



Status of multidrug resistant tuberculosis (MDR-TB) among the Sahariya tribe of North Central India

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KEYWORDS

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Summary

Background: The incidence/prevalence of tuberculosis (TB) is reported to be high in the Sahariya tribe of North Central India. The outbreaks of different drug-resistant isolates of *Mycobacterium tuberculosis* emphasized the need for continuous monitoring of resistance to anti-tuberculosis drugs. This study aimed to assess the profile of multidrug resistant TB among the Sahariya tribe and their non-tribal neighbors for first line drugs through field-based investigations.

Methodology: A total of 274 sputum positive pulmonary TB individuals were enrolled and studied for their drug susceptibility profile by the proportion method.

Results: A total of 21 cases from Sahariya and 6 from non-tribes were identified with MDR-TB. Thus Sahariya tribe showed a 1.95-fold increased risk of developing

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drug resistance than non-tribes. Significant differences were observed for developing drug sensitivity between Sahariya males and females when analyzed for resistance developed to any drug and overall drug resistance vs. sensitive isolates, respectively. A 4.46-fold risk was found for MDR-TB among the smokers of Sahariya tribe, whereas, the non-tribes did not show any significant association.

Conclusion: The drug susceptibility profile developed in the present study indicates that drug-resistant tuberculosis is emerging as a serious public health concern in Sahariya tribe. Urgent and effective control measures and better management policies are needed for the prevention of MDR-TB in the tribe.

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Introduction

Globally, tuberculosis (TB), caused by *Mycobacterium tuberculosis* remains a major cause of death. Each year, approximately 8.6–9.4 million people develop TB (incident cases) and approximately 1.5 million deaths occur due to TB among HIV negative individuals, whereas, 0.4 million deaths occur from HIV associated TB throughout the world. In 2013, the approximate proportion of TB cases from Asia was 56%, while, it was only 29% from Africa. India is at the top among six high burden countries with maximum number of incident cases (2.0–2.3 million) [1]. In Sahariya tribe, the overall prevalence of tuberculosis is 1518/100,000 people [2]. Multidrug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) tuberculosis are emerging as a greater threat and causing higher mortality rates. The MDR-TB is defined as resistance against the two most potent anti-tuberculosis drugs, namely, isoniazid (INH) and rifampicin (RIF). XDR-TB is attributed to the clinical isolate that is multidrug resistant and has also developed resistance to fluoroquinolones and to one of the injectable drugs, such as amikacin, kanamycin or capreomycin. Polydrug resistance refers to *M. tuberculosis* isolates that are resistant to more than one of the first line drugs, other than isoniazid and rifampicin. Monodrug resistance specifies the isolates that are resistant to at least one anti-tuberculosis drug [3]. Detection of drug-resistant tuberculosis is of major importance to determine the ill effects of these strains on the health of affected individuals [4]. Several studies have reported an increase in the number of drug-resistant TB cases in India [4–7]. In this study, our aim was to investigate the status of multidrug resistant tuberculosis in a primitive

tribe of Central India, the Sahariya tribe, which is reported to have a very high incidence and prevalence of pulmonary tuberculosis [2,8–10]. This can be attributed to various social determinants of TB, such as mal-nutrition, overcrowding, indoor air pollution, young age, and clinical risk factors, like diabetes mellitus, smoking, alcohol consumption, immunosuppressive conditions, and socio-economic as well as behavioral factors, all play an important role [11]. Development of drug resistance in *M. tuberculosis* isolates may worsen the TB scenario and its management. Therefore, this study aimed to determine the situation of MDR-TB in the Sahariya tribe and their non-tribal neighbors in order to develop future strategy for the effective control of TB.

Materials and methods

Study population and study design

A total of 274 individuals, 185 from the Sahariya tribe and 89 from the non-tribal population, were screened through field-based investigations from the Gwalior and Sheopur districts of Madhya Pradesh. The participants were first screened for symptoms. Sputum sampling was conducted under the supervision of a clinician. The individuals who had a confirmed diagnosis of TB, based on Ziehl-Neelsen (ZN) staining, were recruited in the study after obtaining their informed consent. The sampling methods and protocols employed in the study were approved by the Institutional Ethics Committee, Jiwaji University, Gwalior. The power of the study was calculated to be 90–95%, using a power and sample size calculator

(www.stat.ubc.ca/~rollin/stats/ssize/b2.html).

The calculated sample size was 90 sputum positive samples for both the Sahariya tribe and non-tribes. In Sahariya, the incidence and prevalence of TB is very high, hence, we collected as many samples as possible to define the etiology of drug resistance in this tribe. The non-tribe samples were collected as controls. All the patients, either newly diagnosed or previously diagnosed, were enrolled under DOTS (Directly Observed Treatment Short Course) chemotherapy program as per RNTCP (Revised National Tuberculosis Control Programme) guidelines.

Sample collection

The sputum samples from the individuals confirmed for TB were collected in sterile sputum vials (Hi-Media, Mumbai), and transported to the laboratory, where they were processed for mycobacterial culture using modified Petroff's method and incubated on Lowenstein-Jensen media at 37°C for approximately 8 weeks [12]. The *M. tuberculosis* in the culture was identified by biochemical assays [13].

Drug susceptibility testing

The drug sensitivity tests for the first line drugs were performed using the standard proportion method [14]. The first line drugs, viz., isoniazid, rifampicin, ethambutol and streptomycin, were incorporated in the Lowenstein-Jensen media at a final concentration of 0.2 µg/ml, 40 µg/ml, 2 µg/ml and 4 µg/ml, respectively, and then incubated at 37°C. A two-third loopful of *M. tuberculosis*, representing an approximately 4 mg of moist weight of bacterial mass was scrapped, and transferred to a screw cap tube containing 4–6 glass beads and 400 µl of distilled water. The colonies were vortexed for 30s to produce a uniform suspension, followed by addition of sterile distilled water and then left for approximately 15 min to permit the coarse particles to settle down. The opacity of the suspension was matched to obtain a concentration of 1 mg/ml of *tubercle bacilli* by matching it with 1 McFarland standard. From this standard inoculum, 4 serial dilutions of inoculum at concentrations of 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴, designated as S1, S2, S3 and S4, respectively, were made. The serial dilutions were inoculated in the drug-containing and drug-free media and PNB (Para Nitro Benzoic Acid) slope. The standard laboratory strain H37Rv was utilized as the control. The growth was read after 28 and 42 days and recorded as 3+ (confluent growth), 2+ (>100 colonies), actual number of colonies (1–100 colonies) and negative (if there

was no growth). The highest counts obtained on drug-containing and drug-free media were used to calculate the proportion of resistant bacilli. Strains with 1% bacilli resistant to two drugs, namely, isoniazid and rifampicin, were graded as multidrug resistant (MDR) [14].

Statistical analysis

The resistance ratio was calculated for each drug in all the isolates [14]. The percentages of monodrug resistance, multidrug resistance and polydrug resistance were calculated for Sahariya and non-tribal populations. A Chi-square test with Yate's correction was applied to identify any association between the drug-sensitive isolates to multidrug resistance, resistance developed to any drug, overall developed drug resistance and smokers/non-smokers vs. MDR-TB/non-MDR-TB in both the population groups using Graph Pad Prism 6 software (www.graphpad.com). A *P*-value of <0.05 was considered statistically significant.

Results

The mean age ± standard deviation (SD) between Sahariya (38.26 ± 13.67) and the neighboring non-tribal population (39.74 ± 15.49) was non-significant (*P*=0.4226). No statistically significant difference was observed in age between males (*N*=140, 39.2 ± 13.93) and females (*N*=45, 35.3 ± 12.53) of Sahariya tribe (*P*=0.0961). However, among the non-tribal population, the age was found to differ significantly (*P*=0.0103) among males (*N*=72, 41.7 ± 14.69) and females (*N*=17, 31.1 ± 16.29).

A comparison between MDR-TB and drug sensitivity did not reveal any significant association among different groups of Sahariya and the non-tribal population. Further comparison between resistance to any drug and drug sensitivity among different groups of Sahariya tribe and the non-tribal population showed significant difference between Sahariya males and females (*P*=0.0074, OR=0.3368, 95% CI=0.1579–0.7181) (Table 1).

When the overall drug resistance was compared to the sensitive isolates for both the populations, Sahariya tribe showed a 1.95-fold greater risk to develop drug resistance than their non-tribal neighbors (*P*=0.0315, OR=1.959, 95% CI=1.095–3.503). Further stratification showed significant association of developing drug sensitivity among Sahariya males compared to Sahariya females (*P*=0.0102, OR=0.3879, 95% CI=0.1951–0.7711). However,

Table 1 Chi-square analysis for the distribution of multidrug resistant isolates, resistance that was developed to any drug and overall drug resistance vs. sensitive isolates in the Sahariya tribe and the non-tribal population.

MDR-TB vs. sensitive isolates					
Groups	MDR-TB N = 27 (%)	Sensitive N = 187 (%)	OR ^a	95% CI ^b	P-value
Sahariya tribe	21 (15)	118 (85)	2.047	0.7876–5.318	0.2011
Non-tribal	6 (8)	69 (92)			
Sahariya male	15 (13)	97 (87)	0.5412	0.1879–1.559	0.3950
Sahariya female	6 (22)	21 (78)			
Non-tribe male	5 (8)	56 (92)	1.161	0.1247–10.80	0.6781
Non-tribe female	1 (7)	13 (93)			
Sahariya male	15 (13)	97 (87)	1.732	0.5974–5.022	0.4399
Non-tribe male	5 (8)	56 (92)			
Sahariya female	6 (22)	21 (78)	3.714	0.4003–34.46	0.4359
Non-tribe female	1 (7)	13 (93)			
Resistance developed to any drug vs. sensitive isolates					
Groups	Resistance to any drug N = 60 (%)	Sensitive N = 187 (%)	OR ^a	95% CI ^b	P-value
Sahariya tribe	46 (28)	118 (72)	1.921	0.9850–3.748	0.0753
Non-tribal	14 (17)	69 (83)			
Sahariya male	28 (22)	97 (78)	0.3368	0.1579–0.7181	0.0074*
Sahariya female	18 (46)	21 (54)			
Non-tribe male	11 (16)	56 (84)	0.8512	0.2073–3.495	0.8826
Non-tribe female	3 (19)	13 (81)			
Sahariya male	28 (22)	97 (28)	1.470	0.6795–3.178	0.4273
Non-tribe male	11 (16)	56 (84)			
Sahariya female	18 (46)	21 (54)	3.714	0.9116–15.13	0.1109
Non-tribe female	3 (19)	13 (81)			
Overall drug resistance vs. sensitive isolates					
Groups	Overall drug resistance N = 87 (%)	Sensitive N = 187 (%)	OR ^a	95% CI ^b	P-value
Sahariya tribe	67 (36)	118 (64)	1.959	1.095–3.503	0.0315*
Non-tribal	20 (22)	69 (78)			
Sahariya male	43 (31)	97 (69)	0.3879	0.1951–0.7711	0.0102*
Sahariya female	24 (53)	21 (47)			
Non-tribe male	16 (22)	56 (78)	0.9286	0.2657–3.245	0.8361
Non-tribe female	4 (24)	13 (76)			
Sahariya male	43 (31)	97 (69)	1.552	0.8006–3.007	0.2523
Non-tribe male	16 (22)	56 (78)			
Sahariya female	24 (53)	21 (47)	3.714	1.049–13.16	0.0691
Non-tribe female	4 (24)	13 (76)			

Bold values highlights the significant differences.

* $P < 0.05$ is considered statistically significant.

^a OR, odds ratio.

^b CI, confidence interval.

no such association was observed in either the non-tribal population ($P=0.8361$, $OR=0.9286$, 95% $CI=0.2657-3.245$) or between males of Sahariya tribe and non-tribes ($P=0.2523$, $OR=1.552$, 95%

$CI=0.8006-3.007$) or between the females of both the groups ($P=0.0691$, $OR=3.714$, 95% $CI=1.049-13.16$) (Table 1). A 4.46-fold risk was observed for smokers of Sahariya tribe

Table 2 Chi-square analysis for smokers and non-smokers vs. MDR and non MDR-TB in the Sahariya tribe and the non-tribal population.

Groups	MDR-TB N = 27 (%)	Non-MDR TB N = 247 (%)	OR ^a	95% CI ^b	P-value
<i>Sahariya tribe</i>					
Smokers	17 (18)	80 (82)	4.463	1.439–13.84	0.0109*
Non-Smokers	4 (5)	84 (95)			
<i>Non-tribe</i>					
Smokers	1 (9)	10 (91)	1.460	0.1543–13.81	0.7563
Non-Smokers	5 (6)	73 (94)			

Bold values highlights the significant differences.

* $P < 0.05$ is considered statistically significant.

^a OR, odds ratio.

^b CI, confidence interval.

to develop MDR-TB ($P=0.0109$, $OR=4.463$, $95\% CI=1.439-13.84$), whereas, no significant association could be observed for the non-tribes ($P=0.7563$, $OR=1.460$, $95\% CI=0.1543-13.81$) (Table 2).

The possibility of developing multidrug resistance in a particular age group among the Sahariya tribe and non-tribal population was also analyzed which did not reveal any significant association (Table 3).

When drug resistance to any one of the four drugs used in chemotherapy and multidrug resistance were compared among new smear positive (NSP) cases of Sahariya and the non-tribal patients, the number of individuals developing drug resistance to isoniazid alone or in combination with rifampicin were observed to be higher in Sahariya (INH = 14%, INH + RIF = 11%) than in the non-tribal population (INH = 7%, INH + RIF = 7%). The resistance developed to isoniazid alone in retreated and treatment after default (TAD) or defaulter patients, revealed a high percentage of isoniazid resistance in retreated and TAD patients of Sahariya (16% and 11%, respectively), but, in non-tribal population the only isoniazid alone resistance was found negligible, when compared (Table 4).

Fewer numbers of isolates, who developed resistance in different combinations of isoniazid in relapse and TAD cases, were observed in both the population groups. In Sahariya tribe, the maximum number of MDR-TB cases ($N=16$, 11%) was observed among new smear positive cases, which showed primary drug resistance. The number of cases among retreated and defaulter (TAD) categories were very few. However, a high percentage of individuals (cases) developing primary resistance was observed among patients of both, the Sahariya tribe and the non-tribal populations. However, the

number of MDR-TB for retreated and treatment after default cases in the non-tribal population was very few ($N=5$, 7%). The resistance to isoniazid was more commonly observed, followed by rifampicin, ethambutol and streptomycin. In the Sahariya tribe, resistance to isoniazid (H) and isoniazid plus rifampicin (HR) constituted 14% and 11%, respectively, whereas, for the non-tribal population, it was only 7% in both cases (Table 4).

Discussion

India has the highest MDR-TB burden with 99,000 estimated incident TB cases. This indicates that the prevalence of MDR-TB is slowly growing up in the country [15]. This is the first study to reveal the MDR-TB situation in a Central Indian tribe, Sahariya and their non-tribal neighbors. The prevalence of TB is already reported to be very high (1518/100,000 people) in Sahariya [2]. The crude prevalence of TB is also observed to be high in the tribes (29.9%) as compared to the non-tribal population (21.4%) [8]. The present study indicates a increased rate of MDR-TB in the Sahariya tribe than in the neighboring non-tribal population, although statistically no significant association could be observed.

A comparison between resistance to any drug and sensitive isolates revealed that Sahariya males are less prone to any drug resistance (or more sensitive to any drug) than females. The Sahariya tribe had a 1.9-fold higher risk of developing overall drug resistance than non-tribes. Further, gender based stratification also showed that Sahariya females were at a higher risk of overall drug resistance than males (or vice-a-versa, i.e., overall, males are

Table 3 Age group vs. pattern of multidrug resistance among Sahariya tribe and their non-tribal neighbors.

Characteristics	Sahariya tribe	Non-tribe	OR ^a	95% CI ^b	P-value
<i>Age (years)</i>					
15–24	6	1	2.000	0.1913–20.91	0.9532
25–34	7	1	2.500	0.2429–25.73	0.7782
35–44	3	1	0.8333	0.07041–9.864	0.6123
45–54	4	1	1.176	0.1059–13.07	0.6431
55–64	0	2	0.04186	0.001702–1.030	0.0621
>64	1	0	0.9512	0.03436–26.33	0.4959

^a OR, odds ratio.

^b CI, confidence interval.

less prone to drug resistance/or more sensitive to drugs).

A high percentage of women developing drug resistance among new smear positive cases was observed for Sahariya tribe. Females were also found to have a high percentage of MDR-TB, resistance developed to any drug and overall drug resistance when compared in both the population groups. Similar studies from Georgia also revealed that females were at 1.36 [16] to 1.6 [17] fold higher risk of developing MDR-TB. On the contrary, Faustini et al. reported tuberculosis and MDR-TB to be more common among men [18]. In Central Nepal also, males were observed to have more MDR-TB (65.5%) more than females (34.5%) [19]. However, it is suggested that in males, the association between previous treatment and MDR-TB may be modified [17]. The reason for the association of female gender with MDR-TB remain, unclear. A likely possibility is that women exert an important influence on health habits in the family because of their social role as nurturer and care takers of the young, old and sick members of the family. Attending men and others with MDR-TB, carrying the social stigma of not leaving the home, poor health care facilities in the rural areas and low ventilated houses increase the risk of transmission of drug resistant bacteria in them. A 4.4-fold increase in risk was observed in smokers and tobacco chewers of Sahariya tribe to develop MDR-TB. However, no significant association of smoking was observed among the non-tribal patients. This observation is in accordance with an earlier report that smoking and alcohol consumption increases the risk of TB in Sahariya tribe [20]. Smoking and alcohol abuse at working places and more significantly, at an early age, are also predisposing factors for high prevalence of TB in Sahariya men [8,20,21].

Further, the prevalence of TB (0.382) was reported higher in Sahariya males (20–40 years) than in Sahariya females (0.142) [21] as well as in non-tribal males [8]. One of the most likely reasons for the increased incidence of TB and

MDR-TB in males of Sahariya could be because they start smoking at a very early age as compared to non-tribes. While, working as laborers they share the same environment and smoking sticks with infected individuals. The high rate of MDR-TB in this community may also be due to several reasons, such as their remote habitation, often difficult to reach to a health care facility, or poor or total absence of required health care facilities, lack of clarity of medical instructions, poor life style, malnutrition, drug mal-absorption, non-adherence to recommended treatment regimen or the treatments, followed by interference of private practitioners in the rural areas who usually do not follow the standard regimen of DOTS or the duration of chemotherapy as per the RNTCP criteria [22,23]. Thus, all or most of the above factors, including their social behavior, when compared with their non-tribal neighbors, could be significantly contributing to such a high rate of MDR-TB in Sahariya.

An earlier study on Sahariya has reported statistically significant increase in the prevalence of TB with age and in males ($P < 0.001$) [2]. It was also suggested that presumptive MDR-TB patients below 26 years of age have a 2.9-fold higher risk of developing MDR-TB than TB patients in the age group more than 26 years old [24]. However, the present study did not find any significant association between Sahariya tribe and the non-tribal population when stratified on the basis of age groups.

In Sahariya tribe, the East African Indian (EAI3_IND/ST11) strain is found more prevalent (MS communicated), which has been described as an ancient strain [25]. The EAI3_IND is less prevalent in North India (32%) but, is more (52%) prevalent in Central India and is significantly high in South India (80%) [26–29]. In non-tribes, however the CAS1_Delhi is the predominant strain (MS communicated). Earlier studies also reported this strain to be most common in Northern India [28–31]. The Beijing strain was observed strongly associated with drug resistance [32,33]. However, in our study; it was completely absent in the Sahariya population;

Table 4 Prevalence of drug resistance among *M. tb* isolates from previously treated cases according to the drug and the type of case from the Sahariya tribal and non-tribal population.

Drug resistance	Sahariya tribe (N = 185) N (%)		Non-tribal (N = 89) N (%)	
	NSP (N = 140)	Retreated (N = 18) (N = 27)	NSP (N = 72)	Retreated (N = 7) (N = 10)
Resistance to any drug	31 (22)	4 (22)	10 (14)	1 (14)
MDR Isolates	16 (11)	2 (11)	5 (7)	1 (14)
Resistance to only one drug				
R	6 (4)	0	2 (3)	1 (14)
H	20 (14)	3 (16)	5 (7)	0
Resistance in different combinations of drugs				
H+R	16 (11)	2 (11)	5 (7)	1 (14)
H+S	2 (1)	1 (6)	1 (1)	1 (14)
H+E	2 (1)	0	1 (1)	0
H+R+S	0	1 (6)	0	0
H+R+E	0	0	0	0
H+R+S+E	0	0	0	0
Sensitive	87 (62)	12 (67)	56 (78)	4 (57)
		19 (70)	118 (64)	9 (90)
			69 (78)	

Abbreviations: E, ethambutol; H, isoniazid; R, rifampicin; S, streptomycin.

whereas, in non-tribes, only 1 Beijing and 2 Beijing like strains were observed. This indicates that EAI3_IND and CAS1_Delhi strains may be developing drug resistance more slowly and may pose a major threat to Sahariya in future.

In Sahariya, 11% of MDR-TB cases were observed in the NSP, retreated and TAD categories of patients. The percentage of drug resistance in NSP cases was higher than the national average, *i.e.*, 2.1% of MDR-TB cases [34]. In the non-tribal population, 7% of MDR-TB cases were found in NSP, whereas, 14% were retreated and TAD cases. Our finding on a high rate of MDR-TB cases for new smear positive cases in Sahariya corresponds well with a study from Kashmir, where primary and secondary drug resistance was observed at 36.5% and 63.4%, respectively [35]. The rates of MDR-TB have been reported to range from 4% (Dashoguz, Turkmenistan) to 22.7% (Samara, Russia), with primary drug resistance [36,37] and from 18% (in Dashoguz, Turkmenistan) to 54–60% (in Republic of Lithuania and Arkhangelsk, Russia) for acquired drug resistance [36,38,39]. An increase in multidrug resistant cases has also been reported from Estonia (12.2%), Tomsk, the Russian Federation (13.7%) and Kazakhstan (14.2%) [40]. In contrast, in a recent study on Sahariya in the Gwalior and Shivpuri districts, Bhat and colleagues observed 3% of NSP cases with MDR-TB and 12–17% in retreated cases [41]. The prevalence of multidrug resistant TB infection has been observed to vary widely across different geographical locations with a high rate of infection reported from Nepal (48%), Gujarat, India (34%), New York, USA (30%) and South Korea (15%) [42]. A study from Northern India revealed continuous and increasing trend of MDR-TB infection, *i.e.*, 36.4%, 36.7%, 39.1% and 40.8% in the years 2007, 2008, 2009 and 2010, respectively [43]. In another study from Northern India, on the prevalence of MDR-TB infection, out of 196 cases, 40 patients (20.4%) had MDR-TB [44]. Recently, Yimer and colleagues from Amara region of Ethiopia, reported a 16.9% overall frequency of resistance to any drug, while the proportion of monodrug, polydrug and MDR-TB were 12.6%, 3.9% and 0.9%, respectively [45].

TB has remained a major public health problem in Sahariya tribe since the time of first report published in 1996 on TB prevalence in this tribe [46]. The living conditions of Sahariya tribe are adverse. They live in forests, remote areas and under improper sanitary and unhygienic conditions, with lack of safe drinking water and more importantly, at a long distance from necessary health care facilities. Forced migration in search of work and non-compliance of patients to the chemotherapy provided under RNTCP programme could be the other

strong reasons for the increased rate of MDR-TB in them. The present study can be used as baseline data for MDR-TB among Sahariya tribe and the non-tribal population and focuses the need of active interference to control the transmission of MDR-TB cases, especially in rural and remote areas.

Our study has certain limitations too. It is a field-based investigation; the tribal people sometimes do not reveal the exact history of the disease or if they had taken DOTS earlier or not. In addition, they often follow treatment procedures from private practitioners who do not adhere to the standard treatment regimen of DOTS. Another limitation is that the HIV status was not examined in the study population.

Conclusion

In conclusion, the present investigation indicated that drug-resistant tuberculosis is emerging as a serious public health concern in the Sahariya tribe. An adequate monitoring of the treatment regimens for MDR-TB in the tuberculosis control program is needed among tribal communities, such as Sahariya, particularly in new smear positive cases (before it aggravates further). The present observations may be considered as alarm for a more serious condition that may occur in future. An urgent and effective strategy needs to be designed for the prevention of MDR-TB, which is essential to check the increasing trend of TB incidence/prevalence in this tribal population. Further, research is also required to determine the transmission dynamics of drug-resistant strains in this tribe compared to its non-tribal neighbors.

Author's contribution

Ravi Prakash: *M. tb* culture, drug sensitivity tests, manuscript preparation and statistical analysis; Dilip Shakya: biochemical assay, drug sensitivity tests; Vinod K. Gupta: sample collection from Gwalior region; Sanjay Jain: sample collection from Sheopur region; D.S. Chauhan: acquisition and interpretation of data; P.K. Tiwari: study design and manuscript writing; V.M. Katoch: drafting and revising the article.

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Competing interests

None declared.

Ethical approval

The sampling method and experimental protocols were approved by the Institutional Ethics Committee of Jiwaji University, Gwalior. Subjects gave their informed consent.

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