

Drug resistance pattern of *Mycobacterium tuberculosis* in seropositive and seronegative HIV-TB patients in Pune, India

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Accepted March 4, 2005

Background & objectives: Tuberculosis is the commonest opportunistic disease in persons infected with human immunodeficiency virus (HIV). Emergence of drug resistant isolates of *M. tuberculosis* highlights the need for continuous monitoring of drug resistance to anti-tuberculosis drugs. Considering the reported high prevalence of drug resistance in HIV infected tuberculosis patients, we studied the anti-tuberculosis drug resistance pattern of *M. tuberculosis* in HIV seropositive and seronegative tuberculosis patients in Pune, Maharashtra, India.

Methods: A total of 70 *M. tuberculosis* isolates, 30 from HIV seropositive and 40 from HIV seronegative tuberculosis patients with no previous history of anti-tuberculosis treatment, were isolated from sputum samples on Lowenstein-Jensen (LJ) medium, confirmed by conventional biochemical tests and stored at -70°C. They were revived by subculturing on LJ medium and tested for drug resistance against four first-line antitubercular drugs by BACTEC Mycobacterial growth indicator tube 960 (MGIT 960) system.

Results : Of the 30 isolates from HIV infected patients, 10 per cent were resistant to isoniazid (H), 6.6 per cent to streptomycin (S), 6.6 per cent to ethambutol (E) and 10 per cent were multi drug resistant (MDR). Of the 40 *M. tuberculosis* isolates from HIV uninfected individuals, 10 per cent were resistant to H, 2.5 per cent to S, 2.5 per cent to E, and 2.5 per cent isolates were MDR.

Interpretation & conclusion : The prevalence of drug resistant *M. tuberculosis* isolates among HIV seropositive tuberculosis patients was similar to that of HIV seronegative TB patients, indicating HIV infection may not be associated with drug resistant tuberculosis. However, considering the results from other studies and a high prevalence of HIV-TB infection in the country, monitoring of drug resistance in *M. tuberculosis* isolates needs prioritization to ensure success in national tuberculosis control programme.

Key words Drug resistance - BACTEC MGIT 960 - HIV - *Mycobacterium tuberculosis*

Tuberculosis (TB) is still a major health concern worldwide and the main cause of death by a single infectious agent, namely *Mycobacterium tuberculosis*. The disease spreads more easily in

overcrowded settings and in the conditions of malnutrition and poverty; characteristics typical of developing countries¹. India contributes nearly 25 per cent of the global burden of tuberculosis. Development

of drug resistance in *M. tuberculosis* isolates may adversely impact the management of the disease. Some recent developments, such as, emergence of multi drug resistant bacilli resulting from inadequate therapies and indiscriminate use of antibiotics and HIV/AIDS pandemic resulting in accumulation of pool of individuals who are more susceptible to TB have worsened the TB scenario². A high HIV seroprevalence among newly diagnosed TB patients has been reported in India^{3,4}.

An increasing prevalence of multidrug resistance (MDR) in several parts of the world including India has been one of the major reasons for declaring tuberculosis (TB) control as a global emergency by WHO^{5,6}. Several outbreaks of MDR-TB^{7,8} necessitated the continuous surveillance of drug resistance, not only for effective treatment of TB patients but also for initiating adequate public health measures. The present study was undertaken with the objective of comparing the anti tuberculosis drug resistance pattern of *M. tuberculosis* isolates from HIV seropositive and seronegative tuberculosis patients in Pune, India.

Material & Methods

Strain isolation : As a part of an ongoing study on efficacy of directly observed treatment-short course (DOTS) in HIV seropositive and HIV seronegative tuberculosis patients in Pune, consecutive patients attending TB clinics of Talera General Hospital and Yashvantrao Chavan Memorial Hospital of Pimpri-Chinchwad Municipal Corporation, Pune, between September 2000 and July 2004 were screened for enrollment in the study. Those who had no previous history of tuberculosis treatment and were diagnosed as having pulmonary tuberculosis by sputum smear microscopy using Ziehl Neelsen's acid fast staining and / or on radiological findings were enrolled. A total of 175 patients were enrolled during the study period. Seventy *M. tuberculosis* isolates, 94.7 per cent (36/38) from smear positive and 24.8 per cent (34/137) from smear negative patients were obtained and included for the present analysis. Of these, 30 isolates were from HIV seropositive and 40 from HIV seronegative tuberculosis patients. The sputum samples were processed by modified Petroff's method, inoculated on Lowenstein-Jensen (LJ) medium (Hi-Media Laboratories Ltd, Mumbai) and the isolates were identified by conventional biochemical methods⁹. The isolates were stored at

Table. Drug resistance pattern of *M. tuberculosis* isolates to four primary anti-tuberculosis drugs

	Total (n=70)	HIV + ve (n=30)	HIV - ve (n=40)
Any resistance			
H	19 (27.1)	9 (30)	10 (25)
S	14 (20)	7 (23.3)	7 (17.5)
E	5 (7.1)	3 (10)	2 (5)
R	4 (5.7)	3 (10)	1 (2.5)
Mono- resistance			
H	7 (10)	3 (10)	4 (10)
S	3 (4.3)	2 (6.6)	1 (2.5)
R	0	0	0
E	3 (4.3)	2 (6.6)	1 (2.5)
Multi drug resistance			
HR ± S ± E	4 (5.7)	3 (10)	1 (2.5)

H, Isoniazid; S, streptomycin; E, ethambutol; R, rifampicin
Values in parentheses are percentages

-70°C and revived prior to anti-tubercular susceptibility testing (AST).

Preparation of inocula: Isolates were revived on LJ medium and incubated at 37°C in ambient air; colonies no older than 14 days were suspended in 4 ml of Middlebrook 7H9 broth (Hi-Media Laboratories Ltd, Mumbai), which was adjusted to a McFarland standard of 0.5. One milliliter of this suspension was diluted with 4 ml of sterile saline (1:5 dilution), 0.5 ml of this dilution was used to inoculate into each of the drug containing Mycobacterial growth indicator tubes (MGITs; Becton Dickinson Microbiology systems, Sparks, MD, USA). One hundred microliters of 1:5 dilution was pipetted into 10 ml of sterile saline to obtain a final dilution of 1:500; 0.5 ml of this diluted suspension was used to inoculate into an MGIT growth control (GC) tube without drug.

Drug solutions: For drug susceptibility testing, 4 ml of sterile distilled water was added to a lyophilized vial of the drugs to prepare a stock solution (Becton Dickinson Microbiology systems, Sparks, MD, USA). Stock solution (100 µl) was added to an MGIT. The final critical concentrations were 1.0 µg/ml for streptomycin (S), 0.1 µg/ml for isoniazid (H), 1.0 µg/ml for rifampicin (R) and 5.0 µg/ml for ethambutol (E). These concentrations are equivalent to critical drug concentrations recommended by Center for Disease Control and Prevention (CDC) Atlanta¹⁰.

Drug susceptibility testing : To each 7 ml MGIT tube, 0.8 ml MGIT 960 growth supplement (Becton Dickinson Microbiology systems, Sparks, MD, USA) and 100µl of the drug stock solution were aseptically added, and finally 0.5 ml of the suspension containing *M. tuberculosis* was added to each MGIT. For each isolate, a growth control (GC) tube with growth supplement and without drug was included. 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 (Becton Dickinson Microbiology systems, Sparks, MD, USA) 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. Susceptibility test results were reported automatically.

Quality control: Fully susceptible H37Rv (ATCC 27294) reference strain of *M. tuberculosis* was used as control, additionally one isolate resistant to streptomycin and isoniazid, previously tested at this centre, was also included.

Statistical analysis : Fischer's exact test and Chi-square test with continuity correction were employed to test the proportions of anti-tubercular drug resistance in HIV seropositive and seronegative tuberculosis patients.

Results & Discussion

Among the 70 isolates, mono resistance to H, S, R and E was found to be 10, 4.3, 0 and 4.3 per cent isolates, respectively. Isolates resistant to isoniazid (H), streptomycin (S), ethambutol (E) or rifampicin (R) in combination with other drugs were 27.1, 20, 7.1 and 5.7 per cent respectively (Table). Drug resistance to at least one drug was found in 13 (43.3%) isolates from HIV seropositive and 12 (30.0%) isolates from HIV seronegative tuberculosis patients. The prevalence of multidrug resistance (resistance to at least isoniazid and rifampicin) in HIV uninfected tuberculosis patients was found to be low (2.5%) compared to that in HIV infected tuberculosis patients (10%). The prevalence of polyresistance (resistance to two or more drugs, but not both isoniazid and rifampicin) was found to be 10 per cent in HIV infected and 12.5 per cent in HIV uninfected tuberculosis patients. Neither individual drug resistance nor the MDR or polyresistance was found to differ significantly in the HIV infected and uninfected TB patients. The turnaround time for the AST by BACTEC MGIT 960 ranged between 5 days 16 h to 12 days 20 h with a median time of 8 days and 8 h.

Emergence and spread of drug resistant *M. tuberculosis* is a serious threat to tuberculosis control programme because patients with drug-resistant bacilli respond less readily to therapy than those with sensitive bacilli, resulting in preferential spread of the drug resistant bacilli in the community. 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^{11,12}. However,

due to exorbitant cost, this system will only be available at few select centres and hence the majority of the sites will have to rely on conventional methods for drug susceptibility studies.

Estimates of initial drug resistance carried out at the Tuberculosis Research Centre, Chennai showed that primary resistance to isoniazid was 15.0 per cent to streptomycin 11.8 per cent, and to both isoniazid and streptomycin was 7.7 per cent during the period 1993-1996; resistance to isoniazid was reported to vary from 3.2 per cent in Pune (1992-1993) to 32.9 per cent in Kolar (1987-1989) and resistance to H and R has been observed to increase over the past four decades¹³. We found that prevalence of resistance to isoniazid and Streptomycin was 10 and 4.3 per cent respectively and that to both drugs was 11.4 per cent. The issue of whether infection with HIV is a risk factor for drug resistant tuberculosis still remains unanswered since results of some studies supported this hypothesis while some did not. A study conducted in New York city¹⁴ revealed that HIV infected TB patients were significantly more likely to develop resistance to at least one drug (37 versus 19%) and MDR (19 versus 6%) than those without HIV infection.

In our study, we did not find any difference in drug resistance between HIV seropositive and seronegative tuberculosis patients, which is in accordance with the findings from other studies in Europe^{15,16}. Some studies have reported lower prevalence of anti tuberculosis drug resistance in HIV seropositive tuberculosis patients compared to that in HIV seronegative patients^{17,18}.

In conclusion, no significant difference was seen in anti-tuberculosis drug resistance in HIV seropositive and seronegative tuberculosis patients. In view of the conflicting results from other studies and high prevalence of HIV-TB in our country, we feel that monitoring of anti-tuberculosis drug resistance pattern in TB patients in general and HIV seropositive tuberculosis patients in particular, would provide important data, which may be crucial for the National Tuberculosis Control Programme.

Acknowledgment

The authors gratefully acknowledge Indian Council of Medical Research, New Delhi for the financial support provided for HIV/TB Task Force study at National AIDS Research Institute, Pune and by authorities of Talera General Hospital and Pimpri-Chinchwad Municipal Corporation, Pune for permission for conducting this study.

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