Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20175698

Original Research Article

A study on pattern of initial and acquired drug resistance for isoniazid and rifampicin in A.F.B. positive sputum smears of pulmonary tuberculosis patients at a Medical College in North Eastern Uttar Pradesh, India

Ravi K. Tiwari[1], Jamal Haider[1]*, Amitabh D. Shukla[2], Prem P. Gupta[3]

[1]Department of Pharmacology and Therapeutics, B.R.D. Medical College, Gorakhpur, India
[2]Department of Pulmonary Medicine (TB & Chest), M.L.N. Medical College, Allahabad, Uttar Pradesh, India
[3]Department of Pharmacology and Therapeutics, A.I.I.M.S., Patna, Bihar, India

Received: 10 November 2017
Accepted: 02 December 2017

***Correspondence to:**
Dr. Jamal Haider,
Email: jamalatbrd@gmail.com

ABSTRACT

Background: Despite availability of good quality anti-tubercular drugs and its administration through Directly Observed Therapy Short Course (DOTS) strategy of Revised National Tuberculosis Control Programme (RNTCP), tuberculosis remains a major cause of morbidity and mortality in India. The emergence of drug resistance necessitates the timely detection of susceptibility of anti-TB drugs. This can help in appropriate modification in treatment strategies.
Methods: A total of 50 patients of pulmonary TB with AFB positive sputum smears attending the OPD of TB and Chest department of B.R.D. Medical College, Gorakhpur were included. Patients were grouped based on history into new (cat-I) and previously treated patients (cat-II). Cat-II patients were further subdivided into defaulter, treatment failure and relapse groups. The culture and DST of AFB positive sputum smears of these patients was done in VersaTREK™®. At the end of study, patients were grouped according to age, sex, category and drug sensitivity pattern for Isoniazid (INH) and Rifampicin (RIF) viz mono resistance (resistance to either INH or RIF) or multi drug resistance (M.D.R.) and the resultant data were analysed.
Results: Of the total 50 patients included in this study, 18 (36%) patients were sensitive to both the drugs INH and RIF, of which 11 (22%) were of cat-I and 7 (14%) of category-II. Twenty-two (44%) patients were resistant to INH only of which 8 (16%) were of cat-I and 14 (28%) of cat-II. One (2%) case of cat-I showed resistance to RIF only, while M.D.R. type of resistance is seen in 1 (2%) patient of cat-I and 8 (16%) patients of cat-II. Pattern of resistance to both INH and RIF together (i.e. M.D.R. type) showed significant difference between cat-I and cat-II.
Conclusions: Most of the patients showing resistance to INH, RIF or both INH and RIF (M.D.R.) belonged to category-II (previously treated) patients.

Keywords: DST, Isoniazid, MDR-TB, Resistance Pattern, Rifampicin

INTRODUCTION

Tuberculosis remains a major health concern in developing world like India. Multidrug drug resistant (M.D.R.) and extensively drug resistant (X.D.R.) TB constitutes a serious threat for the effective control of the disease, stressing the need for rapid detection of sensitivity/resistance pattern to first and second line anti-TB drugs. Conventional methods based on solid culture media for culture and drug susceptibility testing (DST) in Mycobacterium tuberculosis have traditionally relied on slow and cumbersome procedures requiring about 8 to 10 weeks to produce the results.[1]

Several new approaches have been proposed and tested in last few years for the rapid and timely detection of drug

susceptibility/resistance in M. Tuberculosis, e.g. BACTEC TB 460 system, MGIT (Mycobacterial growth indicator tube), BacT/Alert 3D System and VersaTREK™, all are since faster growth is usually obtained in liquid medium.[2]

Keeping this in mind, the present study was planned for culture and drug susceptibility testing (DST) and to determine pattern/prevalence of drug resistance to two first line anti-tubercular drugs-INH and RIF in sputum positive cases of pulmonary TB.

METHODS

This study was conducted after approval by the institutional research ethics committee. Period of study was between December 2009 to June 2010. Patients of pulmonary TB attending the OPD of TB and Chest department of B.R.D. Medical College and associated Nehru Hospital, Gorakhpur were subject of study. Informed consent was obtained from the patients for sample collection and enrolment in this study. The entire patient's related information was taken from the hospital information system (HIS) to know whether the patient was new or previously treated with anti-tubercular treatment. Fifty patients of pulmonary TB having sputum smear positive for AFB belonging to either new cases (cat-I) or previously treated cases (cat-II) were selected randomly in this study. The following patients were excluded from this study- extra pulmonary TB cases, sputum smear negative pulmonary TB cases and the patients of pulmonary TB having other major organ diseases like diabetes, kidney or liver disease. The culture and DST of sputum smears was done on VersaTREK™.

VersaTREK™:[3]

A rapid, automated, non-radiometric, culture and DST method based on liquid culture medium. This method is advantageous than conventional solid media (L J medium) based methods, because VersaTREK™ decreases the total turnaround time for culture and DST considerably (about 3 to 10 days for culture and 1 to 2 weeks for DST). [3] VersaTREK™ system is manufactured by TREK diagnostic system, Cleveland, OH, USA. It has FDA clearance since 1999 for mycobacteria drug susceptibility testing. Besides culture and DST of mycobacteria, VersaTREK™ can be used for blood/sterile body fluids culture for aerobic and anaerobic bacteria.

Mycobacteria detection method by VersaTREK™[3]

The VersaTREK™ system monitors the changes of headspace pressure of the specimen myco bottle (with the help of pressure transducers located in the system). A typical positive response for mycobacterial growth is a net decrease in headspace pressure, indicating oxygen consumption. It is an automated system, when the algorithm for mycobacteria is activated and the growth pattern matches with the unique algorithm, a positive signal occurs and the user is notified by a configurable audible alarm and visual LED indicator.

Requirements for mycobacterial culture and DST by VersaTREK™[3]

VersaTREK™ myco bottle, VersaTREK™ GS (growth supplement), VersaTREK™ PVNA (antibiotic supplement containing polymyxin B, vancomycin, nalidixic acid and amphotericin B), connectors, adaptors, mycobacteria susceptibility kit (containing INH and RIF in the concentration of 0.4 µg/ml and 1.0 µg/ml respectively)), refrigerator, biosafety cabinet, masks, gloves, apron, glassware, autoclave, vortex mixer, centrifuge with aerosol free sealed centrifuge cups, centrifuge tubes (50 ml), McFarland standard (1.0), sterile syringes. and needles. The method was as per manufacturer's guidelines. Isoniazid and rifampicin were provided in the powder form from the manufacturer's drug susceptibility kit. The final concentrations of isoniazid and rifampicin were isoniazid 0.4 microgram/ml. and rifampicin 1.0 microgram/ml.

Statistical analysis

Percentage, mean and standard deviations were calculated. Test of significance, Chi-square test and `p' value was used to compare categorical data. Confidence interval of 95% was taken for test of significance.

RESULTS

Out of 50 patients, 34 (68%) were males and 16 (32%) were females. Higher number of patients belonged to category-II (previously treated group) with 29 (58%) patients, while category-I (new patients) included only 21 (42%) patients. This difference was not found significant (Table 1).

Table 1: Category and sex-wise distribution of patients.

Category of TB patients	Male		Female		Total	
	No.	%	No.	%	No.	%
Category-I (New patients)	15	30	06	12	21	42
Category-II (Previously treated patients)	19	38	10	20	29	58
Total	34	68	16	32	50	100
χ^2 at 1.d.f. = 0.19559; 'p' value >0.05						

Sex wise distribution of male and female patients showed 15 (30%) male patients and 6 (12%) female patients in category-I, while 19 (38%) male and 10 (20%) female patients in category-II. Maximum patients belonged to age group of 16-30 years i.e. 24 (48%) patients, followed by the age group of 31-45 years i.e. 18 (36%) patients. In the age group 0-15 years, only 2 (4%) patients and in the age

group 46-60 years only 6 (12%) patients were found (Table 2).

There was no significant difference in distribution of cases of Pulmonary Tuberculosis in both males and females of all four age groups that were studied. Also, no significant difference was found in the mean age of males (32.647±11.342 years) and the mean age of females (27.187±10.2269 years). Overall mean age of combined group was 33.214±12.586 years and it was significantly different from the mean age of females, that is on the average, females were being affected at an early age than males. Among both new (Category-I) and previously treated patients (Category-II), the age distribution was nearly same (χ^2 at 1.d.f. = 0.38366) (Table 3).

Table 2: Age and sex wise distribution of patients

Sex	Age groups (in years)								Total		Mean Age±SD	'Z' and 'p' value
	0-15		16-30		31-45		46-60					
	No.	%	No.	%	No.	%	No.	%	No.	%		
Male	01	02	14	28	14	28	05	10	34	68	32.647± 11.342	1.6994 (>0.05)
Female	01	02	10	20	04	08	01	02	16	32	27.187±10.2269	1.9693 (<0.05)
Total	02	04	24	48	18	36	06	12	50	100	33.214± 12.586	
χ^2 at 2.d.f. = 2.001634 (p>0.05)												

Table 3: Age and categories of patients.

Category of patient	Age groups (in years)								Total	
	0-15		16-30		31-45		46-60			
	No.	%	No.	%	No.	%	No.	%	No.	%
Category-I (New patients)	-	-	12	24	07	14	02	04	21	42
Category-II (Previously treated patients)	02	04	12	24	11	22	04	08	29	58
Total	02	04	24	48	18	36	06	12	50	100
χ^2 at 1.d.f = 0.38366; 'p' value >0.05										

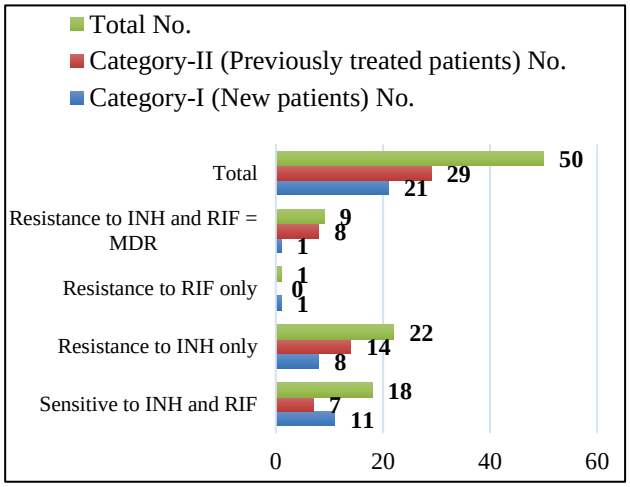

Figure 1: Sensitivity/ resistance for isoniazid (INH) and rifampicin (RIF).

As shown in Figure 1, of the total 50 patients included in the study, 21 (42%) belonged to cat-I and 29 (58%) belonged to cat-II. Total 18 (36%) patients were sensitive to both drugs INH and RIF, of which 11 (22%) were of cat-I and 7 (14%) of cat-II. Twenty-two (44%) cases were resistant to INH only, of which 8 (16%) were of cat-I and 14 (28%) were of cat-II. Only 1 (2%) case of cat-I showed resistance to RIF only. M.D.R. type of resistance pattern (i.e. resistance to both INH and RIF) was seen in 1 (2%) patient of cat-I and 8 (16%) patients of cat-II. Sensitivity of both the drugs (INH and RIF) in cat-I and cat-II was found to be significantly different (p<0.05) and this fact is also shown by the significant difference to resistance for both drugs (INH and RIF) between cat-I and cat-II (p<0.05).

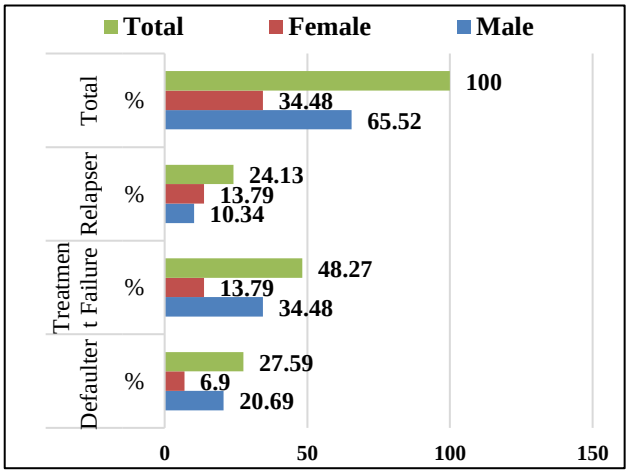

Figure 2: Distribution of Category-II patients according to sex in different types of subgroups.

No significant relationship was found between the Sex of patients and incidence of defaulter, treatment failure or relapse (χ^2 at 1.d.f = 2.09719; p>0.05). These findings showed that either sex was equally probable to become Defaulter, Treatment Failure or Relapse (Figure 2).

Table 4 shows that out of the total 29 patients of the previously treated (i.e. cat-II) group, 7 (24.14%) patients were sensitive to both INH and RIF, of which 2 (6.9%) were defaulters, 4 (13.79%) were treatment failure and 1 (3.45%) was a relapse. Fourteen (48.27%) patients were resistant to INH only, of which 4 (13.79%) were defaulters and 5 (17.24%) each were treatment failure and relapses. M.D.R. type of resistance (i.e. resistance to both INH and RIF) was found in 8 (27.59%) patients of cat-II, of which 2 (6.9%) were defaulters, 5 (17.24%) were treatment failure and 1 (3.45%) was relapse. No significant difference was found between the sensitivity pattern and the belongings of patients, i.e. defaulter, treatment failure or relapse subgroups (χ^2 at 1d.f. = 1.9808; 'p'>0.05) in cat-II patients.

Table 4: Sensitivity pattern in Category-II according to type of subgroups.

Sensitivity/ resistance pattern	Type of subgroup						Total	
	Defaulter		Treatment failure		Relapse			
	No.	%	No.	%	No.	%	No.	%
Sensitive to both INH and RIF	02	06.90	04	13.79	01	03.45	07	24.14
Resistance to INH only	04	13.79	05	17.24	05	17.24	14	48.27
Resistance to RIF only	-	-	-	-	-	-	-	-
MDR = Resistance to both INH and RIF	02	06.90	05	17.24	01	03.45	08	27.59
Total	08	27.59	14	48.27	07	24.14	29	100.0
χ^2 at 1.d.f = 1.9808; 'p'>0.05								

DISCUSSION

The current global concern in the treatment of tuberculosis (TB) is the emergence of resistance to the two most potent anti-TB drugs, i.e. isoniazid (INH) and rifampicin (RIF).

Though drug resistance in TB has frequently been reported from India, most of the available information is localized, sketchy or incomplete. A review of the few authentic reports indicate that there is no clear evidence of an increase in the prevalence of initial resistance over the years. However, a much higher prevalence of drug resistance among previously treated cases has been reported from several regions, though based on smaller number of patients. [4] A strong TB control programme and continuous surveillance studies employing standardized methodology and rigorous quality control measures will serve as useful parameters in the evaluation of current treatment policies as well as the management of MDR-TB cases.[5]

The present study was undertaken to do culture and DST of AFB positive sputum smears of pulmonary TB patients to know the pattern of sensitivity/resistance for the two most potent bactericidal anti-TB drugs, INH and RIF, and to plan and propose, if possible change in the treatment strategy of these patients based on results obtained at the end of intensive phase.

According to standard 10 of ISTC, in new patients (cat-I), if the specimen obtained at the end of intensive phase (2nd month), is smear positive, sputum smear microscopy should be obtained at the end of third month.[6] In its recommendations WHO has mentioned that DST should be done in the following condition- "in new patients, if the specimen obtained at the end of 3rd month is smear positive, sputum culture and DST should be performed."[7] For previously treated (cat-II) patients WHO recommends that- "specimen for culture and DST should be obtained at or before the start of treatment, and DST should be performed for at least isoniazid(INH) and rifampicin (RIF)."[7]

Conventional DST on solid egg or agar based media was a standard technology and is still utilized in many countries worldwide. One disadvantage of conventional DST is long turnaround time needed for culture and sensitivity. Rapid liquid culture-based techniques have been established that can detect growth dependent changes such as CO_2 production (BACTEC460 and MB/BacT) or oxygen consumption (MGIT and VersaTREK).[8-11]

VersaTREK™ (formerly ESP culture system II) is an automated non-radiometric DST method. Very few studies are available on the performance of VersaTREK™.[8,11,12] In a comparative study between Bactec MGIT 960 system with VersaTREK™ for firstline drug susceptibility testing of *M.tuberculosis*, the VersaTREK™ system showed an overall agreement of 98.5% with the results obtained with MGIT 960 system.[13] The kappa index was 1.0 for isoniazid (INH) and rifampicin (RIF). Their results indicated that VersaTREK™ system is a validated methodology for DST of *M.tuberculosis*.

In this study, most of the patients showing resistance to INH, RIF or both INH and RIF (M.D.R.) belonged to 16-30 years of age group, followed by 31-45 years of age group, both in cat-I and cat-II. Also, the resistance was seen more frequently among males as compared to females. In this study, resistance to INH only was seen in 38.1% patients of cat-I. A study in Kolar district (1987-89) of Karnataka had reported initial drug resistance of 32.9% for INH.[5]

In May 2016 W.H.O. issued guidance that people with TB resistant to rifampicin (RR-TB) with or without resistance to other drugs should be treated with an MDR-TB treatment regimen.[14] In previous global reports, estimates on the burden of drug resistant TB have focused on MDR-TB (defined as resistance to rifampicin and isoniazid, the two most effective anti-TB drugs). Globally in 2015 an estimated 3.9% (95% confidence interval[CI]:2.7-5.1%) of new cases (cat-I) and 21% (95% CI: 15-28%) of previously treated cases(cat-II) had MDR/RR-TB. In the global tuberculosis report W.H.O. has also mentioned that the countries with the largest number of MDR/RR-TB (45% of the global total) are China, India and the Russian Federation. Our study showed initial resistance to both INH and RIF (i.e. MDR) in 4.76% cases in new patients (cat-I). NTI Bangalore, conducted drug resistance surveillance (DRS) in four districts of Mysore, Hoogly, Mayurbhanj and also in Bangalore city, where MDR-TB level amongst patients with no history of previous treatment (cat-I) was observed to be 1.2%, 3.0%, 0.7% and 2.2% respectively (NTI 2003).[15-17] In our study MDR type of resistance was found in 27.58% patients of category-II. So, in the opinion of WHO, if their MDR is not detected early and treated with second line drugs, these patients will suffer poor outcomes and spread MDR in their communities. Studies undertaken by TRC during 1997-2000 in different regions revealed the incidence of MDR-TB to vary from 25-100% among previously treated patients (cat-II).[18,19] A study done in year 2002-2006 on the prevalence of drug resistance in previously treated cases of pulmonary TB at Dehradun (Uttarakhand), showed INH resistance, RIF resistance and MDR to be 62.22%, 57.22% and 57.22% respectively.[20]

However, these studies were not designed to obtain a true picture of drug resistance in sputum smear positive cat-I and cat-II patients in these areas and are based on a very small number of patients (as in our study), i.e. are not a representative sample. Therefore, the presently available data on MDR-TB level should be interpreted with caution.

Since our study was based on a very small number of patients, there is a need for further such studies in this area to obtain a true picture of drug resistance among new and previously treated pulmonary TB patients. This in turn will help in taking timely decision to get better chance of cure and preventing the development and dissemination of further resistance in TB control programmes like RNTCP-DOTS in India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Palomino JC. Antibiotic Resistance and Drug Susceptibility Methods, 29th Annual Congress, Plovdiv, Bulgaria. July 2008:15-8.
2. Claudio P, Armando O, Luca B, Claudio S. Current Perspectives on Drug susceptibility testing of Mycobacterium tuberculosis complex: The Automated Non-radiometric systems. J of Cli Mic. Jan 2006;44(1):20-8.
3. TREK Diagnostic system. Thermo-Fisher Diagnostic Private Limted; Technical. Available at: http://trekds.com/techDocsVT/techInsert/TI_VersaTREK_Myco_Susceptibility_Kit_L-TDST014-8.pdf. Accessed 19 June 2017.
4. Behera D. Drug Resistant Tuberculosis in India- Is it a matter of concern? Indian J of Tuberculosis. 2007;54(3):105-9.
5. Paramasivan CN. An overview of drug resistant tuberculosis in India. Indian J Tuberc. 1998;45:73-81.
6. Tuberculosis Coalition for Technical assistance. International standards for tuberculosis care (ISTC), 2nd Ed., Tuberculosis Coalition for Technical assistance, The Hague,2009. Available at: http://www.who.int/tb/ISTC_Report_2ndEd_Nov2009.pdf Accessed 19 June 2017
7. Treatment of Tuberculosis: WHO Guidelines, 2009, 4th Edition, Geneva, World Health Organization. 2009 Available at http://apps.who.int/iris/bitstream/10665/44165/1/9789241547833_eng.pdf?ua=1andua=1
8. La Bombardi. Comparison of ESP and BACTEC system for testing susceptibilities of Mycobacterium tuberculosis complex isolates to Pyrazinamide. J. Clin. Microbiol. 2002;40:2238-9.
9. Angeby KA, Werngren J, Toro JC. Evaluation of BacT/ALERT 3D system for recovery and drug susceptibility testing of Mycobacterium tuberculosis. Clin Microbiol infect. 2003;9:1148-52.
10. Scarparo C, Ricordi P, Ruggiero G. Evaluation of the fully automated BACTEC MGIT-960 system for testing susceptibilities of Mycobacterium tuberculosis to pyrazinamide, streptomycin, isoniazid, rifampicin and ethambutol and comparison with the radiometric BACTEC 460 TB method. J. Clin Microbiol. 2004;42(3):1109-14.
11. Ruiz P, Zerolo FJ, Casal MJ. Comparison of Susceptibility testing of Mycobacerium tuberculosis using the ESP culture system II with that using the BACTEC method. J Clin Microbiol. 2000;38(12):4663-4.
12. Bergmann JS, Woods GL. Evaluation of the ESP culture system II for testing Susceptibilities of Mycobacterium tuberculosis isolates to four primary

antitubercular drugs. J. Clin Microbiol. 1998;36:2940-3.
13. Espasa M, Salvado' M, Vicente E, Tudo' G, Alcaide F, Coll P et al. Evaluation of the VersaTREK system compared to the BACTEC MGIT 960 system for firstline drug susceptibility testing of Mycobacterium tuberculosis. J Clin Microbiol. 2012;50(2):488-91.
14. WHO treatment guidelines for drug resistant tuberculosis (2016 update) (WHO/HTM/TB/2016.04). Geneva: WHO; 2016. Available at: http://www.who.int/tb/areas-of-work/drug-resistant-tb/MDR TB guidelines2016.pdf. Accessed 19 June 2017.
15. Mahadev B, Jagota P, Srikantaramu N, Ganeshwaran M. Surveillance of drug resistance in Mysore district. Karnataka, NTI Bulletin. 2003;39(3,4):5-10.
16. Mahadev B, Kumar P, Agarwal SP, Chauhan LS and Srikantaramu N. Surveillance of drug resistance of anti-tuberculosis drugs in districts of Hoogli in West Bengal and Mayurbhanj in Orissa. Indian J Tub. 2005;52:5-10.
17. Vijay S, Bala Sangameshwara VH, Jagannatha PS, Kumar P. Initial drug resistance among tuberculosis patients under DOTS programme in Bangalore city. Ind J Tub. 2004;51:17-21.
18. Paramasivan CN, Bhaskaran K, Venkataraman P, Chandrasekaran V, Narayanan PR. Surveillance of drug resistance in tuberculosis in the state of Tamil Nadu. Ind J Tuberc. 2000;47:27-33.
19. Paramasivan CN, Venkataraman P, Chandrasekaran V, Bhat S, Narayanan PR. Surveillance of drug resistance in two districts of South India. Int J Tuberc Lung Dis. 2002;6:479-84.
20. Rawat J, Sindhwani G, Dua RJ. Five-year trend of acquired antitubercular drug resistance in patients attending a tertiary care hospital at Dehradun (Uttarakhand). Lung India. 2009;26:106-8.

Cite this article as: Tiwari RK, Haider J, Shukla AD, Gupta PP. A study on pattern of initial and acquired drug resistance for isoniazid and rifampicin in A.F.B. positive sputum smears of pulmonary tuberculosis patients at a Medical College in North Eastern Uttar Pradesh, India. Int J Basic Clin Pharmacol 2018;7:191-6.