ORIGINAL ARTICLE

Drug susceptibility profiles of *Mycobacterium tuberculosis* isolates from Gulbarga, South India

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Abstract   *Aim of the work:* Drug resistance surveillance is a useful tool to assess the effective functioning of tuberculosis (TB) control program. This study was undertaken to know the first line anti tuberculosis drug susceptibility profile of *Mycobacterium tuberculosis* clinical isolates from the Gulbarga district of South India.

Methods: Drug susceptibility test was performed for 102 clinical isolates of *M. tuberculosis* belonging to new ($n = 62$), treated ($n = 22$) and unknown treatment category ($n = 18$) of TB. All the isolates were tested for susceptibility to first line anti-tuberculosis drugs by minimum inhibitory concentration (MIC) and resistance ratio method (for streptomycin).

Results: The susceptibility profile of *M. tuberculosis* to all five first line anti-tubercular drugs was found to be 60.78% (62/102). Overall, multi drug resistance (resistance to at least isoniazid and rifampicin) was observed in 8.82% (9/102) isolates and was found to be higher for treated cases (18.18%).

Conclusions: High level of drug resistance observed in new cases for isoniazid, rifampicin and ethambutol suggests need for the implementation of drug resistance surveillance studies in order to document the success of the tuberculosis control program in reducing the level of drug resistance.
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Introduction

Tuberculosis has become a serious health problem since the emergence of HIV and the increasing appearance of drug resistance strains [1]. Drug resistance in tuberculosis is a global problem. Levels of drug resistance serve as an epidemiological indicator to assess the extent of resistant bacterial transmission in the community. However, information on the level of drug

resistance is difficult to obtain at the community level, especially in a large developing country such as India with limited resources [2]. Although drug resistance surveillance is considered a useful tool to assess the success of the TB control program the facilities to collect specimens and also to perform the drug susceptibility test (DST) are not readily available in all parts of the world. Proper collection and transportation of sputum specimens from remote survey settings to a quality controlled TB culture laboratory is crucial to ensure accurate results that can contribute to national and global surveillance of TB drug resistance [3]. In the present study, an attempt was made to collect the sputum specimens from tuberculosis patients attending designated microscopic centers (DMC) of the Gulbarga district and transport them to a TB culture laboratory for the study of anti-mycobacterial susceptibility profiles of *Mycobacterium tuberculosis* to first line anti-tuberculosis drugs.

Materials and methods

Patients and specimens

The study area, Gulbarga district is located in the north-eastern part of the Karnataka state in India. Sputum specimens were collected from clinically suspected pulmonary tuberculosis patients attending DMC and private clinics of this district. The study was undertaken from February 2005 to March 2008. The treatment history of the patients was collected from available data at the DMC. The specimens were collected after obtaining oral consent from the study participants.

Specimen transport

Sterile screw capped universal containers and preservative, cetylpyridinium chloride (1%) with 2% Sodium Chloride (CPC-NaCl) solution was supplied to the DMC for the collection of sputum. Smear microscopy by Ziehl–Neelsen (ZN) method was performed on all sputum specimens at the DMC as per RNTCP guidelines [4]. After smear microscopy, equal volume of CPC-NaCl was added carefully to the remaining portion of the sputum; the tightly capped containers were sealed with parafilm to prevent leakage during transit. Specimens along with the smear report were transported at ambient temperature to the National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL and OMD), Agra, Uttar Pradesh. The laboratory was accredited for culture and susceptibility testing.

Isolation and identification of M. tuberculosis

The specimens were processed by the method described earlier [5]. In brief, the specimens were centrifuged at 3000g for 15 min and after decanting the supernatant, the deposit was resuspended in 1–2 ml of sterile distilled water. A loopful of the suspension was inoculated onto two plain Lowenstein–Jensen's (LJ) medium slopes containing glycerol and one containing pyruvate [6]. The LJ slopes were incubated at 37 °C and were examined weekly for eight weeks. Mycobacterial cultures were identified based on the growth rate, pigment production and standard biochemical tests like niacin utilization, nitrate reduction, catalase activity and susceptibility to *p*-nitro benzoic acid [7].

Drug susceptibility study

Drug sensitivity test was performed by minimum inhibitory concentration (MIC) method for rifampicin (R), isoniazid (H), ethambutol (E) and pyrazinamide (Z) and resistance ratio (RR) for streptomycin (S) [6,8–11]. The anti-TB drugs were procured from Sigma Chemical Co., St. Louis, USA, Novartis India Pvt. Ltd., Mumbai for preparing the drug containing LJ media. In the case of pyrazinamide, the pH of the medium was adjusted to 5.5 using 1 N HCl. A standard bacterial suspension (4 mg/ml) was used to inoculate on LJ slants with a loop of 3 mm internal diameter. *M. tuberculosis* H37Rv was used as reference susceptible control in every batch of testing. MIC was determined by using standard criteria of counting the colony forming units and comparing with culture controls. MIC is defined as the lowest concentration of the drug inhibiting the growth of 20 or more colonies at drug concentrations (μg/ml) 64, 1, 4, 100 for R, H, E, Z and RR of 8 or more for S.

Results

Drug susceptibility test was performed for 102 *M. tuberculosis* isolates recovered from sputum specimens of individuals attending the DMC of Gulbarga. The specimens were collected after obtaining oral consent from study subjects with the help of DMC staff (Table 1). The mean age of the patients was 37.2 ± 13.18 year and the male to female sex ratio was almost 3:1. The drug susceptibility profile for new, treated and unknown treatment categories is given in Table 2. Overall, the drug susceptibility pattern showed 60.78% sensitivity to all the drugs tested and any type of resistance was observed in 39.21% of the isolates tested. Among any type of resistance, mono resistance was 25.49% and other pattern of resistance was 4.9%. Apart from these, multiple drug resistance (MDR) i.e., *M. tuberculosis* resistance to at least isoniazid and rifampicin was also recorded in this study and it was found to be 8.82% and was highest for previously treated cases (18.18%). On comparing the number of drugs to which patients were resistant to, 25.49% patients showed resistance to one drug and 7.84% of the patients were resistant to two

Table 1 General characteristics of participants enrolled in the study.

Characteristics	Total
Sex	
Male	76
Female	26
Age group	
< 30 years	36
31–60 years	59
> 60 years	7
Category	
New	62
Treated	22
Unknown	18