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ANTI-TUBERCULOSIS DRUG RESISTANCE IN PREVIOUSLY UNTREATED PULMONARY TUBERCULOSIS PATIENTS IN PUNE, INDIA

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ABSTRACT

Emergence of multidrug-resistant tuberculosis (MDR-TB) has been a cause of concern in both developed and developing countries. Hence, there is a need for continuous monitoring of drug resistance to anti-tuberculosis drugs in tuberculosis patients. A study was conducted to determine the prevalence anti-TB drug resistance in *Mycobacterium tuberculosis* isolates obtained from sputum samples of clinically suspected TB patients with no history of previous anti-TB treatment attending a tertiary care hospital from February 2006 to August 2007 in Pune, India. *Mycobacterium tuberculosis* isolates were subjected to drug susceptibility testing (DST) to four firstline anti-TB drugs namely Isoniazid, Rifampicin, Streptomycin and Ethambutol by Bactec MGIT 960 system being currently used in the Revised National Tuberculosis Control Program (RNTCP). It was found that MDR-TB prevalence in newly diagnosed pulmonary tuberculosis patients in Pune has not increased compared to the earlier data. Adequate data on MDR-TB at national and regional level are not available. Hence, it is essential to conduct nationwide drug resistance surveillance for TB with continuous monitoring of trends to curb the emergence of drug resistance to ensure the success of tuberculosis control programs in the country.

KEYWORDS: **drug resistance; HIV; MDR-TB; *Mycobacterium tuberculosis***

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INTRODUCTION

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide with an estimated 8.5 - 9.2 million incident cases and 1.2 - 1.5 million deaths in the year 2010[1]. The emergence of drug resistant strains of TB particularly multi drug resistant (MDR) is a global threat to tuberculosis prevention and control efforts on the background of the global HIV pandemic. Reports suggest that drug resistance tuberculosis is on the rise worldwide potentially undermining the advances made by the global tuberculosis control programs in the last decade [2]. Poor or suboptimal tuberculosis control programmes in both industrialized and developing countries can lead to emergence of drug resistance, especially if the prevalence of tuberculosis is high [3]. Drug resistant tuberculosis is associ-ated with ineffective treatment, leading to acquired resistance and transmission of drug-resistant strains in the community. MDR TB has made treatment of TB even more difficult as it requires use of costly, toxic and less effective second-line drugs for at least 18 months [4]. With an estimated MDR-TB propo-rtion of 4.8% among incident TB cases globally, almost half a million [489,139 (95% CI 455,093–614,215)] cases of MDR-TB are estimated to emerge world-wide every year [2]. In India, prevalence of MDR-TB among new and re-treatment cases is estimated to be 2.3% and 12% -17% respectively [5].

The situation is further worsened by the epidemic of human immunodeficiency virus (HIV). About one-third of the 33.3 million HIV-positive people worldwide are co-infected with TB and one in four people living with HIV die as a result of TB [6]. The changing patterns of global health events over the last two decades, particularly the spread of HIV and drug-resistant TB, have contributed in posing serious healthcare problems, thus necess-itating the advancement of alternative strategies for effectively combating these re-emerging problems. High HIV sero-prevalence among newly diagnosed TB patients has been previously reported in India [7, 8]. However, recent information on anti–TB drug resistance rates is critical as it could have an impact on the outcome of TB control and it could also help in deciding appropriate treatment strategies at the national level. The level of drug resistance is a performance indicator for the Revised National Tuberculosis Control Programme (RNTCP) in the country. Monitoring trends of anti-tuberculosis drug resistance could have significant policy and program related implications and significance. We report here the anti-tuberculosis drug resistance in *Mycobacterium tuberculosis* strains isolated from newly diagnosed treatment naïve pulmonary tuberculosis patients in Pune, India.

MATERIALS AND METHODS

Clinically suspected tuberculosis patients attending the TB out- patient department of Sassoon General Hospitals, a tertiary care hospital in Pune city were consecutively enrolled in the study. A total of 123 suspected TB patients with no history of prior treatment to anti-TB drugs and who were willing to give consent for sample collection were included in the study between February 2006 and August 2007. Data on demographics, medical history and risk behavior were recorded by the trained staff. Along with the sputum, 5 ml of blood sample was collected and tested for HIV infection by commercially available kits. The study was approved by the institutional ethics committee of the National AIDS Research Institute (ICMR), Pune. Sputum samples obtained from these patients were processed by modified Petroff's method and the sediment was cultured onto Lowenstein–Jensen (LJ) media (prepared using reagents from Hi-Media Laboratories Ltd, Mumbai, India) and incubated at 37ºC for 8 weeks and were identified as *M. tuberculosis* using conven-tional biochemical tests [9]. All the isolates were stored in Middlebrook 7H9 broth with 10% glycerol at -70º C for further testing [10]. The stored isolates were revived on LJ medium prior to drug susceptibility testing. Anti-tuberculosis drug

susceptibility testing (DST) to Streptomycin(S), Isoniazid (H), Ethambutol (E) and Rifampicin (R) was performed on early active growth by the Bactec MGIT 960 system. The standard protocol recommended by the manufacturer for first-line anti TB drugs was followed. In brief, each bacterial suspension was prepared in 4 ml of Middle brook 7H9 broth (Hi-Media Laboratories Ltd, Mumbai) and the turbidity was adjusted to 0.5 McFarland standard. From this suspension, 1.0 ml was diluted with 4.0 ml of sterile saline (1:5 dilution). Half a milliliter of this (1:5) dilution was used to inoculate each of the drug containing Mycobacterial Growth Indicator Tubes (MGITs). Subsequently, 100 microliters of 1:5 dilution was pipetted into 10.0 ml of sterile saline to obtain a final dilution of 1:500; of which 500 microliters was used to inoculate MGIT Growth Control (GC) tubes without drug for each isolate. All the four anti TB drugs were reconstituted by the addition of 4.0 ml of sterile distilled water to a lyophilized vial containing drug (Becton Dickinson). Part of this solution (0.1 ml) was aseptically pipetted into a respective MGIT tube to obtain the final critical drug concentrations of 1.0 µg/ ml for S, 0.1 µg/ ml for H, 1.0 µg/ ml for R, and 5.0 µg/ ml for E in the medium [11].

All the MGIT tubes were labeled appropriately and 0.8 ml of MGIT 960 AST Supplement and 0.1 ml of the drug stock solution were aseptically added, and finally 0.5 ml of the test inoculum was added to each 7-ml MGIT tube. The growth control (GC) tube with supplement and without drug was inoculated with 0.5 ml of GC inoculum. All of the inoculated tubes (four drug-containing tubes and one drug-free tube for each isolate) were placed into the BACTEC MGIT 960 instrument on the same day of inoculation. The relative growth ratio between the drug-containing tube and drug-free GC tube was determined by the system's software algorithm. If the relative growth in the drug-containing tube was equal or exceeding that of the GC tube, the isolate was considered drug resistant; if the relative growth was less than that in the GC tube, the isolate was considered drug susceptible. For quality control, reference strain H37Rv was used.

RESULTS

A total 123 (90 male and 33 female) suspected TB patients were enrolled in the study. All the patients were new cases with no previous history of anti tubercular treatment. Of these, 22 (17.9%) patients were found to be HIV sero-positive. Majority, 83 (67.4%) of them were in the age group of 20 to 40 years. Seventy seven (62.6%) sputum samples were positive for acid fast bacilli (AFB) by Ziehl Neelsen staining. *M. tuberculosis* was isolated from 86 (69.9%) patients, of which 9 were from smear negative TB patients. DST was performed on all the isolates against four first-line anti-TB drugs namely streptomycin, isoniazid, rifampicin and ethambutol, the results are summarized in the Table I.

Table I

Level of drug resistance in Mycobacterium tuberculosis to four first-line anti-TB drugs

Drug Resistance	Number	%
Total number of strains tested	86	
Total number of strains sensitive to all drugs	65	75.5
Any resistance		
H	15	17.4
R	3	3.4
S	12	13.9
E	1	1.1
Resistance to one drug		
H	8	9.3
R	0	0
S	5	5.8
E	1	1.1
Resistance to two drugs		
S, H	4	4.6
Multi Drug Resistance (MDR)		
S, H, R	3	3.4

H: Isoniazid, S: Streptomycin, R: Rifampicin, E: Ethambutol

Of the 86 *M. tuberculosis* isolates, 14 were from HIV seropositive tuberculosis patients. Sixty five (75.6%) were susceptible to all the four anti-TB drugs and 21 (24.4%) including 4 strains from HIV seropositive were resistant to at least one anti-TB drug. Highest drug resistance to any anti-TB drug was found in 15 (17.4%) isolates for isoniazid either alone or in combination with other drugs. Drug resistance of MTB isolates and their correlation with gender, age, HIV status and AFB smear result was not statistically significant as shown in Table II. Overall prevalence of MDR TB was 3.4% with the level of MDR TB in HIV seropositive and seronegative tuberculosis patients was 7.1% (1/14) and 2.7% (2/72) respectively. Any resistance to streptomycin, rifampicin and ethambutol were 13.9%, 3.4% and 1.1%. Mono resistance to S, H, R and E was found to be 5.8, 9.3, 0 and 1.1 per cent respectively. The prevalence of anti-TB drug resistance to one drug, two drugs and three drugs in HIV sero positive and seronegative patients has been given in the Table III.

Table II
Distribution of Mycobacterium tuberculosis isolates based on gender, age, HIV status and AFB smear

		Total Enrollement N =123 (%)	MTB isolates, N=86		p value
			Sensitive (%)	Resistance (%)	
Gender	Male	90 (73.2)	45 (70.3)	19 (29.7)	0.098
	Female	33 (26.8)	20 (90.9)	02 (9.1)	
Age	<20	09 (7.3)	06 (100)	00	0.269
	20 - 59	108 (87.8)	56 (74.6)	19 (25.4)	
	>=60	06 (4.9)	03 (60)	02 (40)	
HIV status	Sero-positive	22 (17.9)	10 (71.4)	04 (28.6)	0.956
	Sero-negative	101(82.1)	55 (76.4)	17 (23.6)	
Sputum AFB	Positive	77(62.6)	57 (74)	20 (26)	0.567
	Negative	46(37.4)	8 (88.9)	01 (11.1)	

Table III
HIV sero-status and anti-TB drug resistance (N=86)

HIV status	Single drug resistance (%)	Two drug resistance(%)	Three drug resistance (%)*	Multi-drug resistance(%)	Total (%)
HIV sero-positive	02 (2.3)	01(1.1)	01 (1.1)	01(1.1)	04(4.6)
HIV sero-negative	12(14)	03(3.4)	02 (2.3)	02(2.3)	17(19.7)
Total	14(16.2)	04(4.6)	03(3.4)	03(3.4)	21(24.4)

= also MDR. None of the M. tuberculosis isolates were resistant to more than 3 anti-TB drugs.

DISCUSSION

In this study, three *M. tuberculosis* isolates were resistant to both isoniazid and rifampicin, which accounts for 3.4% prevalence of MDR-TB among new TB patients in Pune, India and nearly 24.4% among all those strains showed any resistance. In India, prevalence of MDR TB in new cases has been observed to vary from 0.5% to 5.7% in recently published studies [12, 13]. Studies conducted in different regions of the country in patients with no history of previous treatment have shown that the magnitude of any resistance to HR was 2.0%, 2.4%, 3.0% and 3.4% in Ernakulam, Gujarat, Hoogly and Tamilnadu respectively [14,15,16,17]. In the present study, the highest resistance (17.4 %) was found in H, followed by S (13.9%) and R(3.4%). Minimal resistance (1.1%) was observed to E. However, in the previous study from Pune, it

was reported that resistance to any drug was 35.7%, any resistance to H, R, S and E was 27.1%, 5.7 %, 20.0% and 7.1% [13]. Another study conducted in Hoogly, found that resistance to any drug was 16.7%, any resistance to H, R, S and E was 10.3%, 3.0%, 13.7% and 1.9% [17], whereas, a largest population-based survey conducted by Tuberculosis Research Center in Gujarat reported that resistance to any drug was 21.32%, any resistance to H, R, S and E was 11.0%, 2.5%, 15.0% and 1.9% respectively [15]. In our present study any resistance to four first line anti TB drugs were less than we had reported earlier but were similar to the findings reported from Hoogly and Gujarat. Similar studies conducted in two districts of south India, North-Arcot district of Tamil Nadu and Raichur district of Karnataka, showed resistance to H to the extent of 23.4% and 18.7%; resistance to R in 2.8% and 2.5%, and MDR of 2.8% and 2.5% respectively in newly diagnosed TB patients [18]. In a statewide study conducted in Tamil Nadu, the resistance levels have been reported as 15% to H; 4.4% to R and an MDR of 3.4% [19]. A study carried out in Bangalore in the year 2002, reported that resistance to H, R and HR was 13.7%, 2.6% and 2.2% respectively [20].

The risk of DR-TB transmission can be reduced by efficient diagnosis and timely treatment of DR-TB patients [21]. Thus, the DR status of a patient needs to be confirmed before treatment. Better access to drug-susceptibility test results at the time of diagnosis of TB would facilitate appropriate selection of treatment regimens, thereby minimizing the development of DR strains. There is an urgent need to develop new methods for early detection of drug-resistant TB cases and faster DST results so that an early and effective drug therapy can be used to prevent the development of MDR and Extensively Drug resistant (XDR) TB. Furthermore, patients with XDR TB have poor out comes, long infectious periods and limited treatment options. In view of this, to prevent the epidemic of untreatable TB, continuous monitoring of anti-TB drug resistance by rapid detection methods is urgently needed. The MGIT 960 system has been reported to be an accurate, non radiometric alternative to the BACTEC 460TB procedure for rapid susceptibility testing of *M. tuberculosis* to four first-line drugs [22, 23]. However, due to increased cost per test for DST by the MGIT 960 method and high cost of instrument, this test is available only at few selected centres and hence the majority of the sites will have to rely on conventional methods for drug susceptibility studies. Though expensive, early detection and turnaround time can pay rich dividends for control and prevention of TB in general and specifically DR TB. In addition to cost, high level of competency, sophisticated infrastructure and technical expertise required to use these instruments limit the use of MGIT 960 system.

Drug-resistant TB has frequently been encountered in India, and its presence has been known after anti-tuberculosis drugs were first introduced for the treatment of TB. Most previous reports on drug resistance from India are from tertiary level care, and are thus not representative of the TB situation in India. These reports are therefore of little use for the planning purpose of the Government of India's Revised National Tuberculosis Control Programme (RNTCP). RNTCP has been a success in India and has contributed substantially to the success of TB control globally. It is not only important to implement the programme, evaluation of the programme is very important to judge the success of the programme and identify any modifications needed. Different studies on surveillance of drug resistance TB in India have been carried out in different parts of the country which has several limitations in terms of sample size, testing methodology, quality control and meticulous data collection. Hence well planned country wide surveillance on drug resistance TB needs to be done using standard protocol of testing to obtain more reliable estimates of prevalence of drug resistance TB. This may help the National Tuberculosis Control Programme to formulate policies to control and management of drug resistance tuberculosis. Limited information about the prevalence of drug-resistant tuberculosis (TB) has been

reported from India, the country with the world's highest burden of TB.

Although the sample size is small, the results suggest that level of multidrug resistance may pose a significant threat to health and impede the success of TB management in India, if adequate control measures are not implemented in timely manner. Monitoring the trend of resistance should remain a priority for the timely management of TB control programme. HIV prevalence in tuberculosis patients in the present study showed a decrease in HIV sero-positivity (17.46%) in the same clinic where a high prevalence of 28.75% was reported in 2002 [8].

CONCLUSION

The level of initial drug resistance of MTB is an epidemiological indicator to assess the success of the TB control program. National tuberculosis programs are particularly challenged by multidrug-resistant tuberculosis. Anti-tuberculosis drug resistance surveillance is, together with the monitoring of treatment outcome, an essential tool for evaluating the quality of tuberculosis control programmes. Our findings show that prevalence of MDR-TB in Pune has not increased since 2005 and DOTS therapy is effective in treatment of all new cases of pulmonary tuberculosis and preventing the emergence of MDR-TB. The limitation of present study is the small sample size which may not be representative of the population and the samples were from tertiary care hospital setting. Currently, routine surveillance of DR TB is not carried out. In the absence of which, studies like this would help to some extent. As little information on anti-TB drug resistance is available from India, we suggest countrywide anti TB drug resistance surveillance need to be conducted by accredited laboratories using quality assured standard methodology to obtain precise data on anti TB drug resistance in the population. The cost could be minimized by initiating central testing at few laboratories and randomly testing of MTB isolates for drug resistance. This would help Revised National Tuberculosis Control Programme (RNTCP) to develop and implement policies to fight against this white plague.

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Conflict of Interest

Conflict of interest declared none.

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