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*International Journal of Current Research*
*Vol. 10, Issue, 01, pp.63976-63978, January, 2018*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

## RESEARCH ARTICLE

# ISOLATION, CHARACTERIZATION AND DETERMINATION OF DRUG SUSCEPTIBILITY PATTERN OF MYCOBACTERIAL ISOLATES FROM NEWLY DIAGNOSED CASES – A STUDY FROM A TERTIARY CARE CENTRE IN SOUTH INDIA

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**ARTICLE INFO**

***Article History:***
Received 19th October, 2017
Received in revised form
05th November, 2017
Accepted 21st December, 2017
Published online 19th January, 2018

***Key words:***
Mycobacterium Tuberculosis,
Primary drug Resistance,
Non-Tuberculous Mycobacteria.

**ABSTRACT**

Tuberculosis continues to be an important public health problem in India and globally. Drug resistance, especially multi-drug resistance to antituberculous drugs and co-infection of human immunodeficiency virus with mycobacteria are the two most important hurdles in the management of tuberculosis. In this study, we isolated and identified mycobacteria from clinical specimens of patients with tuberculosis using conventional methods and tested their drug susceptibility using the proportion method on Lowenstein-Jensen (LJ) medium. Of the 100 mycobacterial isolates from newly diagnosed cases, 3% were non tuberculous mycobacteria (NTM). Among the isolates of Mycobacterium tuberculosis, primary drug resistance to isoniazid and rifampicin were 5.2% and 2.1% respectively. The initial anti tuberculosis drug resistance in this study is low and more studies with larger sample size are needed in future to ascertain the true prevalence of drug resistance.



**Citation: Smitha Mary Rockey and Muralidharan, S., 2018.** "Isolation, characterization and determination of drug susceptibility pattern of mycobacterial isolates from newly diagnosed cases – a study from a tertiary care centre in South India", *International Journal of Current Research*, 10, (01), 63976-63978.

## INTRODUCTION

Despite effective diagnostic modalities and therapeutic tools, tuberculosis continues to be an important communicable disease in India as well as world wide. The rise in the immunocompromised patient population, the emergence of drug resistant strains and the association of tuberculosis with HIV infection are major reasons which has made tuberculosis a disease of great public health concern in this era. As a result of inadequate and inappropriate treatment, drug resistance in Mycobacteria is becoming a major issue. So drug susceptibility testing of the isolates of M. Tuberculosis is important in implementing proper treatment regimes. Though drug resistance in tuberculosis has frequently been reported from India, most of the available information is localized and incomplete (Paramasivan, 2004). With this in background, this study was aimed at primary drug susceptibility pattern of mycobacteria isolated from patients of suspected tuberculosis attending a tertiary care centre in south India.

## MATERIALS AND METHODS

The study was conducted from December 2005 to April 2007, in the Department of Microbiology, at St.John's Medical College, Bangalore, a tertiary care referral center in south India. Clinical specimens submitted to the Microbiology laboratory from patients with a high index of suspicion of having tuberculosis were taken for the study. Clinical data of the cases were collected from the respective case records. The data analyzed included age, sex, clinical features, past history of tuberculosis and other associated conditions like HIV infection. Since we aimed at studying the primary drug resistance, only newly diagnosed cases were included in the current study. New cases were patients who did not have a history of anti-tuberculous treatment or had anti-tuberculous therapy for less than a month. The presence of acid-fast bacilli in a clinical specimen was detected by Ziehl-Neelsen method of acid fast staining. Culture was done on Lowenstein- Jensen medium after decontamination of specimens using Petroff's method. Speciation of mycobacteria was done by conventional biochemical tests and drug susceptibility testing was done by the proportion method (Canetti *et al*., 1969).

## RESULTS

During the study period from December 2005 to December 2007, we received 2368 clinical specimens from suspected cases of tuberculosis. The specimens included sputum (83.2%), urine (8.9%), cerebrospinal, pleural and peritoneal fluids (2.99%), pus (2%), bronchoalveolar lavage (0.76%), gastric aspirate (0.71%) and biopsy specimens (0.8%).

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Out of 2368 clinical specimens, 416 specimens (17.6%) were positive for acid-fast bacilli (AFB), when examined microscopically after staining by the Ziehl-Neelsen method. These AFB positive specimens were processed for culture after decontamination. First 100 consecutive culture positive new cases were taken for further study. These isolates were obtained from sputum in 91 cases, from lymph node aspirates in 4 cases, from urine in 3 cases and one each from the bronchoalveolar lavage and pleural fluid. Analysis of patient data showed that, 61 (61%) patients were males and 39 (39%) were females. The mean age of the subjects was 42.71 (±16.58) years, ranging from 11 to 85 years. Majority of patients were in the age group of 21 to 50 years (60%). The most common presenting complaints were fever (100%) and cough (91%). Other clinical features included weight loss (20%), hemoptysis (4%), dysuria (4%), cervical swelling (2%). The associated conditions were diabetes mellitus (30%) and HIV infection (11%). 4% had history of chronic obstructive pulmonary disease. 30% were smokers, while 7% patients were chronic alcoholics. The mean CD4 count of the 11 patients with HIV infection was 262 cells/ $mm^3$ (150-400 cells/ $mm^3$ ).

The median initial resistance to isoniazid, according to the three global anti tuberculosis drug surveillances (1994-1997, 1996-1999, 1999-2002) were 7.3 %, 6.2 % and 5.7 % respectively (WHO-IUALTD, 2003). Studies on anti tuberculosis drug resistance conducted by Tuberculosis Research Centre (TRC), Chennai from Wardha district of Maharashtra revealed an initial resistance of 15% for isoniazid (Paramasivan, 2004). Mahadev *et al* reported an initial resistance of 10.3% to isoniazid from Hoogli, West Bengal (Mahadev *et al*., 2005). Paramasivan *et al* reported an initial isoniazid resistance of 6.6% from Pondicherry. (Mahadev *et al*., 2005). A study from Mayurbhanj, Orissa, showed an initial isoniazid resistance of 2.5% (Mahadev *et al*., 2005). In our study, the initial resistance to isoniazid was found to be 5.2 %. According to the global anti tuberculosis drug surveillance (1999-2002), the initial resistance to rifampicin ranged from 0% to 15.6%.[4] Paramasivan *et al* reported an initial rifampicin resistance of 2.8% and 2.5% from North Arcot, Tamil Nadu and Raichur, Karnataka respectively (Paramasivan *et al*., 2002). A previous study from Bangalore reported an initial rifampicin resitance of 2.6% (Sophia *et al*., 2004). In our study the initial rifampicin resistance is 2.1%.

**Table 1. Initial anti tuberculous drug resistance – global data**

| Study | Year | Isoniazid resistance | Rifampicin resistance | Multi-drug resistance |
|---|---|---|---|---|
| WHO-IUALTD surveillance (Espinal, 2001) | 1994-1997 | 7.3 % | 1.8 % | 1.4 % |
| WHO-IUALTD surveillance (WHO-IUALTD, 2003) | 1996-1999 | 6.2 % | 1.2 % | 1 % |
| WHO-IUALTD surveillance (WHO-IUALTD, 2003; Espinal et al., 2001) | 1999-2002 | 5.7 % | 1.4 % | 1.1 % |

**Table 2. Initial anti-tuberculous drug resistance in India**

| Study | Year | Isoniazid resistance | Rifampicin resistance | Multi-drug resistance |
|---|---|---|---|---|
| Chandrashekharan et al. (1992) (Bangalore) n=588 | 1992 | 17.3 % | 2.9 % | 1.4 % |
| Paramasivan et al. (1993) (Pondicherry) n=1841 | 1993 | 6.0 % | 0.9 % | 0.7 % |
| Malhotra et al. (2002) Jaipur) n=122 | 2002 | 13.6 % | 6.8 % | 4.5 % |
| Sophia et al. (2004) (Bangalore) n=271 | 2004 | 13.7 % | 2.6 % | 2.2 % |
| Mahadev et al. (2005) (Hoogli) | 2005 | 10.3 % | 3.0% | 3.0 % |
| Present study n=97 | 2006 | 5.2 % | 2.0 % | 2.0 % |

The 100 isolates from the newly diagnosed cases were speciated. Out of which 97 were identified as M.tuberculosis, on the basis of slow growth and typical colony morphology on Lowenstein Jensen medium, lack of growth on PNB containing medium, positive nitrate and niacin tests. The mean time taken for growth of M.tuberculosis in our study was 26 days (range - 20 to 66 days). The isolates of M.tuberculosis were subjected to drug susceptibility testing for rifampicin and isoniazid. Drug susceptibility testing of M. tuberculosis isolates showed that five isolates were resistant to isoniazid (5.2%) and two (2.1%) were resistant to rifampicin. Two isolates resistant to rifampicin were also resistant to isoniazid (2.1%). Of the three non-tubercular mycobacteria (NTM) identified one was M. kansasii and two were M. fortuitum. The two isolates of M.fortuitum were from sputum specimens, one from a 35 year old man with pulmonary symptoms and history of alcoholism and the other from a 38 year old man with COPD and chronic alcoholism. The isolate of M.kansasii was from the sputum of a 55 year old man with COPD.

## DISCUSSION

Drug resistance in tuberculosis has been reported since early days of anti tubercular chemotherapy.

The prevalence of primary MDR ranges from 0% to 14.2% (median 1.1%) (WHO-IUALTD, 2003). Studies in Jabalpur, Madhya Pradesh, showed an initial multi-drug resistance of 1.1% (Paramasivan, 2004). Paramasivan *et al*. reported a prevalence of initial MDR-TB of 3% in the studies conducted in Tamil Nadu (Paramasivan, 2000). Studies on M.tuberculosis isolates from Mysore showed an initial MDR of 1.2% (Paramasivan, 2004). Sophia *et al*, in 2004 reported an initial multi-drug resistance of 2.2% from Bangalore (Sophia *et al*., 2004). Two other studies from Bangalore have reported an initial MDR-TB of 3% and 1.14% (Chandrasekharan *et al*., 1990; Chandrasekharan, 1992). In our study, it was found to be 2.1%. As most of the Indian data are from referral centers, it may not represent the true community prevalence of drug resistance. Initial drug resistance is found to be less while acquired resistance is much higher in specialized settings. In this study we have attempted to isolate *M. tuberculosis* from newly diagnosed tuberculosis patients and study the anti-tuberculosis drug susceptibility of these strains in our laboratory, during a limited time period. The initial drug resistance was found to be low in our study. More studies with larger sample size have to be conducted in the future to find out exact prevalence of initial drug resistance.

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