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Full Length Research Paper

Susceptibility pattern among pulmonary and extrapulmonary isolates of *Mycobacterium tuberculosis* in north India

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Drug resistant tuberculosis (TB) is an increasing problem worldwide. We retrospectively identified *Mycobacterium tuberculosis* isolates from treatment failure pulmonary TB (362), and extrapulmonary TB (338) patients (18-70 years) during a 4 year period (June, 2002-July, 2006). Drug susceptibility testing was carried out for the first line anti-tuberculosis drugs using standard proportion method. Of the treatment failure pulmonary TB, and extrapulmonary TB isolates, 51.9 and 47.3% were pan susceptible, respectively. The rest of the isolates were resistant to at least one of the drugs. Overall, multidrug resistance (MDR) was detected in 16.6% isolates and was higher in treatment failure pulmonary TB as compared to extra-pulmonary TB cases. Periodic monitoring of the level of drug resistance in both pulmonary and extrapulmonary TB is important to assess the true picture of drug resistance in *M. tuberculosis,* and is hence crucial for robust implementation of effective TB control programs.

Key words: Anti-tuberculosis drug susceptibility, *Mycobacterium tuberculosis*, India.

INTRODUCTION

The global magnitude of multi-drug resistant tuberculosis (MDR-TB) has not been well described; however, India harbors the highest number of TB cases and many of them are MDR-TB (WHO, 2009). According to WHO, nearly 50% of the world's burden of MDR-TB cases is in India and China (WHO, 2011). The predisposing factors in developing countries for acquiring pulmonary tuberculosis (PTB) include close contact to smear-positive PTB patients, malnourishment and poverty (Hernandez-Garduno and Perez-Guzman, 2007). Data on the burden of drug resistance in TB are vital to calculate the resource requirements and monitor the nation-wide TB control programs. WHO recognized the importance of the trends of MDR and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* as barriers to the achievement of the WHO's Global Plan's objectives by 2015, at the 2007 World Health Assembly. At least one case of XDR-TB has been reported from 58 countries as of March 2010, and it is estimated that about 5.4% of MDR-TB cases are XDR-TB. Prior studies from India have revealed significant resistance in *M. tuberculosis* isolates (Jain et al., 1992; Paramasivan et al., 1998; Paramasivan and Venkataraman, 2004). There are few reports from India showing increased drug resistance among isolates from pulmonary and extrapulmonary TB patients (Paramasivan et al., 1993; Santha et al., 2006; Sachdeva et al., 2002). Drug resistance, especially MDR, is expected to be particularly high among isolates from treatment failure PTB cases (WHO, 2009). This retrospective study was hence undertaken to assess the prevalence of drug resistance to first line anti-tuberculosis drugs in *M. tuberculosis* isolates from treatment failure PTB and extrapulmonary

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TB patients from June, 2002 to July, 2006, in north India.

MATERIALS AND METHODS

All treatment failure PTB and extrapulmonary TB patients with positive culture results were included in the study. Cultures of sputum samples and extrapulmonary samples were performed at the Mycobacteriology laboratory of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. A treatment failure cases was defined as a patient previously treated for TB, who is started on a retreatment regimen after previous treatment has failed.

Specimens

Three consecutive early morning sputum samples (5-10 ml) were collected in sterile disposable containers. In patients suspected to have urinary TB, first morning whole urine sample was obtained on three occasions. Collection of other samples was as for other bacterial cultures with proper sterile technique and in closed sterile containers. The specimens were transported to the laboratory and processed on the same day. Sputa and other possibly contaminated samples were first decontaminated and digested using the NALC-NaOH method to decrease the commensal bacterial contamination. All fluid samples were processed from the sediment obtained after centrifugation at 3000 rpm for 30 min. All these procedures were conducted according to CDC guidelines (Kent and Kubica, 1985). For culture, two Lowenstein–Jensen (LJ) slants were inoculated and incubated at 37°C and observed at weekly intervals for 6-8 weeks. *M. tuberculosis* was identified by its growth rate, colony morphology, colour, niacin production, catalase production at 68°C, nitrate reduction, and a negative arylsulfatase test. The isolates were stored at -70°C, and revived prior to anti-tubercular drug susceptibility testing (DST).

Drug susceptibility testing

DST was carried out for first line anti-tuberculosis drugs, namely rifampicin (40 mg/L), isoniazid (1 mg/L), ethambutol (2 mg/L) and streptomycin (4 mg/L) using standard proportion method according to center for disease control and prevention (CDC) guidelines (Kent and Kubica, 1985). An isolate was considered resistant if percentage resistance was >1%.

Statistical analysis

The DST results were analysed by the χ^2 test. A two-sided *p*-value of less than 0.05 was considered statistically significant. The data was analyzed using the SPSS version 15.0 software.

RESULTS

A total of 700 isolates, 362 isolates of *M. tuberculosis* from treatment failure PTB cases and 338 from extrapulmonary specimens were analyzed. Among these, the majority of specimens (71%) were from 15-41 years age group, followed by 14% from pediatric age group (6-14 years), 9% from 41-59 years, and 6% from patients > 60 years of age. The extrapulmonary isolates were obtained from patients with lymphadenitis (125/338, 36.9%), pus and aspirates (78/338, 23.1%), CSF (55/338, 16.27%), urine (40/338, 11.8%), synovial/ joint fluids (17/338, 7%), endometrial biopsy (13/338, 3.8%), ascitic fluid (8/338, 2.4%), and pericardial fluid (2/338, 0.6%).

One hundred and seventy four (48.1%) isolates from treatment failure PTB cases and 178 (52.7%) from extrapulmonary TB specimens showed resistance to at least one of the drugs tested. The monoresistance of *M. tuberculosis* isolates to isoniazid, rifampicin, streptomycin and ethambutol were 1.7, 6.4, 6.9 and 0.05% respectively. There was no statistically significant difference in the rate of resistance between pulmonary and extrapulmonary isolates. Overall, multidrug resistance was detected in 116 (16.6%) isolates. There were 76 (20.9%) MDR-TB isolates in treatment failure PTB cases and 40 (11.8%) in the extrapulmonary TB isolates (Table 1).

DISCUSSION

This study reports a high level drug resistance among treatment failure PTB (48.1%) and extrapulmonary TB cases (52.7%) from north India. This is in agreement with Dam et al. (2005) who studied 263 treatment failure PTB cases and reported resistance to be 42.5%. Such high levels of resistance have also been reported from other countries like Uzbekistan and Turkmenistan (Cox et al., 2004). Also, a high multidrug resistance (resistance to isoniazid and rifampicin) was seen in both pulmonary (20.9%) and extrapulmonary isolates (11.8%). Expectedly the rate of resistance was higher for the treatment failure PTB isolates. Similar rates of resistance have been reported from New Delhi where the incidence of MDR-TB was found to be 14% in treatment failure cases (Dam et al., 2005). Studies undertaken by the Tuberculosis Research Centre, Chennai during 1997-2000 from different parts of the country that is, Tamil Nadu, North Arcot and Raichur districts as well as in Wardha and Jabalpur revealed the incidence of MDR-TB to vary from 25-100% (Paramasivan et al., 1993; Santha et al., 2006). The prevalence of MDR tuberculosis has been shown to vary widely over different regions of the world, with highest rates being found in Nepal (48%), New York City (30%), Bolivia (15%) and South Korea (15%) (Cohn et al., 1997). Our findings also corroborate the facts that tuberculosis is more common in the young adults (71% of specimens were from 15-41 years age group) and that the most common presentation of extrapulmonary tuberculosis is tubercular lymphadenitis (36.9%) (Cowie and Sharpe, 1997).

The high prevalence of MDR-TB in our study could be attributed to the fact that it was carried out at a tertiary care hospital where patients are being referred from different parts of North India, that is, from 6 states (Punjab, Haryana, Chandigarh, western parts of Uttar Pradesh, Jammu and Kashmir, and some parts of Rajasthan). These patients are usually partially treated,

Table 1. Drug resistance in pulmonary (treatment failure) and extrapulmonary tuberculosis isolates.

Susceptibility to first line anti-tuberculosis drugs	Pulmonary isolates (treatment failure) n = 362 (%)	Extrapulmonary isolates n=338 (%)	Total isolates n=700 (%)
Pan susceptible	188 (51.9)	160 (47.3)	358 (51.1)
Any resistance	174 (48.1)	178 (52.7)	342 (48.9)
Monodrug resistance			
H	6 (1.7)	15 (4.4)	21 (3.0)
R	23 (6.4)	27 (8.0)	50 (7.1)
S	25 (6.9)	33 (9.8)	58 (8.2)
E	2 (0.05)	7 (2.1)	9 (1.3)
Total monodrug resistance	56 (15.5)	82 (24.3)	138 (19.7)
H and R resistant			
H+R	19 (5.2)	25 (7.4)	44 (6.3)
H+R+E	1 (0.03)	2 (0.6)	3 (0.4)
H+R+S	25 (6.9)	7 (2.1)	32 (4.6)
H+R+S+E	31 (8.6)	6 (1.8)	37 (5.3)
Total MDR (HR±S ±E)	76 (20.9)	40 (11.8)	116 (16.6)
H and other resistant			
H+S	8 (2.2)	14 (4.1)	22 (3.1)
H+E	2 (0.05)	2 (0.6)	4 (0.6)
H+S+E	3 (0.1)	6 (1.8)	9 (1.3)
R and other resistant			
R+S	19 (5.2)	23 (6.8)	42 (6.0)
R+E	1 (0.03)	2 (0.6)	3 (0.4)
R+S+E	2 (0.05)	4 (1.2)	6 (0.9)
Any drug resistance			
Any H resistance	95 (26.2)	77 (22.8)	172 (24.6)
Any R resistance	121 (33.4)	96 (28.4)	217 (31.0)
Any S resistance	120 (33.2)	98 (29.0)	218 (31.1)
Any E resistance	49 (2.5)	34 (10.1)	83 (11.9)

E (Ethambutol), H (Isoniazid), MDR (multidrug resistant), R (Rifampicin), S (Streptomycin).

and secondly, non compliance among patients is a major factor contributing to high resistance to anti-tuberculosis drugs. The increase in drug resistance among extrapulmonary isolates cases warrants further attention towards the treatment of patients with extrapulmonary TB. With the emergence of XDR-TB from various parts of the world, and with no new drugs in our armamentarium against TB, there is an urgent need to consolidate the existing national TB control programs and focus on critical issues like early diagnosis, judicious and timely use of anti-tubercular drugs, and adherence to the treatment, to decrease the morbidity and mortality in those who are suffering from TB, as well as for containment of the spread of the disease in the community. The task is huge, as countries of the developing world like Afghanistan, Pakistan, India, Zimbabwe, Uganda, and many others are still facing challenges in the effective introduction, implementation and expansion of directly observed treatment strategy (DOTS) short course (Ibrahim et al., 2002).

In conclusion, high drug resistance to *M. tuberculosis* warrants periodic assessment of the trends of drug resistance to anti-tuberculosis agents, which is an essential component of the crusade against tuberculosis. Surveillance methods for collection of data on drug resistance need to be more systematic and uniformly standardized, and should be conducted across wide regions to obtain true picture of the problem, as any emergence of resistance to these drugs is a direct reflection of the failure of TB control programs.

Continuous data on drug resistance helps in designing the best control practices and assessing the performance of these programs over time, in order to incorporate necessary adjustments in the approach towards control of this deadly disease.

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