Short Communication

Correspondence

M. Bose

mridulabose@hotmail.com

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Drug-sensitivity profile of clinical *Mycobacterium tuberculosis* isolates – a retrospective study from a chest-disease institute in India

T. Dam,^{†‡} M. Isat[†] and M. Bose

Department of Microbiology, VP Chest Institute, University of Delhi, Delhi 110 007, India

Multi-drug-resistant tuberculosis (MDR-TB) is a major public-health problem, because treatment is complicated and patients remain infectious for months or years, despite receiving the best available therapy. To gain better understanding of MDR-TB, a retrospective study was initiated to determine the level of drug resistance among patients in a chest-disease institute in India. Two hundred and sixty-three isolates from treatment-failure pulmonary tuberculosis patients (20–70 years) were studied. Drug-sensitivity testing was performed by the modified-proportion method. First- and second-line drugs, along with two quinolone drugs (ofloxacin and ciprofloxacin), were tested. Patients included in this study did not improve with therapy; however, 151 isolates (57.5%) were susceptible to all four first-line antituberculosis drugs. This study reports low resistance to fluoroquinolones among the strains present in these patients.

Introduction

Multi-drug-resistant tuberculosis (MDR-TB) is an emerging problem of great importance to public health worldwide. The modern era of tuberculosis has recently been characterized by a rise in the number of cases of MDR-TB, which causes higher mortality rates than drug-sensitive tuberculosis. Detecting antibiotic resistance among *Mycobacterium tuberculosis* strains is becoming increasingly important with the global recognition of drug-resistant strains and their adverse impact on clinical outcome. The global magnitude of MDR-TB has not been well described; however, India harbours the highest number of tuberculosis cases and many of them are MDR-TB (Udwadia, 2001). Several reports showed increased drug resistance among pulmonary tuberculosis patients in India (Mathur *et al.*, 2000; Hemvani *et al.*, 2001; Paramsivan *et al.*, 2002). This retrospective study was undertaken to assess the extent of drug-resistant *M. tuberculosis* strains harboured among a group of treatment-failure pulmonary tuberculosis patients in a chest-disease institute in India. We report the results of *in vitro* drug-sensitivity testing of *M. tuberculosis* isolates from these patients.

Methods

Two hundred and sixty-three sputum samples were collected from 181 (68.8%) males and 82 females showing acid-fast bacilli (AFB) by direct smear, even after 12 months therapy over a period of 3 years (1996–1998). Care was taken to avoid reinclusion of any isolate. Patients were selected from the Vallabhbhai Patel Chest Institute outpatient department clinic, Delhi, India. These patients (based on their previous prescription and records) were treated by any four first-line drugs [rifampicin (RIF), isoniazid (INH), streptomycin (SM), ethambutol (EMB) or pyrazinamide] in different regimens. Sputum samples were collected from patients before they were put on further therapy.

Bacteriological procedure. Three consecutive morning sputum samples (5–10 ml) were collected in McCartney bottles and processed on the same day. Petroff's method was used to decontaminate and increase the bacillary concentration (Balows *et al.*, 1991). NaOH (4%) was used to kill any other contaminants in this procedure. Two Lowenstein–Jensen (LJ) slants were inoculated and incubated at 37 °C for 6–8 weeks. A smear was prepared from each of the processed samples on a grease-free slide and stained by carbol fuchsin using the Ziehl–Neelsen technique. Slides were checked for AFB under a microscope.

Drug susceptibility. The agar-dilution proportion method was used to perform drug-sensitivity testing using 7H10 Middlebrook medium (Difco) (Balows *et al.*, 1991). Lyophilized drugs were reconstituted aseptically in water. The stock was diluted in such a manner that a 5 µl aliquot contained the requisite amount of each drug. The drug concentrations used in this study are shown in Table 1.

Preparation of inoculum for drug sensitivity. Several spade-fulls (2–5 mg) of growth were scraped from LJ slants, transferred to a sterile screw-cap tube containing six to eight glass beads and 3 ml normal saline (0.85%) and mixed well on a vortex mixer. Turbidity was matched against McFarland standard no. 1. Inoculum (100 µl) was added to each plate, containing 5 ml 7H10 Middlebrook medium with

[†]These authors contributed equally to this work.

[‡]Present address: Room 204, Mudd Hall, 3400 N Charles Street, Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA.

Abbreviations: AFB, acid-fast bacilli; EMB, ethambutol; INH, isoniazid; MDR-TB, multi-drug-resistant tuberculosis; RIF, rifampicin; SM, streptomycin.

Table 1. Drug concentrations used in *in vitro* drug-sensitivity testing

Drug	Drug concentration (µg per disc)	Final concentration (µg ml ⁻¹)
Isoniazid	5.0	1.0
Rifampicin	25.0	5.0
Streptomycin	50.0	10.0
Ethambutol	50.0	10.0
Pyrazinamide	100	20.0
Kanamycin	25.0	5.0
<i>p</i> -Aminosalicylic acid	10.0	2.0
Amikacin	30.0	6.0
Thiacetazone	25.0	5.0
Ethionamide	25.0	5.0
Cycloserine	100.0	20.0
Ofloxacin	10.0	2.0
Ciprofloxacin	10.0	2.0

drug in each quadrant. *M. tuberculosis* strain H37Rv was used as control in all sets of experiments. The inoculated plates were incubated at 37 °C in an atmosphere of 10 % CO₂. Results were recorded after 3 weeks. Each drug-sensitivity test was carried out at least three times. Values are expressed as means and SD (Fig. 1).

Results

Two hundred and sixty-three sputum samples from 181 males and 82 females (20–70 years) were studied. The drug-sensitivity profiles of 13 drugs were evaluated. Out of 263 isolates, 112 showed resistance to first-line drugs (INH, RIF, SM and EMB) and the remaining 151 were sensitive to all four first-line drugs. Percentages of resistance to individual antituberculosis drugs, along with ofloxacin and ciprofloxacin, are shown in Fig. 1. Among the first-line drugs, INH and RIF resistance was 21.43 and 27.03 %, respectively. Out of 263 isolates, 37 (14 %) showed multi-drug resistance (resistance to INH and RIF).

Interestingly, all ofloxacin-resistant strains were also resistant to ciprofloxacin. Isolates that were resistant only to INH and RIF (*n* = 6) were 100 % sensitive to ofloxacin and ciprofloxacin (Table 2). Eleven isolates were resistant to SM or EMB in addition to INH and RIF, and demonstrated 5.7 and 2.7 % resistance to ciprofloxacin and ofloxacin. The

remaining 20 isolates out of the 37 MDR-TB strains were resistant to all four first-line drugs and showed 10 and 16 % resistance to ofloxacin and ciprofloxacin, respectively.

Discussion

This retrospective study regarding drug-resistance patterns among treatment-failure tuberculosis cases has generated valuable information in the context of the drug-resistant tuberculosis situation in India. Resistance observed in this study was 42.5 %. A high degree (14 %) of MDR-TB was observed among these patients. These patients claimed to have antituberculosis therapy without improvement; however, 151 (57.5 %) isolates were sensitive to all four first-line drugs (INH, RIF, SM and EMB) that were tested. Although non-compliance is a major factor for poor outcome of therapy, this study underscores the presence of MDR-TB strains among these patients.

Shah *et al.* (2002) studied 482 previously treated pulmonary tuberculosis patients and reported that resistance to INH and INH plus RIF was 12.86 and 15.77 %, respectively. In our study, resistance to INH and INH plus RIF was 20.18 and 16 % (six out of 37), respectively. Although INH resistance was higher than in the study by Shah *et al.* (2002), INH plus RIF resistance was comparable. Vijay *et al.* (2002) reported that resistance to INH, either alone or in combination with another drug, was highest, at 27.4 %. SM resistance (23 %) observed in their study corroborated with the present report. Resistance against RIF (26.73 %) observed in the present study is higher than the RIF resistance (15.5 %) reported in a previous study (Shah *et al.*, 2002).

This study shows that resistance is relatively low in *in vitro* testing, although these patients did not improve with therapy. Non-compliance among patients is an important factor in this regard. Secondly, we observed the highest percentage of resistance (23.51 %) against cycloserine among the second-line drugs; however, these patients never received any drug from the reserve group. Thus, the reason for this resistance may be partially attributed to cross-resistance among drugs, e.g. cross-resistance of ethionamide with INH. This study reports low resistance to fluoroquinolones among the strains tested in this study. This study underlines the necessity of direct observed therapy in Indian patients.

Fig. 1. Resistance to individual drugs used in this study.

Table 2. Pattern of multi-drug resistance to antituberculosis drugs, along with ofloxacin and ciprofloxacin resistance, among treatment-failure pulmonary tuberculosis patients

Drug regimen	Percentage of all resistant cases (<i>n</i> = 263)	No. resistant (<i>n</i> = 37)	Subset also resistant to:	
			Ofloxacin	Ciprofloxacin
INH+RIF	2.2	6 (16 %)	0	0
INH+RIF+SM	2.2	6 (16 %)	1 (2.7 %)	2 (5.7 %)
INH+RIF+EMB	1.9	5 (13 %)	1 (2.7 %)	2 (5.7 %)
INH+RIF+SM+EMB	7.6	20 (54 %)	4 (10 %)	6 (16 %)

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