RESEARCH NOTE

DRUG RESISTANCE AMONG *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM PRIVATE CLINICS AND A DOTS CENTER IN DELHI, INDIA

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Abstract. The aim of this study was to ascertain the incidence of drug resistance of *Mycobacterium tuberculosis* isolates from patients in Delhi, India, being treated with DOTS and in private clinics, since a large proportion of patients with tuberculosis in India seek help from private healthcare sectors. Sputum samples were collected from 60 cases of tuberculosis attending a DOTS center and 42 patients from private clinics. Of these, 35 patients from the DOTS center and 12 patients from private clinics had a second sputum sample collected following two months of therapy. The isolated *M. tuberculosis* strains were assayed for isoniazid (INH), rifampicin (RIF), streptomycin (SM) and ethambutol (EMB) susceptibility by the proportion method. The frequencies of multidrug resistance (MDR) in the *M. tuberculosis* strains obtained from those treated with DOTS and in private centers were 12.7% and 5% ($p>0.5$), respectively. Isolates obtained after two months of therapy showed a similar rate of MDR (12.5%) at the DOTS center. although the number of patients followed-up at the private centers was small, none of these had MDR after two months of therapy. Future studies including a larger number of patients at private centers are needed to further evaluate the prevalence of drug resistance in *M. tuberculosis* from private clinics.

Keywords: *M. tuberculosis*, drug resistance profiling, India

INTRODUCTION

The WHO report on Global Tuberculosis Control ranks India as the world's most heavily affected country (Dye, 2006). The average prevalence of all forms of tuberculosis in India is estimated to be 5/1,000. The prevalence of smear positive cases is 2.27/1,000 and the average annual incidence of smear positive cases is 84 per 100,000 annually (Chakraborty, 2004).

The Revised National Tuberculosis Control Programme (RNTCP) has been attempting to combat tuberculosis since 1993. The RNTCP began large scale nationwide implementation of Directly Observed Treatment – Short Course (DOTS) in 1998 and has since expanded rapidly (Dewan *et al*, 2006). However, the city of Delhi, with

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a population of 13 million and an active RNTCP, has a substantial number of patients not obtaining treatment at a DOTS center. This suggests a large number of unreported TB cases are being managed by the private sector. The main reasons patients attend a private center include better geographical access, shorter waiting times, flexible hours, greater availability of drugs and greater confidentiality (Uplekar *et al*, 1998; Arora and Gupta, 2004). Thus, the success of tuberculosis control in India depends in a large part on private doctors. No studies have evaluated the practices of private doctors in diagnosing and treating patients with tuberculosis in India and its implications on the development of drug resistance. Very few studies have been carried out to determine drug resistance profiles of *M. tuberculosis* isolates obtained from patients attending DOTS centers (Aparna *et al*, 2009, Paramasivan *et al*, 2010). A single study from South India reported the prevalence of multidrug resistant tuberculosis in a tuberculosis unit where DOTS was implemented through a public-private mix (PPM) (Anuradha *et al*, 2006). The present study determined the frequency of drug resistant *M. tuberculosis* isolates in two groups of patients: one attending a DOTS center under the RNTCP and the other obtaining treatment from private clinics in Delhi, India.

MATERIALS AND METHODS

Sputum samples were collected from 102 patients with tuberculosis between January 2006 and February 2007. Simple random sampling was performed following the diagnosis of patients during the recruitment period. Of these, 60 samples, were collected from patients undergoing Category I treatment (2 months of isoniazid, rifampicin, pyrazinamide, ethambutol; followed by 4 months of rifampicin and isoniazid) at Rajan Babu TB Hospital (RBTB), Delhi, India. This hospital also has an "on campus" DOTS Center serving Delhi. Forty-two patients with tuberculosis were selected from four private clinics in Delhi, India. The latter were on self administered short course antituberculous therapy. Only adult patients ≥18 years of age were enrolled in the study. None of the patients had been on treatment for >1 week at the time of enrollment. Thirty-five patients from the RBTB hospital and 12 patients from private clinics were followed up after 2 months of therapy. Informed consent was taken from each patient prior to collection of samples, following clearance from the Institutional Ethics Committee. Sputum samples were submitted to direct microscopy after Ziehl-Neelsen staining, to detect acid-fast bacilli (AFB), and cultured on Lowenstein Jensen (LJ) medium in duplicate according to the protocols recommended by the RNTCP, India (Central Tuberculosis Division, 2001). The culture isolates were further identified to the species level by the niacin test (Konno *et al*, 1966), nitrate reduction (Lutz, 1992) and catalase tests (Kubica *et al*, 1966).

Drug susceptibility testing was performed at the Department of Microbiology, Vallabhbhai Patel Chest Institute, Delhi, using the proportion method. The drug concentrations tested were 4 mg/l for streptomycin (SM), 0.2 mg/l for isoniazid (INH), 40 mg/l for rifampicin (RIF) and 2 mg/l for ethambutol (EMB). The LJ slants were incubated at 37°C and observed at 28 and 42 days of incubation (Canetti *et al*, 1967).

RESULTS

Demographic and laboratory data showed the majority of patients from the

Table 1
Results of drug susceptibility testing from initial culture positive samples obtained from DOTS and private centers

S.No.	Drug susceptibility profile	DOTS center *N*=47 (%)	Private center *N*=20 (%)
1	Resistance to any drug	33 (70)	9 (45)
2	Resistance to one drug		
	S	8 (17)	0
	I	1 (2)	0
	R	0	0
	E	1 (2)	1 (5) #
3	Resistance to more than one drug (excluding MDR)		
	I+S	6 (12.7)	0
	I+E	1 (2)	2 (10) #
	S+E	2 (4)	1 (5) #
	R+S	4 (8.5)	1 (5) #
	R+E	1 (2)	2 (10) #
	I+S+E	3 (6.4)	1 (5) #
4	Resistance to more than one drug (MDR-TB)		
	I+R	1 (2)	0
	I+R+S	1 (2)	1 (5) #
	I+R+E	1 (2)	0
	S+I+R+E	3 (6.4)	0

S, streptomycin; I, isoniazid; R, rifampin; E, ethambutol; MDR, multidrug resistance
$p \geq 0.05$ for all variables

DOTS center (n=44) and private clinics (n=30) were aged 20-40 years. All samples from the DOTS center were positive for AFB, while 34 samples (81%) from the private centers were AFB positive. Forty-seven samples (78.3%) and 20 samples (47.6%) from the RBTB Hospital and private clinics were culture positive, repectively.

Resistance to INH (36%), RIF (23%) and SM (57.4%) was observed more often in the *M. tuberculosis* isolates obtained from the DOTS center than among those obtained from the private clinics (20% each). Resistance to EMB was seen more in the *M. tuberculosis* isolates obtained from the private centers (35%) than in those obtained from the DOTS center (25.5%). Although not statistically significant, a higher proportion of MDR *M. tuberculosis* was found in isolates from the DOTS center (12.7%) than in the private clinics (5%) (p>0.5). None of the isolates were mono-resistant to RIF (Table 1).

Eight out of 35 cases (22.8%) followed up from the DOTS center and 2 of 12 cases (16.6%) from the private clinics were smear and culture positive. The number of patients available for follow-up was significantly higher at the DOTS center (p<0.01). The frequencies of resistant strains among the 8 isolates followed-up from the DOTS center, were 5 (62.5%), 3 (37.5%), 2 (25%) and 1 (12.5%) for SM, INH, RIF and EMB, respectively. Of the two *M. tuberculosis* isolates obtained at follow-up from the pa-

tients from the private clinics, there was no difference in the drug susceptibility profile between the initial and follow-up isolates. One isolate was resistant to INH, SM and EMB and the second strain was sensitive to all four first line antituberculous drugs.

DISCUSSION

Although reliable statistics are unavailable, a large number of patients with tuberculosis in India go to private clinics because of easy access and time constraints (Uplekar *et al*, 1998; Dewan *et al*, 2006). This may influence the outcome of tuberculosis control. No studies in India have reported drug susceptibility profiles of patients undergoing private treatment. One study analyzed the incidence of fluoroquinolone (FQ) resistant *Mycobacterium tuberculosis* at a private hospital from 1995 to 2004 and concluded that FQ resistance had increased exponentially from 3% in 1996 to 35% in 2004 (Agrawal *et al*, 2009). However, there are no comprehensive reports on the drug resistance profile of *M. tuberculosis* to first line antituberculous agents from other private hospitals in India. Moreover, very few studies have reported the drug resistance profile of *M. tuberculosis* isolates from patients being treated at DOTS centers. Most of these studies were from southern India (Anuradha *et al*, 2006; Aparna *et al*, 2009; Paramasivan *et al*, 2010). The present study was undertaken to investigate the drug resistance profile of patients from Delhi, one group attending a DOTS center and another group attending private clinics.

Of the 102 patients studied, drug susceptibility profiles for the 2 groups showed 12.7% of cases (6/47) from the DOTS center and 5% of cases (1/20) from the private clinics were multidrug resistant though the difference was not statistically significant ($p>0.5$) (Table 1).

In our study a significantly higher number of cases showed monoresistance to antituberculosis drugs at the DOTS center than the private center ($p<0.01$) (Table 1). Similar to our earlier study (Varma-Basil *et al*, 2004), none of the isolates in our study were monoresistant to RIF. SM monoresistance (17%) at the DOTS center was more common than monoresistance to any other single drug ($p<0.05$). EMB resistance was significantly associated with resistance to other drugs ($p<0.001$). EMB resistance due to mutation at the *embB* codon is associated with an increased resistance to other drugs (Hazbon *et al*, 2005). This may explain why EMB resistance was associated with resistance to other drugs.

Thirty-five of 47 patients (74%) obtaining treatment at the DOTS center and 12 of 20 patients (60%) getting treatment in private clinics could be followed up. The decrease in the number of patients follow-up compared to the number studied initially was due to non-compliance of patients or movement of patients out of the Delhi area without giving a change in address. The difference between the DOTS and private patients at follow-up was statistically significant ($p<0.05$). Patients going to the DOTS center were generally more complaint with follow-up than the private clinic patients. Private clinic patients tended to be more suspicious of the study and less likely to share a history of their disease. They felt tuberculosis was a taboo subject and did not want their family members to know about the disease. This can have a major impact on tuberculosis control since such patients would not be expected to take precautionary measures to prevent tuberculosis spread in their environment, possibly leading to an

increase in the number of tuberculosis cases.

Drug susceptibility testing revealed one isolate was susceptible to all four antituberculosis drugs tested initially, but developed resistance to SM and RIF during the course of treatment. The patient from whom this isolate was obtained obtaining treatment from the DOTS center. It is possible the patient was not compliant with treatment.

No significant difference was observed in the drug resistance profile for *M. tuberculosis* isolates between the DOTS center and the private clinics. At follow-up, only 23% (8/35) and 17% (2/12) of patients from the DOTS center and the private clinics, respectively, had positive cultures. There was a 77% cure rate at the DOTS center and an 83% cure rate at the private clinics, which is close to the RNTCP recommendations of a cure rate of 85% among registered new smear positive pulmonary tuberculosis cases. Our results could be due to the fact that our patients belonged to a region where private practitioners were aware of tuberculosis treatment under DOTS. The small number of patients in our study could have biased our results. Investigating patients treated privately in remote areas with a larger study population could provide further information.

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