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ORIGINAL ARTICLE

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

Philip Raj Abraham [a,1], Prashant Upadhyay [b], Jaya Faujdar [b], Ravindranath Gangane [c], Subhashchandra M. Gaddad [a], Vishnu Datta Sharma [b], Channappa T. Shivannavar [a,*]

[a] *Department of Microbiology, Gulbarga University, Gulbarga 585 106, India*
[b] *Department of Microbiology and Molecular Biology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (NJIL and OMD), Agra 282 001, India*
[c] *Department of Microbiology, Mahadevappa Rampure Medical College, Gulbarga 585 105, 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.

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

* Corresponding author at: Department of Microbiology, Gulbarga University, Gulbarga 585 106, Karnataka, India. Tel.: +91 8472 263297 (O), +91 8472 248267 (R), +91 9481640497 (Cell).
E-mail address: ctshiv@gmail.com (C.T. Shivannavar).
[1] Present address: ICMR-Postdoctoral Research Associate, Laboratory of Molecular and Cell Biology, Center for DNA Fingerprinting and Diagnostics, Opp M.J. Market, Nampally, Hyderabad 500 001, India.
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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 3000*g* 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

Table 2 Drug susceptibility profile of *M. tuberculosis* in Gulbarga.

Drug susceptibility profile	New cases		Previously treated		Unknown treatment category		Total	
	No.	%	No.	%	No.	%	No.	%
Total No. of cases tested	62	100	22	100	18	100	102	100
Sensitive to all five drugs	38	61.29	11	50	13	72.22	62	60.78
Any resistance	24	38.70	11	50	5	27.77	40	39.21
Isoniazid (H)	10	16.12	7	31.81	4	22.22	21	20.6
Rifampicin (R)	7	11.29	5	22.72	3	16.66	15	14.70
Ethambutol (E)	10	16.12	4	18.18	2	11.11	16	15.68
Pyrazinamide (Z)	5	8.06	3	13.63	1	5.55	9	8.82
Streptomycin (S)	–	–	2	9.09	–	–	2	1.96
Mono resistance	17	27.41	7	31.81	2	11.11	26	25.49
Isoniazid (H)	5	8.06	3	13.63	2	11.11	10	9.80
Rifampicin (R)	2	3.22	1	4.54	–	–	3	2.94
Ethambutol (E)	7	11.29	1	4.54	–	–	8	7.84
Pyrazinamide (Z)	3	4.83	2	9.09	–	–	5	4.90
Multi drug resistance	3	4.83	4	18.18	2	11.11	9	8.82
H + R	3	4.83	1	4.54	–	–	4	3.92
H + R + E	–	–	–	–	2	11.11	2	1.96
H + R + E + S	–	–	2	9.09	–	–	2	1.96
H + R + E + Z	–	–	1	4.54	–	–	1	0.98
Other patterns	4	6.45	–	–	1	5.55	5	4.90
H + E	1	1.61	–	–	–	–	1	0.98
H + S	–	–	–	–	–	–	–	–
H + Z	1	1.61	–	–	–	–	1	0.98
R + E	1	1.61	–	–	–	–	1	0.98
R + E + Z	1	1.61	–	–	–	–	1	0.98
R + Z	–	–	–	–	1	5.55	1	0.98

Note: The combination of drugs which is not mentioned in this table had 0% resistance.

drugs while, 2.94% of patients were resistant to three and four drugs. However, none of the isolates was totally resistant to all the five drugs tested in this study (Fig. 1).

Discussion

The level of drug resistance is known to provide an epidemiological indicator to assess the extent of resistant bacterial transmission in the community as well as success of the TB control program. In the present study we have made an attempt to know the drug susceptibility profile of *M. tuberculosis* among different categories of TB patients in Gulbarga.

The drug susceptibility profiles of *M. tuberculosis* among the new cases showed susceptibility to S for all the isolates tested where as it was observed to be 13.7% in Hooghly [12] and almost 15% in Wardha district, which was reported to be the highest among the studies conducted in India [13]. The pattern of resistance to H was 16.12% while the resistance reported from the Kolar district of the same state was almost double (32.9%) [14]. The resistance pattern for R and E was observed to be higher in this study (11.29% and 16.12% respectively) as compared to similar studies conducted in India [15]. Hence, care should be taken to prevent the development of drug resistance when handling new cases of TB during the course of treatment. MDR was also observed in new cases and it was 4.83%. However; highest percentage of MDR (13.2%) in India was reported from Lucknow during the study conducted in between 2000 and 2002 with a sample size of 318 [16].

Among the previously treated cases, it is interesting to note that other pattern of resistance was not observed. The isolates

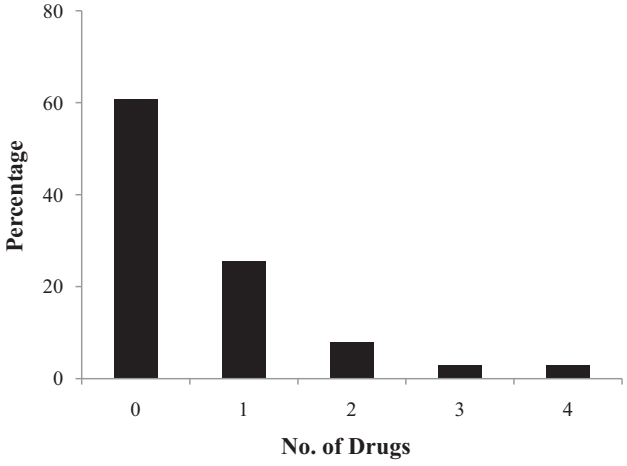

Figure 1 Frequency of drug resistance in *M. tuberculosis* isolates of Gulbarga. Among the five anti-TB drugs tested, the number of drugs to which the patients were resistant to was calculated for 102 *M. tuberculosis* isolates. It was found that 60.28% of the isolates showed susceptibility to all the drugs and remaining isolates showed resistance to one to four drugs; none of the isolates was found to be resistant to all the five drugs tested.

exhibited either mono or multiple drug resistance patterns. On the other hand, resistance observed was higher for H and R (31.81% and 22.72% respectively) and resulted in prevalence of MDR. Regarding prevalence rate of MDR, the literature from India and other developing Asian countries shows a wide

variation. According to the WHO fourth Global Project [17], prevalence rate of MDR-TB among previously treated cases in India is 17. 2%; while, we observed 18.18% which is comparable to previous Indian studies (14–49%) [18]. It is also observed that among the MDR, half of the isolates (9%) showed resistance to all four antitubercular drugs (H + R + E + S) tested in this study which indicates the failure of ATT. Hence, preventive measures such as motivating the patients for strict adherence to the prescribed drug therapy and also avoiding the shifting of treatment are essential.

The treatment category of 18 patients was unknown as the specimens from these patients were collected from private diagnostic centers and hence, they were separately categorized. However, the drug resistance pattern of this category shows higher resistance to H and R and also MDR was observed in this category which resembles the susceptibility profile of previously treated cases suggesting that the patients belonging to this category might have received ATT and discontinued the therapy. Hence, treatment history of the patients should be properly collected before the initiation of ATT for appropriate treatment and also to avoid misuse of the drugs.

When considering the overall specimens tested in this study, the combined resistance for any drug and multiple drugs was observed to be higher than that reported in Raichur, a neighboring district of Gulbarga where drug resistance survey was conducted in 1999 [19]. Although, RNTCP was implemented in Gulbarga during 2000, poor personal as well as public health awareness may increase the incidence of most infectious diseases including tuberculosis. New smear positive case detection rate and treatment success rate shows this district is far from reaching the global target set by WHO (new smear positive case detection rate $\geqslant$70% and treatment success rate of $\geqslant$85%). The emergence of drug resistance in *M. tuberculosis* isolates of this area may be due to any of the factors described by Paramasivan et al. [20]. Hence, considering the population of Gulbarga and also migration of people to other states, drug resistance survey in this district will be helpful in effective management of TB. This study also demonstrates that the specimens from this area can be transported to any Indian reference laboratories for undertaking the drug resistance surveillance in this part of the country.

The major limitation of this study is the number of samples included in each category which is due to the insufficient data collection. The isolates grouped under UTC showed the pattern of resistance which is similar to treated cases. Another limitation of this study is the methodology followed for DST. Generally for drug resistance surveys the proportion method is followed. However, this is a preliminary study and our intention was to study the drug susceptibility profile of *M. tuberculosis* to first line anti TB regimens. This information will serve as useful baseline data in future studies.

Source of support

None.

Conflict of interest

None.

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