

## Situation of drug resistant tuberculosis in *Saharia* tribe of central India

J. Bhat, V.G. Rao, R. Yadav, M. Muniyandi, R. Sharma, C. Karfarma & C. Luke

*National Institute for Research in Tribal Health (ICMR), Jabalpur, India*

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**Background & objectives:** The information on multidrug resistant tuberculosis (MDR-TB) situation amongst *Saharia*, one of the Particularly Vulnerable Tribal Groups (PVTGs) in Madhya Pradesh, India, is not available. Hence, this study was undertaken to find the situation of MDR-TB amongst *Saharia* PVTG in two districts of Madhya Pradesh.

**Methods:** Community based cross-sectional TB prevalence surveys were conducted among *Saharia* PVTG in Gwalior and Shivpuri districts of Madhya Pradesh. Chest symptomatics were identified from the individual registered for the study. Two sputum samples were collected from each of the eligible individuals, transported to the laboratory, and were examined by Ziehl-Neelsen (Z-N) smear microscopy and solid medium culture methods. Drug susceptibility testing of the isolates was done by indirect proportion method on solid medium.

**Results:** MDR rate was 2.2 per cent of new cases and 8.2 per cent among the previously treated cases of TB in Gwalior while it was two per cent among the previously treated cases in Shivpuri district.

**Interpretation & conclusions:** Though the prevalence of tuberculosis in these districts was alarmingly high, the MDR rates were more or less similar to national average. However, the findings of this study highlight the need for active intervention so that the MDR-TB is kept under control.

**Key words** Drug susceptibility - MDR - *Saharia* - tribal - tuberculosis

Multidrug resistant tuberculosis (MDR-TB) is a challenge to TB control programmes worldwide. Moreover, MDR patients can turn to extensively drug resistant TB (XDR-TB) which has much limited options for treatment<sup>1</sup>. Globally, 3.7 per cent (2.1-5.2%) of new cases and 20 per cent (13-26%) of previously treated cases are estimated to have MDR-TB. India accounts for an estimated 64,000 patients out of 3,10,000 cases of drug resistant TB estimated to have occurred amongst the notified cases of TB across the globe in a year<sup>1</sup>.

In the early studies undertaken during 1965-1967, resistance to isoniazid ranged from 11-20 per cent, to streptomycin 8-20 per cent and to both drugs 4-11 per cent<sup>2</sup>. Another study showed resistance to isoniazid 15-69 per cent, to streptomycin 12-63 per cent and to both drugs 5-58 per cent<sup>3</sup>. Further, the level of drug resistance was proportionate to the duration of previous treatment<sup>3</sup>. A subsequent study from Chennai, south India, carried out a decade later reported similar results to these two earlier surveys<sup>4</sup>. This indicated that the

prevalence of initial drug resistance has not changed<sup>4</sup>. The drug resistance survey (DRS) study conducted in 1999 in the two districts of Tamil Nadu reported the MDR in new cases as 2.8 per cent and in previously treated cases between 69-100 per cent<sup>5</sup>.

The drug resistance surveys conducted in the States of Gujarat and Maharashtra estimated the prevalence of MDR-TB to be about 3 per cent in new cases and 12-17 per cent in re-treatment cases, and also indicated that the prevalence of MDR-TB was not increasing in the country<sup>6</sup>. India is one of the highest MDR-TB burdened countries in the world with an estimated 99,000 incident MDR-TB cases<sup>7</sup>.

A few studies in the different pockets of the country have been carried out to know the prevalence of TB amongst the tribal population<sup>8-11</sup>. However, information about drug resistant tuberculosis among them is lacking. It has been reported that the *Saharia* - a Particularly Vulnerable Tribal Group (PVTG) in the State of Madhya Pradesh has an alarmingly high prevalence of tuberculosis<sup>12</sup>.

The present study was conducted in *Saharia* PVTG in the two districts of Gwalior and Shivpuri in Madhya Pradesh to know the prevalence of TB and to assess the situation of drug resistance, particularly MDR-TB among them.

### Material & Methods

*Study area*: Cross-sectional surveys were carried out among *Saharia* PVTG in Gwalior and Shivpuri districts of Madhya Pradesh during December 2012 to August 2013. These districts were selected considering the operational feasibility, rapport with community and willingness to support by district authorities. The terrain was difficult, with most of the villages located in remote jungle areas having poor connectivity by road, and remained cut-off from the surrounding areas during the rainy season

*Sampling and sputum collection*: Both male and female individuals aged  $\geq 15$  yr were included. The required sample size for estimation of prevalence was 9200 from each district. Samples were selected by arranging all the villages in descending order of the tribal population and those villages having more than 80 per cent of tribal population were selected. A village was considered as a sampling unit and required number of villages was selected using probability proportional

to the size of each block in the district to cover the estimated sample size. Sputum was collected from all chest symptomatic individuals. (individuals who had symptoms of TB like cough, fever and chest pain), identified by using a questionnaire. This was done by house to house survey.

*Processing of sputum specimens*: In the field, samples were stored in refrigerators and then brought to the National Institute for Research in Tribal Health (NIRTH), Jabalpur, laboratory in a cold box on every second day. The specimens were processed for culture by modified Petroff's method<sup>13</sup>, inoculated on Lowenstein-Jensen (L-J) medium and were examined for growth of *Mycobacterium tuberculosis* once a week for up to eight weeks. Niacin test and growth on para-nitrobenzoic acid was done to confirm the speciation, *i.e.* growth of *M. tuberculosis*. Smears were made from the processed specimens and were stained by Ziehl-Neelsen (Z-N) method and examined by trained technical staff for acid-fast bacilli (AFB)<sup>13</sup>. In addition, all positive smears and 10 per cent random sample from negatives were read once again for quality check. This laboratory is an Intermediate Reference Laboratory (IRL) for Central Tuberculosis Division, Government of India, and participates in External Quality Assurance Programme. Internal quality control procedures were also followed, each batch of drug sensitivity testing (DST) for all drugs tested was accompanied by an H<sub>37</sub>Rv strain (obtained from National Institute for Research in Tuberculosis, Chennai) which was tested along with one strain with known resistance to each drug.

Isolates were subjected to drug susceptibility testing by indirect proportion method on solid L-J medium as per standard methodology<sup>13</sup>. The final concentrations used for drugs were isoniazid 0.2  $\mu\text{g/ml}$ , streptomycin 4  $\mu\text{g/ml}$ , (dihydrostreptomycin sulphate, at a concentration corresponding to 4 mg/ml base), rifampicin 40  $\mu\text{g/ml}$  and ethambutol 2  $\mu\text{g/ml}$ . The drugs were procured from Sigma Aldrich, USA. Briefly, the *M. tuberculosis* isolate was suspended in distilled water and diluted in doubling dilutions. The second and fourth dilutions were used for inoculation on drug containing and drug free medium. The bottles were kept for incubation at 37°C and readings were taken on 28<sup>th</sup> and 42<sup>nd</sup> days. Any isolate with one per cent (the critical proportion) of bacilli resistant to any of the four drugs (rifampicin, isoniazid, ethambutol,

**Table I.** Smear and culture results of the specimens

		Culture +ve	Culture -ve	Culture contaminated
Gwalior	Smear +ve	212	85	0
	Smear -ve	51	722	1
Shivpuri	Smear +ve	131	62	1
	Smear -ve	100	1136	0

and streptomycin) was classified as resistant to that drug<sup>13</sup>. MDR isolates were further processed for DST for ofloxacin (2µg/ml) and kanamycin (30µg/ml).

The detected cases of TB and MDR-TB were referred to the District TB officer for management. The study was approved by Ethics Committee of the NIRTH, Jabalpur.

### Results

Overall 19,409 individuals were screened and sputum was collected from 2,501 symptomatic individuals. In Shivpuri, 9756 individuals were screened and sputum was collected from 1430 individuals while in Gwalior 9653 individuals were screened and sputum was collected from 1071 individuals. In Gwalior district, 263 of the 1071 individuals were culture positive for *M.tuberculosis* while in Shivpuri district 231 of 1430 individuals were positive by culture. The contamination rate for both the areas was 0.3 per cent (Table I). Mycobacteria other than tuberculosis (MOTT) were detected in one per cent of the isolates in Shivpuri while it was 1.8 per cent among Gwalior isolates. Prevalence of TB in Gwalior was 3294 per 1,00,000 while it was 3003 per 1,00,000 in Shivpuri district.

In Gwalior district, 65 per cent of the isolates were sensitive to all the first line drugs while 82 per cent of isolates from Shivpuri were sensitive to all the drugs (Table II). Mono drug resistance was the most common form of resistance. In both the areas, highest resistance was seen for streptomycin followed by isoniazid. Multidrug resistance was more in Gwalior (16 isolates) than Shivpuri (3 isolates). In Gwalior, five MDR isolates were from new cases while remaining 11 were from previously treated cases. Four of these MDR isolates were resistant to all the four drugs. The MDR rate in new cases was 2.2 per cent while it was 8.2 per cent among previously treated cases. All the three MDR isolates from Shivpuri were from previously treated cases making the MDR rate 2 per cent. Of the

19 MDR cases, 15 (79%) were resistant to ofloxacin. None of the isolates was resistant to kanamycin.

### Discussion

Drug resistance in TB bacilli is a big hindrance for TB control programmes all over the world. In spite of this, data on drug resistance are very limited. This study was part of prevalence surveys conducted amongst *Saharia* tribe in Gwalior and Shivpuri districts of Madhya Pradesh. The prevalence of TB was over 3000 per 1,00,000 in both these areas which is alarmingly high. The MDR rate amongst this population was more or less similar to national average of 3 per cent in new cases and 12-17 per cent in previously treated cases<sup>6</sup>. Mono drug resistance was highest for streptomycin followed by isoniazid. Similar findings have been reported earlier<sup>14</sup>. In a study from Gujarat resistance to streptomycin was highest in new cases while in previously treated cases resistance was higher for isoniazid<sup>6</sup>. In other studies also mono drug resistance

**Table II.** Results of DST of the isolates

Drug susceptibility pattern	Gwalior (N=254)	Shivpuri (N=221)
Sensitive to all drugs	166	181
Mono drug resistance		
Isoniazid (H)	13	11
Streptomycin (S)	36	18
Rifampicin (R)	2	2
Ethambutol (E)	2	1
Two drug resistance		
S+H	14	4
H+E	5	1
Three drug resistance		
S+H+E	0	0
S+H+R	7	2
H+R+E	3	0
MDR (H+R)	16	3
Resistance to all four drugs	4	0

was highest for isoniazid<sup>15,16</sup>. The high streptomycin resistance in the present study may be because of usage of streptomycin in the area for treatment of other infections.

According to WHO<sup>7</sup>, more number of MDR cases were detected in 2012 as compared to earlier year and India was one of the three countries which have shown largest increase in MDR-TB detected cases. However, worldwide and in most countries with high burden of MDR-TB, less than one third of the TB patients estimated to have MDR-TB were actually detected in 2012<sup>7</sup>. The situation becomes more complicated in tribal population because of factors like difficult terrain, fewer resources to reach healthcare facility, poverty, ignorance and traditional beliefs, *etc.* Special efforts need to be made to reach such marginalized population to counter this situation.

The major limitation of this study was that the sample size estimation was not done for drug resistance but was calculated for prevalence of tuberculosis.

Though the prevalence of TB was found to be high amongst the *Saharia* tribe, the proportion of drug resistant cases was similar to that of other population. Active intervention in the form of active case detection and treatment compliance by patients are needed to interrupt the transmission and to keep a check on MDR rate in the community.

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