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ORIGINAL PAPER

Mycobacterium tuberculosis strain lineage in mixed tribal population across India and Andaman Nicobar Island

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Abstract

In India, the tribal population constitutes almost 8.6% of the nation's total population. Despite their large presence, there are only a few reports available on *Mycobacterium tuberculosis* (*M. tb*) strain prevalence in Indian tribal communities considering the mobile nature of this population and also the influence of the mainstream populations they coexist within many areas for their livelihood. This study attempts to provide critical information pertaining to the TB strain diversity, its public health implications, and distribution among the tribal population in eleven Indian states and Andaman & Nicobar (A&N) Island. The study employed a population-based molecular approach. Clinical isolates were received from 66 villages (10 states and Island) and these villages were selected by implying situation analysis. A total of 78 *M. tb* clinical isolates were received from 10 different states and A&N Island. Among these, 16 different strains were observed by spoligotyping technique. The major *M. tb* strains spoligotype belong to the Beijing, CAS1_DELHI, and EAI5 family of *M. tb* strains followed by EAI1_SOM, EAI6_BGD1, LAM3, LAM6, LAM9, T1, T2, U strains. Drug-susceptibility testing (DST) results showed almost 15.4% of clinical isolates found to be resistant to isoniazid (INH) or rifampicin (RMP) + INH. Predominant multidrug-resistant (MDR-TB) isolates seem to be Beijing strain. Beijing, CAS1_DELHI, EAI3_IND, and EAI5 were the principal strains infecting mixed tribal populations across India. Despite the small sample size, this study has demonstrated higher diversity among the TB strains with significant MDR-TB findings. Prevalence of Beijing MDR-TB strains in Central, Southern, Eastern India and A&N Island indicates the transmission of the TB strains.

Keywords Clusters · Lineage · Prevalence · *M. tuberculosis* · Spoligotyping · Tribe

Introduction

Indian tribal population is one of the highly neglected groups of people due to geographical and cultural barriers in terms of health and associated pubic health services (Govt of India 2020–21). Tuberculosis (TB) claims about 2 million lives annually and it is the leading cause of mortality worldwide due to a single bacterial agent. The situation is particularly alarming in several developing countries including India, as India accounts for over 30% of the global burden of TB. Besides, the emergence and spread of drug-resistant strains and the synergistic association of these diseases with AIDS are posing major challenges for TB control. Due to the aforesaid reason, the World health organization (WHO) has announced tuberculosis as a Global emergency (WHO 2020). Recent report states that a widespread prevalence of pulmonary tuberculosis (PTB) extensively was seen across the zones and states in India. It indeed surged to conduct several studies at the zonal and various state level across India (Thomas et al. 2021). Preliminary data indicate the prevalence of TB among the tribal population is 703 per

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100,000 populations but there is an urgent need to access the incidence and strain diversity of *Mycobacterium tuberculosis* (Thomas et al. 2015). According to Rao et al. (2019), more than 70% of the tribal population resides in the central Indian states of the country. Madhya Pradesh in central India accounts for about 21.1% of the tribal population with 46 ethnic groups. To comprehend the transmission dynamics of *M. tuberculosis* strains and their distribution, molecular typing plays a significant role to scrutinize outbreaks in health care settings at a specific region/area (Ameke et al. 2021). Based on the WHO guidelines, TB resistance to at least two drugs (Isoniazid and Rifampicin) was characterized as multidrug-resistant tuberculosis (MDR-TB). MDR-TB epidemics were chiefly focused on transmission than drug resistance acquisition during treatment (Kendall et al. 2015; Sharma et al. 2017). Spoligotyping, Restriction fragment length polymorphism (IS6110-RFLP) typing/and Mycobacterial interspersed repetitive unite variable number tandem repeat (MIRU-VNTR) typing are the most used tools for epidemiological strain typing or molecular evolutionary studies of *M. tuberculosis* (Devi et al. 2021). Characterizing *M. tuberculosis* isolates by using Spoligotyping, is a PCR-based reverse hybridization blotting technique established by polymorphism (Gupta et al. 2014; Varma-Basil et al. 2017; Rahul 2019; Desikan 2012). Overall genomic differentiation in *M. tuberculosis* is designated by articulating via spoligotyping patterns which eventually depicts the diversity (Said et al. 2009). Our research intended to identify the types of *M. tuberculosis* strains prevalent across several parts of India, A&N Island tribal groups which seem to report different strains and the associated clusters across India. It is the foremost intervention upon mixed tribal populations across India, A&N Island. Several communal elements such as deprived medical support, pitiable socio-economic, and other medical risk aspects play a significant role featuring the mitigation of TB incidence (Togun et al. 2020). Additionally, the deterioration of the TB situation is mainly due to the progress of drug resistance in *M. tuberculosis* isolates. Our study aimed to estimate the prevalence of *M. tuberculosis* lineages among mixed tribal groups in tribal areas of Central, Southern, Eastern India, and A&N Island.

Materials and methods

Study design

In order to study the prevalence of pulmonary tuberculosis among tribal populations in India from April 2015 to March 2020 in the published work of Thomas et al (2021) the large-scale national level tribal tuberculosis prevalence survey of India was carried, evolving different phase studies aged ≥ 15 years in tribal villages. Based on the population proportional to the estimated size (PPES) method, equal weightage was given to the entire country. Based upon the disease prevalence of 387/100,000 populations (Bhat 2009), 92,038 sample size were projected apparently, the country was divided into 6 zones, each with two or more states. Districts with > 70% tribal prime populations were recorded from each zone and itemized the villages details in these districts besides based on PPES. These states comprising East, West, North, South, Central, and North East of almost 88 villages totally selected from 17 states of India. In order to attain the minimum of 800 individuals selected randomly from village streets (Thomas et al. 2021). Our research report involves Phase I trial of Thomas et al. group, which covered 66 villages from 10 states and A&N island viz., Madhya Pradesh, Chhattisgarh, Jharkhand, Maharashtra, Odisha, Manipur, Tripura, Rajasthan, Telangana, and Nagaland, where in 78 clinical isolates of *M. tuberculosis* were isolated from tribal people living in these places and we have reported and analysed the type of lineages seen among these isolates.

Culture identification

The cultures received from the laboratories were sub-cultured on Lowenstein Jensen Media (LJ) and were identified using the MPT64 antigen detection test, growth on P-nitrobenzoic acid (PNB), and Ziehl–Neelsen staining to confirm *Mycobacterium* (Bacteriology and of. Standard Operating Protocol 2016, http://www.nirt.res.in/pdf/bact/SOP.pdf).

Drug resistance associated mutation

Those cultures identified as *M. tuberculosis*, further subjected to genotypic identification by Genotype MTBDR plus 96 kits to determine the susceptibility for RMP (*rpoB*) & INH (*katG* and *inhA*) by LPA assay (WHO 2007). When the cultures are found to be non-tuberculous mycobacteria (NTM), species identification was performed by a conventional method. Species growth identification done by checking for the growth in the presence of P-nitrobenzoic acid (PNB) by PNB inhibition test and 68 °C catalase test were performed as well (Agarwal et al. 2014).

Spoligotyping

Spoligotyping was performed as described by Kamerbeek et al. (1997). DRa and DRb primers were used for amplifying the direct repeat (DR) region in the genome of the *M. tuberculosis* complex. *M. tuberculosis* H37Rv strain and *M. bovis* BCG P3 chromosomal DNA were used as positive controls. The molecular biology grade water served as the negative control. The commercial membrane pre-coated

with spacer-oligos, which represents the spacer region of known sequences, was used for hybridizing the amplified product. The membrane was incubated with streptavidin-peroxide and ECL to visualize the presence/absence of spacer on X-ray film as black squares. BioNumerics software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) was used for spoligotyping through the neighbor-joining method with Jaccard`s distance and analysed the association between the different states and lineages.

Results

M. tuberculosis clinical isolates distribution

A total of 78 *M. tuberculosis* clinical isolates of several tribal populations among 11 places were studied, samples obtained so far from Phase I study. Out of 11 sampling sites, 30 *M. tuberculosis* cultures were from Madhya Pradesh and Chhattisgarh. Whereas 9 *M. tuberculosis* cultures were from A&N island (Harminder bay at Hut bay), comprises 3 Beijing strains and the respective tribal group was Nicobarese. The remaining 10 Beijing strains were reported in Madhya Pradesh, Jharkhand, Manipur, Nagaland, and Tripura respectively (Fig. 1a). The respective tribal population in Madhya Pradesh were Bheel, Kol, Baiga, Kokru, Korku, Gond, Bhil, Bhilala, Bhoomiya, Barela, Rathore, Barelapa, Rathiya, Shariya, and Bhiala. Similarly, the remaining predominant Chattisgarh site comprises the following tribes namely; Bhaina, Bhena, Gond, Binjwal, Charwaha, Cherwa, Chirwa, Khairwar, Sawara, Nagesiya, and Urao (Fig. 1b).

Lineage dissemination

According to the lineage dissemination of the classification, the strain types were categorized into lineages 1 to 9 (L1 to L9). Based on this system of heterogeneous grouping, the distribution of strains belonging to lineages 1 to 4 were seen prevalent in Madhya Pradesh with more of L1 (n = 10), while L3 (n = 7) ranked next with one each of L4 and L2. In Odhisa, the predominant type was found to be L1 (n = 8). In A&N island L1 (n = 5) predominate followed by L2 (n = 3). In Chhattisgarh L3 lineage lead (n = 5) followed by L1 (n = 3). Whereas, the Northeast states comprising Manipur, Nagaland, and Tripura; overall exhibited L2 (n = 8) strain type followed by L3 (n = 6) (Fig. 2).

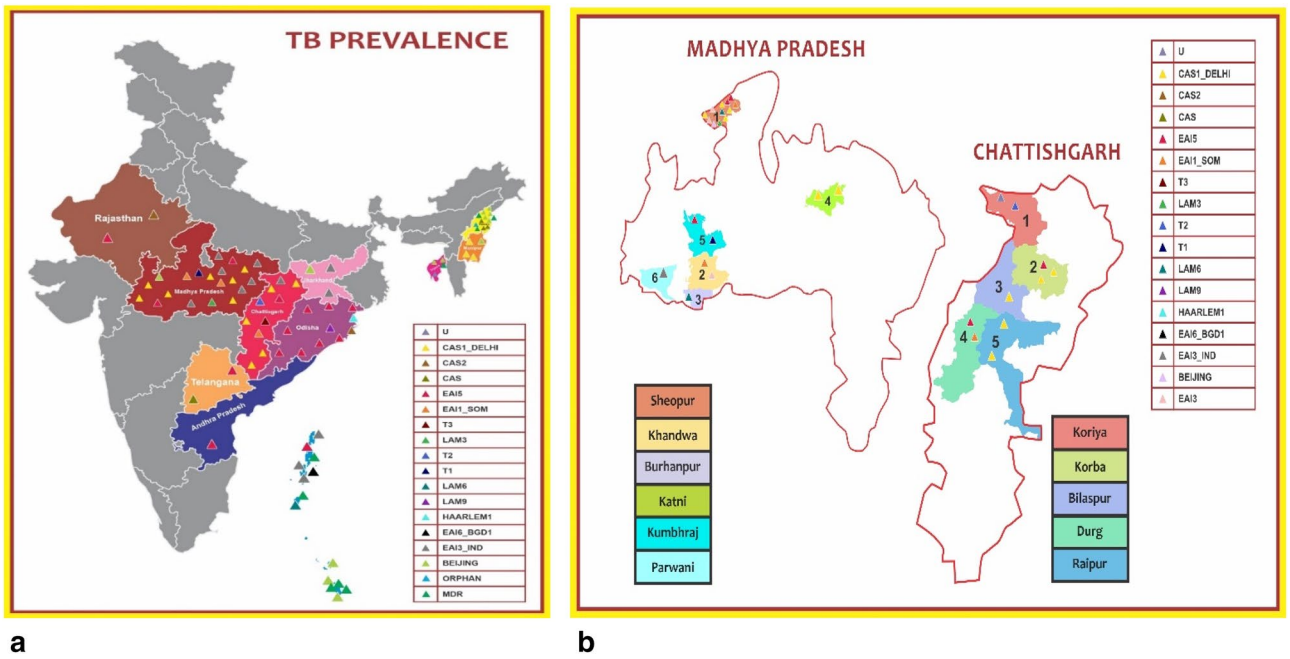


Fig. 1 **a** TB tribal prevalence across India and A&N Island in phase I trial. The 11 highlighted areas depict 10 states and A&N Island; with the distribution of strain lineage in several states. Symbolic representation Of MDR *M. tb* clinical strains prevalence in Madhya Pradesh and A&N Island. Whereas the grey shaded zones were not included in the phase I study report. **b** Tribal population distribution is prime in Madhya Pradesh and Chhattisgarh states. Predominant TB cultures were reported in villages of Madhya Pradesh and Chhattisgarh states highlighted with different symbols. Higher TB prevalence were seen in sheopur from Madhya Pradesh than other villages. Equal *M. tb* clinical strains were seems distributed in Chhattisgarh villages namely Koriya, Korba, Bilaspur, Durga & Raipur

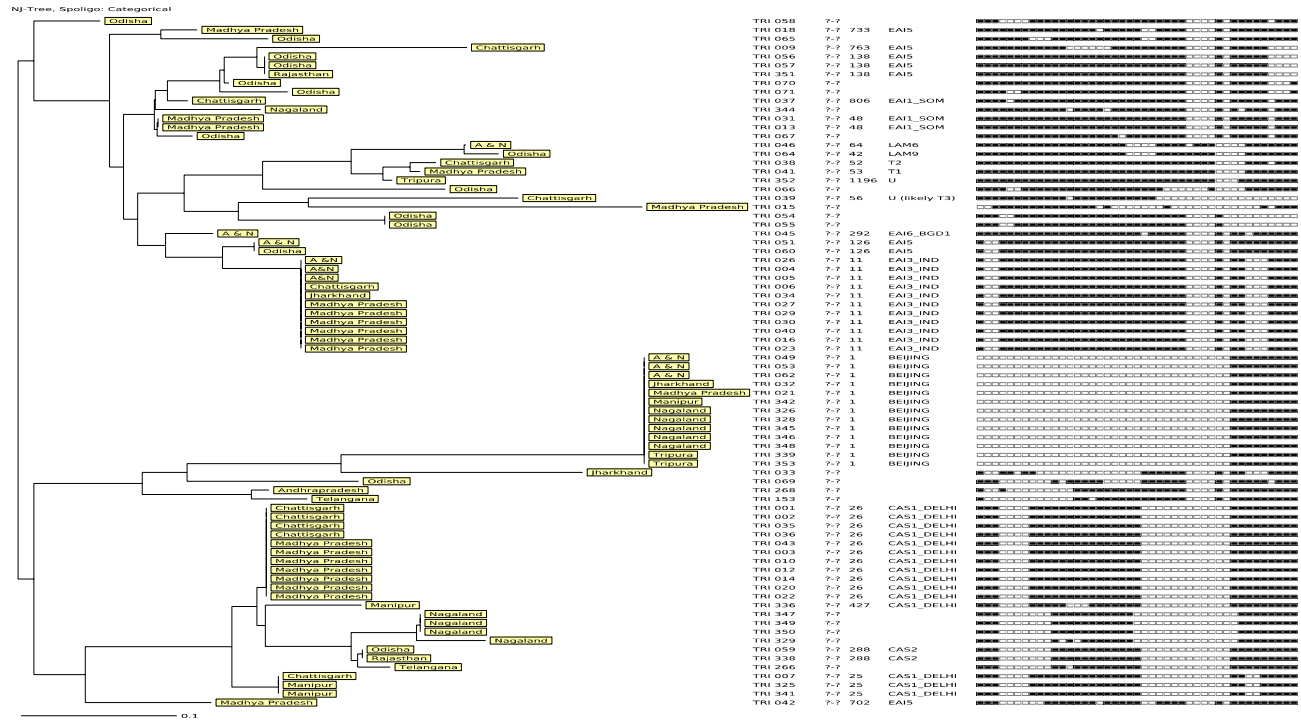


Fig. 2 Phylogenetic tree using Neighbourhood joining method exhibited 10 states and A&N Island distributed into major lineages using unweighted pair group method and arithmetic mean. Similar sequence grouped based on appropriate jaccard's distances. The respective tribal ID correlated with 78 *M. tb* clinical isolates with their shared types. Presence and absence of spacer aligned accordingly

Cluster examination

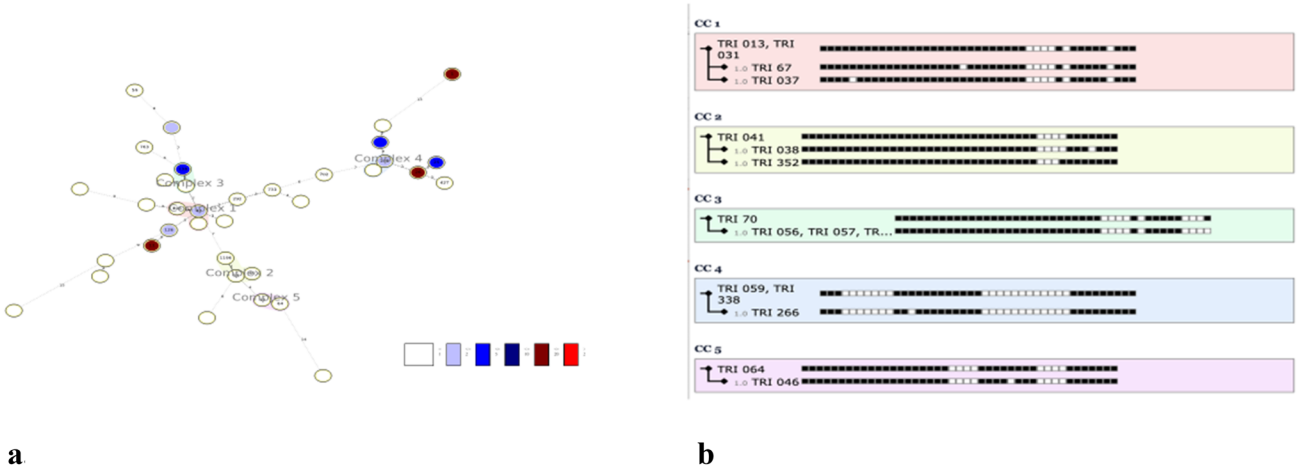
Almost 50 strains (64%) were isolated from men and 28 (26%) were women. The Male TB percentage exceeds the female case among the small sample size. Almost 16 different spoligotype patterns were identified in clinical isolates (n = 78) by the spoligotyping technique. Strains with the same spoligotype were considered as a cluster in the study. These 78 isolates were observed to belong to 8 clusters, ranging from 2 to 20 isolates per cluster, while 25 were found to be unique. Out of these 8 clusters, cluster 1 and cluster 8 were found to be MDR-TB Beijing strains, and cluster 3 was found to be mono resistant CAS1India strain from Madhya Pradesh (Table 1) (Fig. 3a, b). The major spoligotype families observed belonged to Beijing, CAS1_DELHI, EAI3_IND, and EAI5 family of

Table 1 State-wise cluster distribution and Spoligotyping

Clusters	State	Spoligotyping	Shared types	No in cluster
Cluster 1**	A&N	BEIJING	1	2
Cluster 2	A&N	EAI3_IND	11	3
Cluster 3*	Madhya Pradesh	CAS1_DELHI	26	11
Cluster 4	Madhya Pradesh	EAI1_SOM	48	2
Cluster 5	Madhya Pradesh	EAI3_IND	11	7
Cluster 6	Nagaland	CAS	2758	3
Cluster 7	Odisha	EAI 5	138	2
Cluster 8**	Nagaland	BEIJING	1	2

8 cluster grouped based on strains (predominant 3 clusters belongs to the Madhya Pradesh and 2 clusters belongs to A&N island identified as MDR and monoresistant respectively). Shared types presented based on their spacer absence/presence

**Monoresistant*; ***MDR*



a **b**

Fig. 3 **a** Pictorial representation of 5 complex clusters. Strains with similar spoligotyping were depicted as clusters. Each cluster depicts a different strain distribution. 5 clusters with different colours ranging from 2 to 20 isolates per cluster were witnessed on 78 *M. tuberculosis* isolates. **b** Clusters are represented with respective octal codes and their tribal ID. Similar octal code grouped representing complex 5 clusters

Table 2 Shared types of 78 M. *tuberculosis* clinical isolates from the random tribal area across India and A&N Island

S. No	Presence or absence of spacer	Octal Code	*M.tb* Strain	Shared types
1.		703777740003771	CAS1_DELHI (15)	ST26
2.		700377740003771	CAS2 (2)	ST288
3.		700337740003771	CAS	ST2373
4.		700257700001771	ORPHAN	ORPHAN
5.		700377700001771	CAS (3)	ST2758
6.		700775747413771	EAI5	ST702
7.		703777777413731	EAI5	ORPHAN
8.		777775747413771	EAI5	ST733
9.		774377747413771	EAI5	ORPHAN
10.		777777777413731	EAI1_SOM (2)	ST48
11.		757777777413731	EAI1_SOM	ST806
12.		777700777413700	EAI 5 (4)	ST138
13.		777777777760731	T3	ST56
14.		717777777410000	EAI5 (2)	ORPHAN
15.		177775002000131	LAM3	ORPHAN
16.		777777777760731	T2	ST52
17.		777777777760771	T1	ST53
18.		777777607560771	LAM6	ST64
19.		777777607760771	LAM9	ST42
20.		747777774020771	Haarlem1	ORPHAN
21.		777777757413371	EAI6_BGD1	ST292
22.		477777777413771	EAI5 (2)	ST126
23.		477777777413071	EAI3_IND (11)	ST11
24.		000000000003771	BEIJING (13)	ST1
25.		466000037413071	EAI3	ORPHAN
26.		700276037413731	EAI 5	ORPHAN
27.		400033777413771	EAI 5	ORPHAN
28.		440037777413771	EAI 5	ORPHAN

Spacer absence/presence with their respective octal code. Spacer absence/presence represented with their respective octal code. *M. tb* strains with similar octal code were denoted together with their shared types

M. tuberculosis strains (n = 58, 74%), followed by Central Asian strain CAS2 (n = 2, 2.9%), EAI1_SOM (n = 3, 4.3%), and other strains viz; EAI3, EAI6_BGD1, LAM3, LAM6, LAM9, T1, T2, U are present solitary strains and cumulatively contribute 13% (Table 2).

Drug susceptibility testing

The drug susceptibility tests for INH and RMP were carried out for all the 78 clinical isolates. Results exhibited 15.4% (n = 12) of clinical isolates showing resistance to INH or RMP + INH, which are first-line anti-TB drugs. The results of our DST tests identified resistance to INH (mono drug) in 3.8% isolates and multidrug resistance in 11.5% of cases. While 2.6% MDR strain was recorded among the *M. tuberculosis* isolates from women subjects, 9% *M. tuberculosis* isolates from men were found to be MDR (Table. 3).

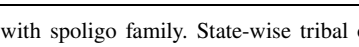
Mutation of *rpoB*, *KatG* and *inhA*

According to the LPA assay, 3 types of mutations were found in the *rpoB* gene among five different tribes. MUT3 (S531L) were found in TRI062 and TRI346, whereas 2 tribal clinical isolates (TRI326, TRI345) showed MUT1 (D516V) and MUT2 were seen in TRI339. Likewise, the *katG* gene exhibited a different mutation; MUT2 (S315T2) in TRI053 whereas other clinical samples showed similar mutations.

Discussion

Epidemiological analysis of the clinical isolates aided by spoligotyping, a PCR-based genotyping method was carried out to understand the lineage-wise distribution of *M. tuberculosis* among the tribal population. In this study, *M. tuberculosis* belonging to L1 to L4 was seen in various ratios among the states. The dominance of *M. tuberculosis* lineages that are more prone to acquire drug resistance complicates the TB control program to a greater extent in that region. We observed predominance of L1 in Odhisha, Andaman/Nicobar Island, and Madhya Pradesh; L2 was the dominant strain type in North-Eastern states, while L3 was found more in numbers at Chhattisgarh. Notably, L2 and L3 are omnipresent in all the reported states which implicates that the emergence of drug-resistant phenotypes in these tribal areas is more likely to occur requiring more stringent control measures focusing on the tribal population. According to Gagneux et al. (2007) *M. tuberculosis* can be classified into lineages 1 (L1) to lineage 7 (L7), wherein L5, L6 and L7 are the types prevalent in African countries. lineages 2 (East Asian) which includes the Beijing family of strains are associated with an increased probability of acquiring drug resistance than L3 and L4 while the least being L1 (Devi et al. 2015; Blouin et al. 2012; Firdessa et al. 2013; Comas et al. 2015; Munsiff et al. 2006). A latest findings about the MDR *M. tuberculosis* complex (MTBC) strains found in African Great lake region, signifies an unknown lineage termed Lineage 8 (L8) (Ngabonziza et al. 2020).

Table 3 INH/RIF phenotypic profile with respective tribal isolate code

S. No	Isolate Code	INH Phenotypic profile	RIF Phenotypic profile	Spoligotype	Share type/Spoligo family	Tribal Origin
1.	TRI 010	Resistant	Sensitive		ST26 /CAS1_DELHI	Madhya Pradesh
2.	TRI 042	Resistant	Sensitive		ST702/EAI5	Madhya Pradesh
3.	TRI 045	Resistant	Sensitive		ST292/EAI6_BGD1	A&N Island
4.	TRI 046	Resistant	Resistant		ST64/LAM6	A&N Island
5.	TRI 049	Resistant	Resistant		ST1/Beijing	A&N Island
6.	TRI 053	Resistant	Resistant		ST1/Beijing	A&N Island
7.	TRI 062	Resistant	Resistant		ST/Beijing	A&N Island
8.	TRI 326	Resistant	Resistant		ST1/Beijing	Nagaland
9.	TRI 336	Sensitive	Resistant		ST26/ CAS1_DELHI	Manipur
10.	TRI 345	Resistant	Resistant		ST1/Beijing	Nagaland
11.	TRI 339	Resistant	Resistant		ST1/Beijing	Tripura
12.	TRI 346	Resistant	Resistant		ST1/Beijing	Nagaland

Spacer presence/absence with shared type correlated along with spoligo family. State-wise tribal origin denoted the predominant MDR and monoresistant strains prevalence

Another study demostartes the presence of L9, *M. africanum*, an african lineage likewise L6 (Coscolla et al., 2021). Our study revealed CAS1_DELHI/ST26 *M. tuberculosis* strains belonging to L3 are circulating in Eastern and Central India (Madhya Pradesh, Manipur, and Assam district). The EAI3_IND/ ST11 mycobacterial strains of L2 prevail among the tribal patients of Madhya Pradesh and A&N Island. Incoherence with our observation, reports of Devi et al. (2015) and Gupta et al. (2014) reported that prevalence of two clades of *M. tuberculosis* isolates belonging to L2 and L3 in Assam and Gwalior and Sheopur districts of North-Central India. Three *M. tuberculosis* strains belonging to EAI1_SOM (Eastern African Indian_Somalia) are reported in Madhya Pradesh districts. Previously, EAI1_SOM has been reported to be highly prevalent in southern regions compared to Northern India (Couvin et al. 2019). This may be due to increased commuting for varied reasons. We observed the occurrence of the CAS family of L3 among tribes of Nagaland, Rajasthan, Odisha, and Telangana (Eastern, central, western, and Southern India). EA1 strain which was previously prevalent only in the Southern Indian region (Brosch et al. 2002) was now found to be distributed in Southern, Eastern, Western, and Central India as well through our current study. Latin American-Mediterranean (LAM) strains of L4 are highly prevalent globally and regionally it constitutes an endemic pattern (Acosta et al. 2019). Similar findings in our study report LAM3, LAM6, and LAM9 in Madhya Pradesh, A&N Island, and Odisha respectively.

Complex clustering was visualized in spoligotyping data analysis. A total of eight clusters was noted by spoligotyping and these clusters were based on the state they are isolated and the type of their family. The number of strains per cluster ranged from 2 to 20. A total of 10 strains are designated as orphans that could not be brought under any clusters were also observed. The strain prevalent in each tribal area was the same as with the strain circulating in that respective state or the region. The number and types of the cluster in Madhya Pradesh were on the higher side and these need further investigation. The data from Madhya Pradesh showed that there exists a considerable amount of transmission among close tribal communities in India accentuating the need for constant screening of these communities to inter-communal spread among the tribes. It has two spoligotype clusters, one with CAS Delhi strain and the other with EAI strains, previous reports have shown a trend of decrease in EAI strains as we travel from south to north and increase in CAS Delhi strains as we travel from south to north of India (Singh et al. 2007). According to the above hypothesis, Madhya Pradesh should have an equal number of these strains and our data perfectly fits into the hypothesis. Since these are common strains in the community, we also predict that these strains have been transmitted from the general population to the tribal population. Similarly, we found that the cluster with Beijing strains (n = 13) is more predominant in the northeast region of India compared to other reports and also in the present study. This could be more related to the region being more geographically closer to china where Beijing strains are common as reported by Devi et al. (2015). The western part of India seems to report a high percentage of Beijing genotype (23%) besides associated with MDR-TB. The highest incidence of MDR-TB among the Beijing family of *M. tuberculosis* strains is also reported in the present study. This study report forecasts the Beijing strain prevalence over the border of eastern India and A&N Island.

Eight MDR (10.25%) *M. tuberculosis* was recognized in this work. Out of twelve drug-resistant isolates reported in this study including MDR-TB, five were from A&N Island which seems to be the foremost report from Nicobarese (ST292/EAI6_BGD1, ST64/LAM6, three from ST1/Beijing). Our report implicates that it would be a major challenge for the patients to travel to Port Blair for the initiation of MDR-TB treatment. It is extremely difficult and also a major risk for MDR-TB transmission. Moreover, three clinical strains were ST1/Beijing from Nagaland and Tripura respectively. The other 4 mono resistant clinical isolates were, 1 ST26 /CAS1_DELHI from Madhya Pradesh and Manipur each; ST702/EAI5 from Madhya Pradesh; ST292/EAI6_BGD1 from A&N respectively. The recent report reveals 4.85% MDR and the percentage of INH resistance was found to be higher (14%) than MDR-TB in the Sahariya tribal population when compared to the non-tribal (6%) population (Prakash et al. 2016).

Conclusion

Based on the literature reviewed, compared to the common population, the prevalence of PTB among the tribal clusters are relatively seen at higher level amid the tribal groups at state and zonal level besides which were left unexplored due to the unapproachable perspective among the tribal populations which was set to call for site-specific interference plans. This study is the most likely first of its kind to report the *M. tuberculosis* strain lineage among mixed tribes belonging to various parts of India and Andaman & Nicobar Island. On the whole, our study sheds light on the increasing drug-resistance prone L2 lineage strains, especially the Beijing strains among the tribal population across the country and the inter-communal spread of TB between the various tribes and regions of the country.

Limitations

The Spoligotyping method used in this study is known to have a problem of homoplasy and this is one of the limitations of this study. Secondary line typing methods (IS6110-RFLP or MIRU-VNTRs) are to be employed to advance this research further. The major limitation of this study is the low sample size which could be addressed in the next phase of this tribal study to arrive at any conclusion in other domicile states of India.

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Author contributions AD: Conceptualization, Methodology, Visualization, Investigation, and supervision. AK: Investigation. SKM: Data curation, writing, original draft preparation. BM: Supervision. SS: Validation and data curation. SR, SA, SV: Project administration. VGR, RY, VP, AJP, TH, VK, KRD, AKIK, PA, PD, AKB: Resources. MD, HK, DR: Review and editing. RM: Supervision, review & editing. BET: Resources and project administration.

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Data availability Available.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This prospective study involving human participants was revised and permitted by the Institutional Ethics Committee of the ICMR-National Institute for Research in Tuberculosis, Chennai, India. The participants provided their written informed approval to take part in this study. Approval Number: NIRT IEC No; 2014005.

Consent to participate The patients/participants provided their written informed approval to take part in this study.

Consent for publication I, give my consent for the publication of identifiable details, which includes photograph(s) and/or case history and/or details within the text ("Material") to be published in the "World Journal of Microbiology and Biotechnology".

References

Acosta CCD, Graciela R, Norma C, Viviana R, Sidra EGV, de Marcia BPM, de Romero NJ, Nora M, Jacobus HDW, Harrison MG, Philip NS (2019) Exploring the "Latin American Mediteerranean" family and the RDRIO lineage in *Mycobacterium tuberculosis* isolates from Paraguay, Argentina and Venezuela. BMC Microbiol 19:1–13

Agarwal A, Dhole TN, Sharma YK (2014) Evaluation of p-nitro benzoic acid (pnb) inhibition test to differentiate *Mycobacterium tuberculosis* complex from non-tuberculosis mycobacteria using microscopic observation of drug susceptibility. Indian J Tuberc 61:232–235

Ameke S, Asare P, Aboagye SY, Otchere ID, Osei-Wusu S, Yeboah-Manu D (2021) Molecular epidemiology of *Mycobacterium tuberculosis* complex in the Volta Region of Ghana. PLoS ONE 16(3):e0238898. https://doi.org/10.1371/journal.pone.0238898

Bacteriology D of. Standard Operating Protocol (2016) National Institute for Research in Tuberculosis. Available from http://www.nirt.res.in/pdf/bact/SOP.pdf. Accessed 10 Oct 2021

Bhat J, Rao VG, Opi PG et al (2009) Prevalence of pulmonary tuberculosis amongst the tribal population of Madhya Pradesh, Central India. Int J Epidemiol 38(4):1026–1032. https://doi.org/10.1093/ije/dyp222

Blouin Y, Hauck Y, Soler C, Fabre M, Vong R, Dehan C, Cazajous G, Massoure P-L, Kraemer P, Jenkins A et al (2012) Significance of the identification in the horn of Africa of an exceptionally deep branching *Mycobacterium tuberculosis* clade. PLoS ONE 7:e52841

Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K et al (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc Natl Acad Sci USA 99(6):3684–3689

Comas I, Hailu E, Kiros T, Bekele S, Mekonnen W, Gumi B, Tschopp R, Ameni G, Hewinson RG, Robertson BD (2015) Population genomics of *Mycobacterium tuberculosis* in Ethiopia contradicts the virgin soil hypothesis for human tuberculosis in Sub-Saharan Africa. Curr Biol 25:3260–3266

Coscolla M, Gagneux S, Menardo F, Loiseau C, Ruiz-Rodriguez P , Borrell S, Otchere DI, Asante-Poku A, Asare P, Sánchez-Busó L, Florian Gehre C, Sanoussi N, Antonio M, Affolabi D, Fyfe J, Beckert P, Niemann S, Alabi SA, Grobusch PM, Kobbe R, Parkhill J, Beisel C, Fenner L, Böttger CE, Meehan, Harris RS, Jong B, Yeboah-Manu D, Brites D (2021) Phylogenomics of Mycobacterium africanum reveals a new lineage and a complex evolutionary history. Microb Genom 7:000477

Couvin D, Reynaud Y, Rastogi N Two tales (2019) Worldwide distribution of Central Asian (CAS) versus ancestral East-African Indian (EAI) lineages of *Mycobacterium tuberculosis* underlines a remarkable cleavage for phylogeographical, epidemiological and demographical characteristics. PLoS ONE 14:e0219706. https://doi.org/10.1371/journal.pone.0219706

Desikan P, Chauhan D, Sharma P, Panwalkar N, Gautam S, Katoch V (2012) A pilot study to determine genetic polymorphism in *Mycobacterium tuberculosis* isolates in Central India. Indian J Med Microbiol 30(4):470–473

Devi KR, Bhutia R, Bhowmick S, Mukherjee K, Mahanta J, Narain K (2015) Genetic diversity of *Mycobacterium tuberculosis* isolates from Assam, India: dominance of Beijing family and discovery of two new clades related to CAS1_Delhi and EAI family based on spoligotyping and MIRU-VNTR typing. PLoS ONE 10(12):e0145860. https://doi.org/10.1371/journal.pone.0145860.20

Devi KR, Jagat P, Rinchenla B, Peggy D, Atanu S, Nitumoni G, Kanwar N (2021) Molecular diversity of *Mycobacterium tuberculosis* complex in Sikkim, India and prediction of dominant spoligotypes using artificial intelligence. Sci Rep 11:7365. https://doi.org/10.1038/s41598-021-86626-z

Firdessa R, Berg S, Hailu E, Schelling E, Gumi B, Erenso G, Gadisa E, Kiros T, Habtamu M, Hussein J et al (2013) Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. Emerg Infect Dis 19:460–463

Gagneux S, Small PM (2007) Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. Lancet Infect Dis 7(5):328–337

Gupta A, Kulkarni S, Rastogi N, Anupurba S (2014) A study of *Mycobacterium tuberculosis* genotypic diversity & drug resistance mutations in Varanasi, North India. Indian J Med Res 139(6):892–902

Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S et al (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 35(4):907–914

Kendall EA, Fofana MO, Dowdy DW (2015) Burden of transmitted multidrug resistance in epidemics of tuberculosis: a transmission modelling analysis. Lancet Respir Med 3(12):963–972

Ministry of tribal affairs Government of India. Annual report 2020–21

Munsiff SS, Li J, Cook SV, Piatek A, Laraque F, Ebrahimzadeh A et al (2006) Trends in drug-resistant *Mycobacterium tuberculosis* in New York City, 1991–2003. Clin Infect Dis 42(12):1702–1710. https://doi.org/10.1086/504325

Ngabonziza JCS, Loiseau C, Marceau M, Jouet A, Menardo F, Tzfadia O, Antoine R, Niyigena EB, Mulders W, Fissette K, Diels M, Gaudin C, Duthoy S, Ssengooba W, André E, Kaswa MK, Habimana YM, Brites D, Affolabi D, Mazarati BP, Catherine de Jong B, Rigouts L, Gagneux S, Meehan CJ (2020) A sister lineage of the *Mycobacterium tuberculosis* complex discovered in the African Great Lakes region. Nat Commun 11:2917. https://doi.org/10.1038/s41467-020-16626-6

Prakash R, Kumar D, Gupta VK, Jain S, Chauhan DS, Tiwari PK et al (2016) Status of muctidrug resistant tuberculosis (MDR-TB) among the Sahariya tribe of North Central India. J Infect Public Health 3:289–297

Rahul G, Rabbind SA, Ravi P, Sanjay J, Pramod KT (2019) Spoligotyping, phenotypic and genotypic characterization of katG, rpoB gene of *M. tuberculosis*. J Infect Public Health 12:395–402. https://doi.org/10.1016/j.jiph.2018.12.009

Rao VG, Bhat J, Yadav R, Sharma RK, Muniyandi M (2019) Declining tuberculosis prevalence in Saharia, a particularly vulnerable tribal community in Central India: evidences for action. BMC Infect Dis 19(1):180. https://doi.org/10.1186/s12879-019-3815-8

Said A, Hadidy G, Gomaa N, Robert C (2009) Strain differentiation of *Mycobacterium tuberculosis* complex isolated from sputum of pulmonary tuberculosis patients. Int J Infect Dis 13:236–242

Sharma A, Hill A, Kurbatova E, van der Walt M, Kvasnovsky C, Tupasi TE et al (2017) Estimating the future burden of multidrug-resistant and extensively drug-resistant tuberculosis in India, the Philippines, Russia, and South Africa: a mathematical modelling study. Lancet Infect Dis 17(7):707–715

Singh UB, Arora J, Suresh N, Pant H, Rana T, Sola C et al (2007) Genetic biodiversity of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in India. Infect Genet Evol 7(4):441–448. https://doi.org/10.1016/j.meegid.2007.01.003

Thomas BE, Adinarayanan S, Manogaran C, Swaminathan S (2015) Pulmonary tuberculosis among tribals in India: a systematic review & meta-analysis. Indian J Med Res 141(5):614–623

Thomas BE, Thiruvengadam K, Vedhachalam CAS, Rao VG, Vijayachari P et al (2021) Prevalence of pulmonary tuberculosis among the tribal populations in India. PLoS ONE 16(6):e0251519. https://doi.org/10.1371/journal.pone.0251519

Togun T, Beate K, Neil GS, Marc L (2020) Anticipating the impact of the COVID-19 pandemic on TB patients and TB control programmes. Ann Clin Microbiol Antimicrob 19:1–6

Varma-Basil M, Nair D, Molecuar epidemiology of tuberculosis (2017) Opportunities & challenges in disease control. Indian J Med Res 146:11–14

World Health Organization (2007) The use of liquid medium for culture and drug susceptibility testing (DST) in low- and medium-income settings. Geneva; Available from https://www.who.int/tb/laboratory/use_of_liquid_tb_culture_summary_report.pdf?ua=1. Accessed 10 Oct 2021

World Health Organization (2020) Global tuberculosis report 2020

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