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Research Article

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

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Abstract: Pulmonary tuberculosis with multi-drug resistant Mycobacterium tuberculosis is a major cause of concern in many of the developing countries. The present study was carried out to study the prevalence of multi-drug resistant tuberculosis (MDR-TB) in clinical isolates at DR.PSIMS & RF general hospital in Gannavaram, which is a tertiary care hospital. Two hundred and fifty seven 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 50 cultural isolates were obtained and subjected to economic variant of proportion method for drug susceptibility against Isoniazid (INH) and Rifampicin(RIF). A total of eight (16%) isolates were found to be resistant against INH and one strian (2%) was found to be resistant against both RIF and INH. No strain was found to be resistant against RF alone. The present study revealed the presence of 2-3% of multi-drug resistant *M. tuberculosis* infection in patients attending DR. PSIMS general hospital. This emphazises the need for strengthening laboratory services for timely diagnosis of MDR TB.
Keywords: Pulmonary tuberculosis, drug susceptibility, proportion method, Isoniazid. Rifampicin

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 [1].

According to World Health Organisation (WHO) 2007 report, more than 80% of all tuberculosis (TB) patients live in Sub-Saharan Africa and Asia. Two out of every five Indians are estimated to be infected with TB bacillus, of these 10% will develop TB at some point during their life time. Over 70% of cases occur in the economically productive age group of 15-45 years. Every year 1.8 million new cases occur in our country, of which almost half are infectious. A patient with infectious pulmonary TB can infect 10-15 persons per year [2].

In 2008 as per WHO report, an estimated 4, 40,000 cases of multi drug resistant tuberculosis (MDR-TB) emerged globally. India and China carry the greatest estimated burden of MDR-TB, together accounting for almost 50% of the total cases. In 2008, MDR-TB caused an estimated 1, 50,000 deaths. An estimated 1.7 million people died from TB worldwide in 2009 [3].The proportion of MDR cases among new cases and previously treated cases of tuberculosis reported globally from 1994 through 2009 ranged from 0 to 28.3% and from 0 to 61.6%, respectively [2, 4].

MDR-TB is posing considerable challenge to global TB control. A laboratory based study was conducted at our institute between May 2010 to October 2012 to know the prevalence of MDR-TB [5].

MATERIALS AND METHODS

The present study was done in the department of Microbiology on 50 cultural isolates of *M. tuberculosis* isolated from 257 sputum samples of suspected tuberculosis patients attending to Tuberculosis unit of Dr. Pinnamaneni Siddhartha

Institute of Medical Science & Research foundation hospital, Chinoutapalli,Vijayawada from June 2010 to October 2012.

INCLUSION CRITERIA

Fifty strains of M.tuberculosis, isolated from sputum samples were included in the study.

EXCLUSION CRITERIA

Isolates of atypical mycobacteria were excluded from the study.

METHODS

The sputum samples were subjected to smear examination for acid fast bacilli by microscopy(Zeihl-Neelsenstaining), decontamination and concentration by Petroff's method, culture on Lowenstein-Jensen medium. Identification of mycobacterial isolates was done by colony morphology, smear examination, Niacin and other biochemical tests [6]. Drug susceptibility testing was done for Isoniazid and Rifampicin (Economical variant of Proportion method).

Smear Examination

Modified Ziehl-Neelsen acid fast staining procedure was performed with positive and negative controls. Three hundred fields were examined to rule out smear negative samples [7].

Petroff's Method

Smear positive sputum samples were subjected to Petroff's method**[**8].

CULTURE ON LOWENSTEIN-JENSEN(LJ)MEDIUM

Sediment obtained in the Petroff's method was inoculated onto LJ medium and incubated at 37^{o} 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. Sixty five isolates morphologically resembling mycobacterium were further subjected to identification [8].

IDENTIFICATION

Dry, rough, raised, irregular colonies with wrinkled surface, creamy white becoming yellowish or buff colored on further incubation were subjected to AFB staining and niacin test. Fifty isolates which were positive for niacin test were identified as *M. tuberculosis* and were subjected to drug susceptibility testing [9].

DRUG SUSCEPTIBILITY TESTING

The 50 isolates of *M. tuberculosis* were subjected to drug susceptibility testing by economical variant of proportion method.

Proportion Method [8]

All strains of *M. tuberculosis* contain 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 µg/ml and 40 µg/ml of the medium respectively, to get 1% critical proportion to determine the drug resistance in this method [8].

Standardization of inoculum

The various dilutions of inoculum: Neat 107-108, 10-2 and 10-4 for inoculation of drug containing medium are prepared in comparison to McFarland's No 1 Standardization of inoculum [8].

Specimen inoculation

For each isolate a total of seven LJmedium slopes (with and without drugs) were inoculated. A loopful of inoculum was streaked onto the LJ media. With neat concentration of 107 to 108 inoculum,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 [8].

Incubation and Reading

Inoculated LJ slopes are incubated at 37^{o}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 inoculum 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 department of Microbiology on 50 cultural isolates of *M. tuberculosis* isolated from 257 sputum samples of suspected tuberculosis patients attending to Tuberculosis unit of Dr.PSIMS & RF hospital (tertiary care hospital) from June 2010 to October 2012. Of the total 50 isolates 43(86%) were from male patients and 7(14%) were from female patients.76% of the patients were in the age group of 20-60yrs.The 50 isolates of *M. tuberculosis* were subjected to drug susceptibility testing by economical variant of proportion method. Forty one(41) isolates were sensitive to both INH and RIF(82%).Eight were resistant to INH alone(16%).Only one strain was resistant to both INH and RIF(2%).

Fig. 1: Drug resistance pattern of the isolates to INH and RIF

Sl.No.	Resistant	Isolates
1	INH alone	08
2	RIF alone	0
3	INH and RIF	01
4	No drug resistance (sensitive)	41
	Total	50

Fig. 1: Microbial Isolates

DISCUSSION

MDR-TB hasbeen 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 257 sputum samples were collected and a total of 50 positive isolates were obtained and the others excluded due to smear negativity and growth of atypical mycobacteria. The fifty (50) pure isolates were subjected to drug susceptibility testing by economical variant of proportion method for INH and RIF. Eight (8 i.e., 16%) isolates showed resistance to single drug (INH). One (2%) isolate showed multi drug resistance (INH+RIF). In 2006 World Health Organization(WHO) survey showed that the global proportion of resistance among all tuberculosis cases is 4.8%. China, India and the Russian Federation are estimated to carry the highest number of MDR-TB cases. China and India carry approximately 50% of the global burden and the Russian Federation a further 7% [10].

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 correlates with the present study which shows single drug resistance of 16% and multi drug resistance in 2%.Cohn and Bustriore viewed and tabulated 63 surveys of resistance to anti-tuberculous 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%, Koria 14.5% [11].

Almeida and Rodrigue in 2002 reported the incidence of multi drug resistance in 150 consecutive Mycobacterium tuberculosisisolates 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 [12]. Deivanayagam and Rajasekaran studied total of 1000 sputum samples from which 618(61.8%) isolates obtained. Four hundred ninty five (495-80.09%) samples were resistant to any one drug. MDR-

TB was detected in 339 patients (54.84%). HIV seropositivity among MDR-TB was 4.42%. Significantly, 245 patients (39.64%) had tubercle bacilli resistant to one or more reserve drugs too (ethionamide, kanamycin and/or ofloxacin). Present study results were on a lower side [13].Ustamujic and Zuticanalyzed and reported drug-resistant tuberculosis in seven years period (2000-2006) in federation of Bosnia and Herzegovina found men were more frequently affected particularly in 2003 (male 29-71%; female 12-29%) which correlates with the present study which shows males were more affected than females and ratio is 86% and 14% [14].

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 [15]. 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) resistance76.9%. The present study showed values on a lower side [16]. Affolabi and Adjagbastudied a total of 470 isolates of *M. tuberculosis* complex from pulmonary tuberculosis (TB)patients. Of these 244 were from new cases and 226from 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 [17].

Abe and Hirano studied on cultures obtained from patients hospitalized at 78 hospitals in different districts of Japan throughout a 6-month period,in 1997**.** the prevalence of primary multi drug resistance (MDR) was 0.8%.Acquired resistance was reportedas19.7% for MDRwhich correlates with the present study[18].

Zwolska and Kopecconducted a prospective survey, collected M.tuberculosisstrains 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 resistance as 0.7% and multi drug resistance as 0.6%. [19]. 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 8 isolates (16%) and multi drug resistance (INH+RIF) in single (01) isolate (2%). They also studied resistance pattern of other drugs like Ethambutol and streptomycin [20].

In another study conducted by Katoch and Malhotraat 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) which also correlates with the present study which shows single drug resistance in 8 isolates(16%) and multi drug resistance (INH+RIF) in single(01) isolate (2%) [21].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. For resource poor countries like India proportion method is ideal which was followed in this study [22].

In another study conducted by ParamasivanandVenkataramanin North Arcot (Tamilnadu) 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 [23].

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.

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