

Study of Prevalence of Drug Resistant Tuberculosis in a Tertiary Care Hospital

DK Shah¹, JK Mishra²

¹Chief Medical Officer, SSH, Banaras Hindu University, Varanasi, ²Professor & Head, Department of Tuberculosis and Respiratory Diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi

***Corresponding Author:**

Email: jkmishra.imsbhu@gmail.com, deepshah2000@gmail.com

Abstract

Pulmonary tuberculosis with multi-drug resistant Mycobacterium tuberculosis is a major cause of concern in the developing countries. The present study was carried out to study the prevalence of multi-drug resistant tuberculosis (MDR-TB) in clinical isolates at Sir Sunderlal hospital in Banaras Hindu University, Varanasi, India, which is a tertiary care hospital. One hundred and eighty nine (189) sputum samples were collected from clinically suspected cases of tuberculosis and subjected to Zeihl-Neelsen stains (ZN) and culture on Lowenstein-Jensen (LJ) medium and 46 cultural isolates were obtained and subjected to drug susceptibility against Isoniazid (INH) and Rifampicin (RIF). A total of nine (19.5%) isolates were found to be resistant against INH and two strains (4.5%) was found to be resistant against both RIF and INH. No strain was found to be resistant against Rifampicin alone. The present study revealed the presence of 4-6% of multi-drug resistant *M. tuberculosis* infection in patients attending Sir Sunderlal hospital in Banaras Hindu University. This emphasizes the need for strengthening laboratory services for timely diagnosis of MDR TB.

Keywords: Pulmonary tuberculosis, drug susceptibility, Isoniazid, Rifampicin

Access this article online	
Quick Response Code:	Website: www.innovativepublication.com
	DOI: 10.5958/2394-2738.2016.00002.9

wrinkled surface, creamy white becoming yellowish or buff colored on further incubation were subjected to AFB staining and niacin test. Forty six isolates which were positive for niacin test were identified as *M. tuberculosis* and were subjected to drug susceptibility testing.

Drug Susceptibility Testing

The 46 isolates of *M. tuberculosis* were subjected to drug susceptibility testing by economical variant of proportion method.

Proportion Method

All strains of *M. tuberculosis* contains some subpopulation of bacilli that are resistant to anti tuberculosis drugs. This method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilutions of the bacilli 10^{-2} and 10^{-4} are inoculated on drug containing and drug free media in order to obtain countable colonies on both media. The ratio of number of colonies observed on the drug containing to drug free medium indicates proportion of resistant bacilli present in the strain. For any isolate if the proportion is less than 1%, the strain is classified as sensitive and above 1% as resistant.

Preparation of drug containing LJ medium

The drug concentration for INH and RIF should be $0.2 \mu\text{g/ml}$ and $40 \mu\text{g/ml}$ of the medium respectively, to get 1% critical proportion to determine the drug resistance in this method.

Standardization of inoculums

The various dilutions of inoculums: Neat 107-108, 10-2 and 10-4 for inoculation of drug containing

Introduction

Tuberculosis continues to be a major health problem in India accounting for an estimated 30% of global tuberculosis burden. At present about one million new smear positive cases are added annually to this figure. Although drug resistant tuberculosis has frequently been encountered in India, the available information is localized. Much of drug resistance encountered in India is diagnosed presumptively based on patient's lack of clinical improvement or relapse of symptoms.

mples were subjected to Petroff's method.

Culture on Lowenstein- Jensen (LJ) Medium

Sediment obtained in the Petroff's method was inoculated onto LJ medium and incubated at 37°C . LJ slants were observed for growth daily for one week, twice weekly for six weeks and once weekly for the next two weeks. Culture negative LJ slants were discarded after 12 weeks. Fifty four isolates morphologically resembling mycobacterium were further subjected to identification.

Identification

Dry, rough, raised, irregular colonies with

medium are prepared in comparison to McFarland's No1 Standardization of inoculums.

Specimen inoculation

For each isolate a total of seven LJ medium slopes (with and without drugs) were inoculated. A loopful of inoculums was streaked onto the LJ media. With neat concentration of 10⁷ to 10⁸ inoculums, one drug free LJ medium is inoculated. With 10⁻²: One drug free, one INH (0.2 µg/ml) and one RIF (40µg/ml) containing LJ media are inoculated. Similarly with 10⁻⁴: One drug free, one INH and one RIF containing LJ media are inoculated.

Incubation and Reading

Inoculated LJ slopes are incubated at 37°C for 42 days and were examined on day 28 and 42 for colonies. Slopes which were positive for growth by 28th day and found to have confluent growth on both drug free and drug containing media, were discarded considering them as resistant strains. If the results on 28th day were "sensitive" for the two drugs or negative for growth, a second reading was taken on 42nd day.

Presence of growth is recorded as

Confluent growth	= 3 +
More than 100 colonies	= 2 +
Countable number of colonies	= 1 - 100 colonies

When the number of colonies in 10⁻⁴ dilution is less than five colonies, the next larger inoculums 10⁻² was read for colonies. Colonies were counted only on the slopes that were readable (up to 100 colonies on the slope). More than 100 colonies was taken as confluent. Dividing the number of colonies in drug containing slopes by that in drug free slopes gives the proportion of resistant bacilli existing in the strain. Below 1% of critical proportion the strain was considered as sensitive and above 1% as resistant. In case growth on the control media is poor even after six weeks i.e., few or no colonies on the 10⁻⁴ bacterial dilution, the tests were repeated.

Results

This prospective study was done in the Institute of Medical Sciences on 46 cultural isolates of *M. tuberculosis* isolated from 189 sputum samples of suspected tuberculosis patients attending to Tuberculosis unit of Sir Sunderlal hospital (tertiary care hospital) from June 2014 to December 2015. Of the total 46 isolates 33 (71.5%) were from male patients and 13 (28.5%) were from female patients. The 46 isolates of *M. tuberculosis* were subjected to drug susceptibility testing by economical variant of proportion method. Thirty five (35) isolates were sensitive to both INH and RIF (76%). Nine strains were resistant to INH alone (19.5%). Only two strain was resistant to both INH and RIF (4.5%).

Discussion

MDR-TB has been influencing the world economy as well as the health of individuals and their family members. The emergence of XDR-TB and TDR-TB together put a challenge to the mankind. Various reasons are proposed for the emergence of drug resistant strains. The active participation of government as well as non-governmental organizations is lacking in some under developed and developing countries like Russian federation, India and China. Unavailability of proper laboratory setup at the gross root level was the most probable reason. In these countries there is scaling up of facilities at tertiary care centers of various states but at the primary care centers these facilities were still lacking. TB as well MDR-TB incidence is still increasing especially in the present HIV era.

In the present study 186 sputum samples were collected and a total of 46 positive isolates were obtained and the others excluded due to smear negativity and growth of atypical mycobacterium. The forty six (46) isolates were subjected to drug susceptibility testing. Nine (9 i.e.; 19.5%) isolates showed resistance to single drug (INH). Two (2 i.e.; 4.5%) isolates showed multi drug resistance (INH+RIF). In 2003 WHO-IUAT (International Union Against Tuberculosis) had reported single drug (INH) resistance in 15.2% cases and multi drug (INH+RIF) resistance in 0.5%, (10) which is higher in the present study which shows single drug resistance of 19.5% and multi drug resistance in 4.5%.

Almeida and Rodrigue in 2002 reported the incidence of multi drug resistance in 150 consecutive Mycobacterium tuberculosis isolates obtained from a rural center (in Sakawar, India) and an urban tertiary care center (in Mumbai, India). The study highlights an alarmingly high percentage of multi drug-resistant *M. tuberculosis* isolates in Mumbai (51%) as compared with that at the rural center (2%). The present study of multi drug resistance correlates with the rural center value. Deivanayagam and Rajasekaran studied total of 1000 sputum samples from which 618(61.8%) isolates obtained. Four hundred ninety five (495-80.09%) samples were resistant to any one drug. MDR- TB was detected in 339 patients (54.84%). Present study results were on a lower side.

Cohn and Bustriore viewed and tabulated 63 surveys of resistance to anti-tubercular drugs that were performed between 1985 and 1994. The rate of primary resistance to INH was 0-16.9%, RIF was 0-3.0%, streptomycin was 0.1%-23.5%, ethambutol was 0-4.2%. The highest rates of multi drug resistant tuberculosis has been reported in Nepal 48.0%, Gujarat, India 33.8%, New York City 30.1%, Bolivia 15.3%, Korea 14.5%.

Saillour and Robert studied the factors related to the outcome of 51 cases of multi-drug resistant tuberculosis (MDR-TB) in 1994 reported to the French National Reference Center were retrospectively

analyzed. The patients (median age, 45 yr) were mainly male (75%). Seventeen (17 i.e., 33%) isolates were reported as resistant only to INH, 1 RIF, 18 (35%) streptomycin (SM), 4 (8%) to ethambutol (EMB), and 12 (24%) to both SM and EMB. Hassan and Musa conducted study for a total of one hundred (100) sputa collected from new untreated and epidemiologically unrelated patients from March 2006 to March 2007. The study reported multi drug resistance as 66.7% and single drug (INH) resistance 76.9%. The present study showed values on a lower side. Affolabi and Adjagba studied a total of 470 isolates of *M. tuberculosis complex* from pulmonary tuberculosis (TB) patients. Of these 244 were from new cases and 226 from previously treated cases. Drug susceptibility testing was performed using the proportion method. They reported MDR in 1.6% new cases. No relation was found between human immunodeficiency virus co-infection and anti-tuberculosis drug resistance. The present study correlates with this value.

Zwolska and Kopec conducted a prospective survey, collected *M. tuberculosis* strains from 3970 tuberculosis patients (2976 newly diagnosed cases and 994 previously treated patients) confirmed by culture between November 1996 and October 1997. Drug susceptibility testing (DST) to Isoniazid (INH), streptomycin, ethambutol and rifampicin (RMP) were performed on Lowenstein-Jensen medium according to the proportion method and using the radiometric Bactec 460 TB system. They reported single drug INH resistance as 2.6%, RIF's resistance as 0.7% and multi drug resistance as 0.6%.

In another study conducted by Katoch and Malhotra at Jaipur during 1997-99 where 164 samples were processed and 122 isolates were subjected to DST and the following results were obtained. Drug resistance towards RIF was 3/44 isolates (6.8%) and to INH was 6/44 isolates (13.6%) and two (2) isolates showed multi drug resistance (INH+RIF). Another study by Krishnamurthy and Rodrigues at Mumbai by means of phage assay and BACTEC 460 TB analyzed 85 samples. The following results obtained 70 were resistant to RIF and 12 were sensitive. Though in the present study for DST (Drug susceptibility testing) it requires 6-8 weeks for isolation and 6 weeks for DST the proportion method is economical than above said phase assay and BACTEC 460 TB.

In another study conducted by Paramasivan and Venkataramanin North Arcot (Tamil Nadu) and Raichur (Karnataka) with sample size of 320 from North Arcot and 314 from Raichur the following results were obtained. In North Arcot mono resistance to INH-23.4%, to RIF-2.8% and multi drug resistance 2.8% and in Raichur for INH, RIF and multi drug resistance (INH+RIF) were found to be 18.7%, 2.5% and 2.5% respectively.

In a study conducted by Mahadev and Kumar in Hoogli in West Bengal and Mayurbhanj in Orissa for

detection of drug resistance during August 2000 to July 2001 where 350 smear positive samples from Hoogli and 343 smear positive samples from Mayurbhanj microscopy centers were collected. Pure isolates were obtained after processing the samples and subjected to DST. The following results were obtained. Multi drug resistance (INH+RIF) seen in one (01) sample in both the areas and mono resistance of INH seen in 6 samples from Hoogli and 3 samples from Mayurbhanj, which correlates with the present study which shows single drug resistance in 9 isolates (19.5%) and multi drug resistance (INH+RIF) in two (02) isolate (4.5%). They also studied resistance pattern of other drugs like Ethambutol and streptomycin.

Conclusion

The present study emphasizes the need for strengthening laboratory diagnosis of MDR-TB and XDR-TB, infection control methods to avoid transmission to health care workers and in Community. Research to be promoted for development of new diagnostic methods, drugs and vaccines for early detection and management of MDR-TB.

References

1. Vasantha M, Gopi PG, Subramani R. Survival of tuberculosis patients treated under dots in a rural tuberculosis unit (TU), South India. *Indian J Tuberc.* 2008;55(2):64-69.
2. Sharma SK, Mohan A. Multidrug Resistant Tuberculosis: a menace that threatens to destabilize tuberculosis control. *Chest*, 2006;130(1):261-272.
3. Paramasivan CN; An overview on drug resistant tuberculosis in India. *Ind J Tub*;1998;45:73-81.
4. Lango DL, Fauci AS, Kaser D, Hauser S L, Janeson J L, Loscalzo J.; Harrison's Principles of Internal Medicine. 18th edition. New York, McGraw Hill, 2012:1340-1377.
5. Pandey A, Madan M, Asthana A K, Kansal R, Das A. Cold acid fast staining method: Efficacy in diagnosis of mycobacterium tuberculosis. *African Journal of Microbiology Research*, 2009;3(9):546-549.
6. Sharma SK, Mohan A. Multidrug resistant tuberculosis. *Indian J Med Res.*, 2004;120(4):354-376.
7. Koneman EW. *Mycobacteria*. In Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition, Philadelphia, Lippincott Williams and Wilkins, 2006:1065-1117.
8. Hassan SO, Musa MT, Elsheikh HM, Eleragi AMS, Saeed NS. Drug resistance in *Mycobacterium tuberculosis* isolates from north-eastern Sudan. *British Journal of Medicine and Medical Research*, 2012;2(3):424-433.
9. Cohn DL, Bustreo F, Raviglione MC. Drug-Resistant Tuberculosis: Review of the Worldwide Situation and the WHO/ IUATLD Global Surveillance Project. *Clinical Infectious Diseases*, 1997;24:121-130.
10. Saillour MF, Roberl J, Jarlier V, Grosset J. Outcome of multi-drug-resistant tuberculosis in France. A Nationwide Case-Control Study. *Am J Respir Crit Care Med.*, 1999;160(2):587-593.
11. WHO. A brief history of tuberculosis control in India. WHO/HTM/TB 2010.
12. Mahadev B, Kumar P, Agarwal SP, Chauhan LS, Srikantaramu N. Surveillance of drug resistance to anti

- tuberculosis drugs in districts of Hoogli in West Bengal and Mayurbhanj in Orissa. *India J Tuberc.*,2005;52:5-10.
13. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA *et al.* Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. *Bull Wld Hlth Org.*,1969;41(1):21-43.
 14. Ustamujic A, Zutic H, Dizdarevic Z, Cukic V, Maglajlic J. Antituberculosis Drug Resistance During Seven Years (2000-2006) in Federation of Bosnia and Herzegovina. *MateriaSocio Medica*, 2009;21(1):43-46.
 15. Deivanayagam CN1, Rajasekaran S, Venkatesan R, Mahilmaran A, Ahmed PR, Annadurai S *et al.* Prevalence of acquired MDR-TB and HIV coinfection. *Indian J Chest Dis Allied Sci.*,2002;44(4):237-242.
 16. Culture of mycobacterium tuberculosis and drug susceptibility testing on solid medium. Central TB division.
 17. Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi,2009:35- 65.
 18. Affolabi D, Adjagba OABG, Kledjo BT, Gninafon M, Anagonou SY, Portaels F. Anti- tuberculosis drug resistance among new and previously treated pulmonary tuberculosis patients in Cotonou, Benin. *Int J Tuberc Lung Dis.*,2007;11(11):1221–1224.
 19. Almeida D, Rodrigues C, Zarir F, Udwadia, Lalvani A, Gothi GD *et al.* Incidence of Multidrug-Resistant Tuberculosis in Urban and Rural India and Implications for Prevention. *Clinical Infectious Diseases*, 2003;36(12):e152–154.
 20. Malhotra B, Pathak S, Vyas L, Katoch V M, Srivastava K, Chauhan D S *et al.* Drug susceptibility profiles of *M. tuberculosis* isolates at Jaipur. *Ind J Med Microbiol.*,2002;20(2):76-78.
 21. Zwolska Z, Augustynowicz-Kopec E, Klatt M. Primary and acquired drug resistance in Polish tuberculosis patients: results of a study of the national drug resistance surveillance programme. *Int J Tuberc Lung Dis.*,2000;4(9):832–838.
 22. Krishnamurthy A, Rodrigues C, Mehta AP. Rapid detection of rifampicin resistance in *M. tuberculosis* by phage assay. *Ind J Med Microbial.* 2002;20(4):211-214.