were estimated to have occurred in India, of whom 0.87 million were infectious cases, thus catering to a fifth of the global burden of TB [1]. About 40% of Indian population is infected with TB bacillus. The present study was carried out in Chennai, India to find out the prevalence of MDR-TB in the local population using BACTEC microMGIT culture system.

Study Population, Design and Methods

The study was carried out after getting the approval from Institutional Research Ethics committee. An informed consent from the patients was obtained after explaining the purpose of the study. The patients were recruited after thorough examination by the Chest clinicians from 3 centres: Institute of Thoracic Medicine, Chetpet, Chennai, Stanley Medical College, Chennai and Madras Medical College, Chennai. The patients were categorized into new [patients without any previous history of anti-tuberculous treatment (ATT)] and control (patients with malignancy and without radiological evidence of tuberculosis). 961 clinical specimens including 801 new cases and 160 control cases were collected during the period December 2009 to September 2011. The specimen and categorywise distribution of the 961 clinical specimens is given in table 1. The mean age of the patients was 37.3 (age range: 18-81) and the male female ratio was 1.5:1 (491- male and 310-female). The specimen was processed for direct smear by Ziehl-Neelsen method and culture for *M. tuberculosis* by BACTEC MicroMGIT culture system as per the manufacturer's instruction.

BACTEC Culture and Drug Susceptibility Testing

Mycobacterial culture was performed by BACTEC microMGIT culture system after standard decontamination procedure using NALC-NaOH [2]. The isolates were confirmed as *M. tuberculosis* by

performing PCR targeting MPB64 gene [3] and IS6110 region [4]. After the confirmation of isolate as *M. tuberculosis*, phenotypic drug susceptibility testing (DST) for the first line drugs streptomycin (S), isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z) was performed by BACTEC microMGIT culture system. Antibiotic stock solutions for S, H, R, E and Z were prepared and kept in aliquots in -20°C until use. The final concentrations used for performing DST were 0.8µg/ml, 0.1µg/ml, 2.0µg/ml, 100µg/ml and 100µg/ml of S, H, R, E and Z respectively.

Clinical Specimen	New	Control
Sputum	474	111
Bronchial wash	80	5
Pleural Fluid	59	9
FNAB	157	27
Pus	24	6
Urine	6	2
Ascitic fluid	1	0
Total	801	160

Table 1: Specimen and category wise distribution of 961 clinical specimens

Results

BACTEC Culture

Out of the 801 new cases, 322 (41.3%) were culture positive for the isolation of *M. tuberculosis* by BACTEC microMGIT culture system. The specimen wise distribution of 322 *M. tuberculosis* isolates was given in table 2. The isolation rate among the new patients was 40.1% (322/801). None of the control was culture positive.

First Line Drug Susceptibility Testing Results

Among the 322 *M. tuberculosis* isolates, 194 (60.2%) were sensitive to all the five first line drugs, 13 (4.03%) were MDR (resistant to H and R with or without resistance to other first line drugs), 62 (19.25%) were polyresistant (resistant to more than two drugs) and 53 (16.45%) were monoresistant. Among the 53 monoresistant strains, 22 were resistant to Z, 19 to S, 9 to H and 3 to E (Table 3). Majority of the resistant strains were from Sputum - 104 followed by FNAB - 13, Bronchial wash - 6, Pleural fluid - 3, and pus aspirates - 2 (Table 4).

MDR-TB among new cases

Out of the 13 MDR-TB patients, 9 were male and 4 were female and mean age was 34.8 (age range: 22-60). The 13 MDR-TB was isolated from 10 sputum, 2 bronchial wash and 1 FNAB from right axillary lymphnode.

Clinical Specimen	New
Sputum	256
Bronchial wash	18
Pleural Fluid	6

FNAB*	36
Pus [§]	6
Total	322

*FNAB from Cervical lymphnode-27, Right neck-4, Axillary lymph node-2, one each from fore head, Right ear and Right supraclavicular node-1
[§]Pus aspirates cervical lymphnode-3, one each from right axillary lymphnode, cold abscess sternal region and right wrist joint

Table 2: Specimen wise distribution of 322 *M. tuberculosis* isolates

Total no. of isolates tested	Susceptible to all the first line drugs	Monoresistant N=53 (16.45%)	Resistant to two or more drugs N= 62 (19.25%)	MDR-TB N=13 (4.03%)
322	194 (60.2%)	Z-22 S-19 H-9 E- 3	SHEZ- 16 SZ- 12 SHE- 6 SHZ- 6 EZ- 6 SEZ- 3 SH- 4 HEZ - 2 HZ- 2 RZ- 2 SR- 2 SE- 1	SHREZ- 9 HR- 2 SHRZ- 1 SHRE- 1

S -Streptomycin, H- Isoniazid, R- Rifampicin, E- Ethambutol, Z- Pyrazinamide

Table 3: Phenotypic drug susceptibility testing results of 322 *M. tuberculosis* by BACTEC MicroMGIT system

The resistance patterns of 13 MDR-TB strains were as follows: 9 were resistant to all the 5 first line drugs, 2 strains were resistant to HR and one each was resistant to SHRZ and SHRE (table 3).

Only 4 of the 13 MDR-TB patients turned up for follow up at the end of 6 months. Three patients were able to give sputum for mycobacteriological investigation and both smear and BACTEC culture for the isolation of *M. tuberculosis* were negative in the sputum collected from the rest 3 patients. For the other patient, FNAB was collected from the right axillary lymphnode during the first visit and the swelling had completely reduced on treatment and the patient was doing well.

Clinical specimen (n=322)	All sensitive	MDR-TB	MR	PR
PULMONARY				
Sputum (256)	152	10	45	49
Bronchial wash (18)	12	2	3	1
Pleural Fluid (6)	3	0	1	2
EXTRA PULMONARY				
FNAB (36)	23	1	3	9

Pus (6)	4	0	1	1
TOTAL (322)	194	13	53	62
MR- Monoresistant, PR- Polyresistant, MDR-TB: Multidrug resistant tuberculosis				

Table 4: Clinical specimenwise of results of phenotypic drug susceptibility testing for First line anti-TB drugs

Discussion

The emergence of drug resistant TB, and particularly MDR-TB, has become a significant public health problem in a number of countries and an obstacle to effective TB control. Drug resistance surveillance conducted in Gujarat and Maharashtra indicated multi drug resistance levels of <3% among new TB cases and 14-17% among previously treated TB patients [5]. But a study from Mumbai had showed a relatively high prevalence of MDR-TB of 24% and 41% among new and treated cases respectively [6].

In the present study, MDR-TB among new cases was 4.03%, which is slightly higher than reported by RNTCP 2010 report and a recent study by Sharma et al [7]. The main drawback of the present study is follow up of the MDR-TB patients was possible only for four (22.2%) patients out of the 13 patients. Among the monoresistant *M. tuberculosis* isolates, least percentage of resistance was detected in Ethambutol (0.35%) and highest percentage in pyrazinamide (2.7%). In the 62 polyresistant strains, 37 (4.6%) were found to be resistant to both streptomycin and pyrazinamide. Thus the present study shows that prevalence of MDR-TB is increased among new cases in Chennai.

The laboratory capacity needs to be urgently strengthened in India as 20% of the world's MDR-TB burden is reported to be from India [8]. This is a pilot study conducted by a non-governmental organization with the support of a private funding agency interested in Public health. This type of study is useful to generate reliable data on the current status of prevalence of tuberculosis.

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