ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence of Beijing genotype of *Mycobacterium tuberculosis* and its role in drug resistance: A study from a tertiary care hospital in Chhatrapati Sambhajinagar, Maharashtra, India.

Gunjal PN[1]*, Iravane JA[2], Shanmugam S[3], Gunjal SP[4], and Dave M[5].

[1]Ph.D. Scholar, Department of Microbiology, Govt. Medical College and Hospital, Chatrapati Sambhajinagar, Maharashtra. And Assistant Professor at the Department of Microbiology, DVVPF's Medical College and Hospital, Ahilyanagar, Maharashtra, India.
[2]Professor and Head, Department of Microbiology, Govt. Medical College and Hospital, Chhatrapati Sambhaji Nagar, Maharashtra.
[3]Head, Dept. of Bacteriology, ICMR-National Institute of Research in Tuberculosis, Chennai, Tamil Nadu, India.
[4]Associate Professor, Dept. of Microbiology, DVVPF's Medical College and Hospital, Ahilyanagar, Maharashtra.
[5]Microbiologist, Culture and Drug Susceptibility Testing Laboratory for *Mycobacterium tuberculosis* (NABL Accredited ISO15189:2012), Dept. of Microbiology, Govt. Medical College and Hospital, Chhatrapati Sambhaji Nagar, Maharashtra, India.

ABSTRACT

The Beijing genotype of *Mycobacterium tuberculosis* (MTB), as it becomes more prevalent, constitutes a serious threat to public health. The correlation with multidrug resistance (MDR) has been established through different studies. Nevertheless, information regarding the spoligotyping of Mycobacterium *tuberculosis* complex (MTBC) isolates from pulmonary and extrapulmonary specimens in Maharashtra is inadequate. This study aims to evaluate the prevalence of the MTB Beijing genotype and its role in drug resistance in the Marathwada region of Maharashtra. Spoligotyping was conducted on 397 MTB isolates; of these, 55 (13.85%) were identified as Beijing genotypes, while 294 (74.05%) were non-Beijing, and 48 (12.09%) isolates were not typable. The study found that of these 55 Beijing genotype isolates, 48 (87.27%) were detected as MDR Beijing genotype isolates. Of the 294 (74.05%) non-Beijing isolates, 98 (33.33%) were detected as MDR isolates.

Keywords: Beijing genotype, *Mycobacterium tuberculosis*

https://doi.org/10.33887/rjpbcs/2024.15.6.90

Corresponding author

INTRODUCTION

The genotypes of *M. tuberculosis* (MTB) associated with the Beijing strain were initially identified in 1995, representing 86% of the tuberculosis isolates from Beijing, China, as well as a significant share of isolates from Mongolia, South Korea, Hong Kong, Malaysia, and Vietnam. In regions such as Finland and India, the occurrence of the Beijing family genotype is comparatively uncommon [1]. The *M. tuberculosis* strains from the Beijing family exhibit an increased capacity for transmission, a greater propensity to cause disease, and a stronger correlation with drug resistance when compared to non-Beijing MTB isolates [2]. Previous studies conducted in Germany, Cuba, Estonia, Russia, and South Africa documented an association between the transmission of drug resistance and Beijing family genotype strains [3]. The strain W, known for its significant drug resistance, has been reported from the United States and is classified within the Beijing family [4].

Reports indicate that outbreaks of multidrug-resistant tuberculosis associated with the Beijing genotype have occurred in various regions globally [4]. Concerns exist regarding the potential tendencies of the Beijing genotype, such as the development of drug resistance, which could spread globally, possibly due to increased virulence [3, 5]. With an increase of 4.5% as compared to 2021, accounting for 10.6 million cases and 1.6 million deaths globally, tuberculosis specifies its significance as one of the deadliest killers. India accounts for 28% of cases, representing two-thirds (68.3%) of the global TB patient count. Accounting for a total of 36% of TB deaths among HIV-negative TB patients. As per the global MDR TB report 2021, the burden increased by 3% with newly diagnosed Rifampicin-resistant TB (RR-TB) cases [6]. In 2022, 63,801 MDR/RR TB cases were diagnosed in India, according to the India TB Report 2023 [7]. The worldwide burden of drug-resistant TB (DR-TB) rose by 3% from 2020 to 2021, with 450,000 new cases of rifampicin-resistant TB (RR-TB) recorded in 2021 [6].

Thus, obtaining a deeper understanding of the clinical and epidemiological significance of the *M. tuberculosis* Beijing/W genotype may aid in developing more effective strategies for tuberculosis control. This study aims to determine the prevalence of the *M. tuberculosis* Beijing genotype, along with its associated patterns of drug resistance, in the Marathwada region of Maharashtra.

MATERIALS AND METHODS

Materials

Specimen Collection: Samples from pulmonary and extrapulmonary TB patients were collected in sterile, wide-mouth containers according to the guidelines of the National Tuberculosis Elimination Program (NTEP) laboratory, previously known as the Revised National Tuberculosis Control and Prevention Programme (RNTCP) [8].

Sample size: 397.

Sampling method: Total consecutive sampling method.

Place of study: Culture and DST Laboratory, accredited by the National Accreditation Board of Laboratories (NABL Accredited Laboratory ISO15189:2012), Department of Microbiology, Government Medical College, Chhatrapati Sambhaji Nagar, Maharashtra, and Department of Bacteriology (NABL Accredited Laboratory ISO15189:2012), ICMR-National Institute of Research in Tuberculosis (NIRT), Chennai, Tamil Nadu, India.

Study period: 2020-2022.

Statement of Ethics

- The Institutional Ethics Committee approved the study.
- Written informed consent was obtained from the participants before specimen collection.
- Patient confidentiality was maintained.

Materials and Methods

A total of 397 specimens were processed according to the standard guidelines of the National Tuberculosis Elimination Programme (NTEP) [8] at the Department of Microbiology, Government. Medical College, Chhat. Sambhajinagar, Maharashtra, for the identification of *Mycobacterium tuberculosis* from the received specimens. The procedures carried out include the N-Acetyl-L-Cystine (NALC)-NaOH decontamination process and Ziehl-Neelsen's (ZN)

Genotype - Line Probe Assay (LPA) MTBDR*Plus* and MTBDR*sl* (Version 2.0)

A total of 397 specimens were screened for drug susceptibility testing, these specimens were processed by the first-line Line Probe Assay (LPA) testing (Genotype MTBDR*Plus* version 2.0, Hain Life Science GmbH) according to the manufacturer's instructions provided with the kit, to determine the resistance status. Specimens with detected resistance to first-line drugs by LPA were then tested using a second-line LPA (GenoType MTBDR*sl* by Hain Lifescience GmbH). Procedures were followed as per the kit instructions to determine the resistance status to second-line drugs, including fluoroquinolones and second-line injectables [9, 10].

Spoligotyping

Genotyping of all isolates was carried out according to the method described by Kamerbeek *et al*. Spoligotyping was performed at the National Institute for Research in Tuberculosis (NIRT), Chennai [11]. Two primers, DRa and DRb, were used to amplify the direct repeat (DR) region of the MTBC. The positive controls were *M. tuberculosis H37Rv* and *M. bovis BCG P3*, while the negative control was molecular grade water. A membrane, pre-coated with spacer-oligos representing the spacer region of unknown sequence, was used to hybridize the amplified products. After incubation with streptavidin-peroxidase, the products were subjected to ECL detection, and the spacers were detected using X-ray films, resulting in black squares.

The drug susceptibility of these isolates was determined using the Line Probe Assay Genotype MTBDR*Plus* and MTBDR*sl* (Version 2.0) for first-line and second-line antitubercular drugs, respectively [8, 12-14).

RESULTS AND OBSERVATIONS

Spoligotyping was conducted on 397 MTB isolates; of these, 55 (13.85%) were identified as Beijing genotypes, while 294 (74.05%) were non-Beijing, and 48 (12.09%) isolates were not typable. The most prevalent genotype was Manu1(24.68%), followed by Orphan (16.62%) & Beijing (13.85%). The "Institute Pasteur de la Guadeloupe" provides access to a global database for *Mycobacterium tuberculosis* genotyping markers through the following website: http://www.pasteur-guadeloupe.fr:8081/SITVIT2/ [15]. Hence, the prevalence of the Beijing genotype was determined to be 13.85%. Table 1 elaborates the spoligotyping analysis of total specimens.

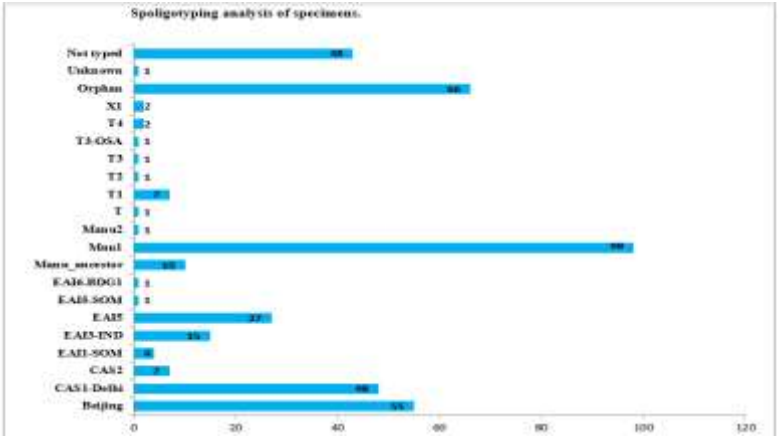

Figure 1: Details of Isolates processed by spoligotyping (n=397).

ISSN: 0975-8585

Table 1: Details of isolates processed by spoligotyping (n=397)

Sr. No.	Spoligotype	SIT No. *	No. of Strains (%)
1	Beijing	1, 1651, 255	55 (13.85)
2	CAS1-Delhi	26, 288, 357, 429, 1344, 1590, 1963	48 (12.09)
3	CAS2	288	7 (1.76)
4	EAI1-SOM	48	4 (1.00)
5	EAI3-IND	11, 1342	15 (3.77)
6	EAI5	236, 340, 776, 934	27 (6.80)
7	EAI5-SOM	735	1 (0.25)
8	EAI6-BDG1	591	1 (0.25)
9	Manu_ancestor	523	10 (2.51)
10	Manu1	100	98 (24.68)
11	Manu2	226	1 (0.25)
12	T	102	1 (0.25)
13	T1	53	7 (1.76)
14	T2	53	1 (0.25)
15	T3	1547	1 (0.25)
16	T3-OSA	627	1 (0.25)
17	T4	40	2 (0.50)
18	X1	119	2 (0.50)
19	Orphan	-	66 (16.62)
20	Unknown	1083	1 (0.25)
21	Not typed		48 (12.09)
22	**Total**		**397 (100)**

The 1st-line LPA was performed on 397 specimens; of these, 55 (13.85%) were Beijing genotypes and 294 (74.05%) were non-Beijing genotypes (excluding 48 non-typable isolates). Table 2 elaborates on the drug resistance pattern of 1st-line LPA-positive 55 (13.85%) Beijing genotypes and 294 non-Beijing genotype isolates. Of the 55 total, 48 (87.27%) were detected as MDR, one isolate showed resistance to rifampicin alone, and two isolates showed resistance to isoniazid alone. Overall, drug resistance was detected in 51 (92.71 %) isolates. Overall sensitivity was detected in 4 (7.27%) of the 55 Beijing isolates.

In cases of non-Beijing 294 isolates, 98 (33.33%) were MDR, 22 (7.71%) were mono-resistant to rifampicin, and 114 (38.77%) were mono-resistant to isoniazid, respectively. While 60 (20.40%) were sensitive to all first-line drugs.

Table 2: Drug resistance pattern of 1st line LPA positive 55 Beijing genotype and 294 non-Beijing genotype isolates.

Resistant Pattern of the isolates	1st line LPA Results of Beijing isolates (n=55) (%)	1st line LPA Results of non-Beijing isolates (n=294) (%)
Multidrug-resistant (MDR)	48 (87.27)	98 (33.33)
Rifampicin Resistant (RR/RS)	01(1.81)	22 (7.71)
Isoniazid Resistant (Hr/SR)	02 (3.63)	114 (38.77)
Sensitive (SS)	04 (7.27)	60 (20.40)
Total	**55**	**294**

Value of $\chi^2 = 56.05$, p = 0.0000000000041, highly significant.

By applying the Chi-square test, there is a highly significant association between the drug resistance pattern of 1st line LPA positive 55 Beijing genotypes with reference to 294 non-Beijing genotype isolates (Beijing vs. non-Beijing).

All these 397 specimens were further processed by 2nd-line LPA. While processing these 397 specimens, 09 (2.26%) specimens were invalid. Hence, 2nd line LPA results could be determined in 388 specimens. Out of these 388 specimens processed by 2nd line LPA, 55 (14.17%) were Beijing genotype and 285 (73.45%) were non-Beijing genotypes (excluding 48 non-typable genotypes). Table 3 elaborates on

the drug resistance pattern of 2nd-line LPA-positive 55 Beijing genotypes and 285 non-Beijing genotype isolates.

Table 3: Drug resistance pattern of 2nd line LPA positive 55 Beijing genotype and 285 non-Beijing genotype isolates.

Resistant Pattern of the isolates	2nd line LPA Results of Beijing isolates (n=55) (%)	2nd line LPA Results of non-Beijing isolates (n=285) (%)
Extensively drug-resistant (XDR)	11 (20.00)	18 (6.31)
Pre-existence of extensively drug-resistant (Pre-XDR) (Fluoroquinolones resistant isolates)	31 (56.33)	67 (23.50)
Pre-extensively drug-resistant (Pre-XDR) (Second-line injectable drug-resistant isolates)	02 (3.63)	03 (1.05)
Sensitive (SS)	11 (20.00)	197 (69.12)
Total	**55**	**285**

Value of χ^2 = 47.665, p = 0.0000, highly significant

By applying the Chi-square test, there is a highly significant association between the drug resistance pattern of 2nd line LPA positive 55 Beijing genotypes with reference to 285 non-Beijing genotype isolates (Beijing vs. non-Beijing).

In cases of 55 Beijing isolates, 11 (20%) were XDR, 31 (56.33%) were pre-XDR, detected with resistance to the fluoroquinolone group of antibiotics. At the same time, 2 (3.63%) were also pre-XDR, showing resistance to second-line injectable anti-TB drugs. While 11 (20%) were shown to be sensitive to all second-line anti-TB drugs.

In cases of the non-Beijing 285 isolates, 18 (6.31%) were XDR, 67 (23.50%) were pre-XDR detected with resistance to the fluoroquinolones group of antibiotics. At the same time, 3 (1.05%) were also pre-XDR, showing resistance to second-line injectable anti-TB drugs. While 197 (69.12%) were shown to be sensitive to all second-line anti-TB drugs.

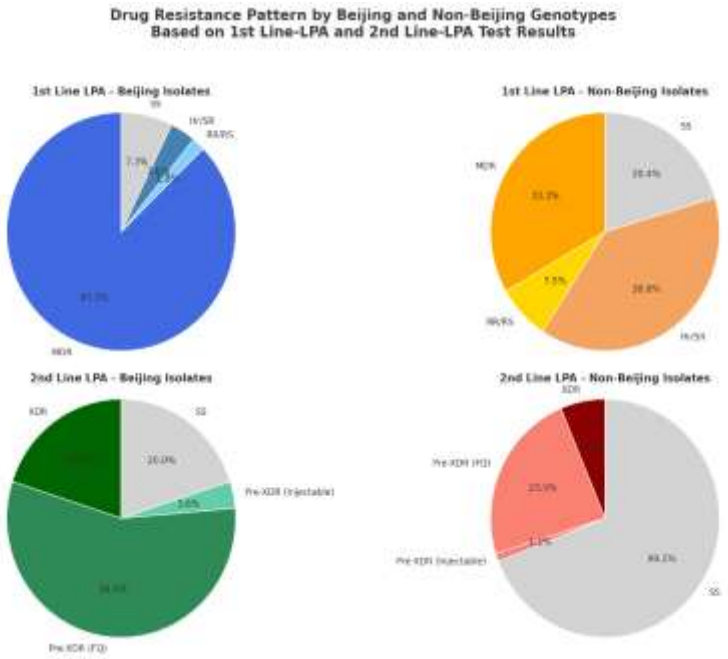

Figure 2: Drug Resistance Pattern by Beijing and Non-Beijing Genotypes Based on GenoType MTBDR*plus* - 1st-Line LAP and GenoType MTBDR*sl* – 2nd-Line LPA Test Results.

ISSN: 0975-8585

DISCUSSION

The Beijing genotypes of *Mycobacterium tuberculosis,* well known, particularly for their extensive geographical distribution, association with drug resistance, and epidemics. They have been notably involved in significant institutional outbreaks and represent an increasing concern in global tuberculosis (TB) management. This study represents one of the few thorough analyses of the prevalence and drug-resistant patterns of Beijing genotype MTB strains, utilizing both first- and second-line LPA data, from the Marathwada region of Maharashtra, thereby greatly enhancing regional epidemiological knowledge, as no previous data yet published from this particular region on drug-resistant MTB as well as Beijing genotype of MTB.

In this study, spoligotyping detected a 13.85% prevalence of the MTB Beijing genotype in this area (55/397) (Table 1/Figure 1); these findings are in accordance with those of Singh AV *et al.* (2021), who reported 11. 26% prevalence of the Beijing genotype in their study [16]. Gupta *et al.* reported in their study that out of a total of 381 MTB isolates, 76 (19.95%) belonged to the Beijing genotype of MTB. Their findings are slightly higher than those of the current study, at 13.84% [17]. Research from Mumbai, as reported by Almeida *et al*. (2005), revealed a higher prevalence of Beijing strains (35%), which differs from the current findings, where Beijing strains constitute 13.85% of the isolates [18]. Mathuria *et al*. also reported an 8.9% identification rate of the MTB Beijing genotype and 91.12% of non-Beijing genotype strains in their study from North India; these findings are also well within the range of our findings [19]. Erie H *et al.* (2017), from Iran, reported 13.9% and 86.1% prevalence of Beijing and non-Beijing genotypes in their study, which is in agreement with our study findings: 13.85% and 74.05% prevalence of Beijing and non-Beijing genotypes, respectively [20]. The absence of data from the Marathwada region of Maharashtra renders our result of a 13.85% prevalence of the Beijing genotype in this area significantly crucial for epidemiological and tuberculosis management considerations.

Multidrug-resistant tuberculosis is defined as exhibiting non-sensitivity for at least rifampicin (RIF) and isoniazid (INH), the two principal first-line anti-TB drugs, and constitutes a significant challenge to tuberculosis management due to its diverse diagnostic and treatment complexities. Mutations in the 81bp rifampicin resistance-determining region (RRDR) of the RNA polymerase β-subunit (*rpoB*) gene have been associated with rifampicin resistance. The prevalence of MDR-TB is increasing globally, including in India, among both newly diagnosed and previously treated patients. It is crucial to verify MDR-TB before starting treatment, as the use of inadequate medications can lead to the proliferation of resistant bacilli, exacerbating the problem of resistance [21]. Consequently, prompt diagnosis and identification of MDR-TB are not just important, but urgent for efficient treatment. Novel, rapid molecular assays for first-line drug resistance can expedite the identification of multidrug-resistant tuberculosis (MDR-TB) and facilitate the modification of treatment. In 2008, the effectiveness of molecular line probe assays, particularly GenoType MTBDR*plus* (Hain Life Science GmbH, Nehren, Germany), led the WHO to recommend their use for the fast screening of patients at risk for MDR-TB [21-23].The GenoType MTBDR*plus* test, a PCR-based amplification and reverse blotting assay, is a crucial tool in the field of tuberculosis. It utilizes specific probes hybridized to nitrocellulose strips for the identification of resistance to RIF and INH. The assay's ability to detect mutations in the *rpoB* gene associated with rifampicin resistance, in the *katG* gene linked to high-level isoniazid resistance, and in the *inhA* regulatory area related to low-level isoniazid resistance, underscores its importance in identifying and managing drug resistance in tuberculosis [21-23].

In this study, we also examined the association between the Beijing genotype and drug resistance. The cases of multidrug resistance were found in 48 (87.27%) isolates of the 55 Beijing MTB genotypes. One isolate (1.81%) was detected as resistant to rifampicin, and two isolates (3.63%) showed resistance to isoniazid, as detected by the GenoType MTBDR*plus* 1st-line LPA test in this study (Table 2/ Figure 2). Barnard *et al.* (2012) from South Africa reported that 71% of cases were identified as MDR MTB by the Genotype MTBDR*plus* (Version 2.0), which aligns with our findings of 87.27% [24]. Singh *et al*. (2020) documented a detection rate of 94%, aligning with our reported ratio of (25). Singhal *et al*. (2016) reported MDR TB at 39.7% in North India, with mono-resistance to rifampicin at 1.8%, comparable to our study's finding of 1.81%. Monoresistance to Isoniazid was reported at 10.7%, exceeding our findings of 3.63% [26]. Moga *et al*. (2023) reported that 35 of 137 MTB isolates from Ethiopia were identified as MDR by Genotype MTBDR*plus* (Version 2.0), representing 25.54%. Additionally, 62 isolates (45.25%) were found to be resistant to Isoniazid. In contrast, our study identified 87.27% of isolates as MDR-TB, with only two strains exhibiting resistance to Isoniazid, and one isolate

was also resistant to rifampicin [27]. Huang *et al*. (2009) reported a total of 95.6% rifampicin-resistant isolates, a figure significantly higher than our finding of 1.81%. Additionally, they noted 81% resistance to INH, which also falls outside the range of our reported ratio. The MDR ratio was 78.5%, consistent with our findings of 87.27% MDR in the Beijing isolates [28].

In our study, the non-Beijing strains were found to be MDR in 98 (33.33%) isolates, 22 (7.71%) were rifampicin resistant, 114 (38.77%) were mono-resistant to isoniazid, and 60 (20.40%) were detected as sensitive to the first-line group of anti-TB drugs (Table 2 / Figure 2). A study from China reported a lower percentage difference between MDR cases of Beijing and non-Beijing genotypes, at 11.3% and 7.4%, respectively [29]. Mathuria *et al*. (2017) from North India reported an overall sensitivity of 75.2%, an overall resistance of 10.6%, and 19.5% of strains from the non-Beijing group with MDR status. Additionally, 23% of the isolates showed resistance to isoniazid, and 20.3% showed resistance to rifampicin in their study group of non-Beijing genotype isolates [19]. Guo *et al*. (2022) reported that resistance to INH was noted at around 10 to 11%, which is in good agreement with our study findings of 12.92% [30]. Seifert *et al*. (2016) reported that 42% of the strains were resistant to RIF, while 43% were detected as having INH resistance. This finding is significantly higher compared to our previous finding of 12.92% resistance noted in INH [31]. Huang *et al.* (2009) reported susceptibility rates of 79%, 15%, and 10% to the non-Beijing strains in their study, respectively [28]. Our study findings are comparatively very high [28]. Our result implies that the Beijing genotypes are linked to drug resistance at a much higher rate than the non-Beijing families.

Table 3 / Figure 2, shows the findings of testing 55 Beijing strains for drug resistance using the 2nd Line-LPA (MTBDR*sl* Version 2.0). The results revealed a concerning pattern, with 11 isolates (20%) being XDR, a significant number that demands further research. Among the 55 Beijing isolates, 31 isolates (56.33%) exhibited resistance to fluoroquinolones. They were classified as pre-XDR, while two isolates (3.63%) showed resistance to Second-Line Injectable Drugs (SLIDs) and were also classified as pre-XDR. A total of 43 isolates (78.18%) exhibited overall drug resistance to all second-line anti-TB drugs. Mono-resistant isolates showed resistance to a single group of second-line anti-TB drugs, with 31 (56.33%) and 2 (3.63%) identified as mono-resistant by the Genotype MTBDR*sl*, respectively.

A Mumbai-based whole genome sequencing study on 1,852 MDR-TB strains found 69.2% pre-XDR (MDR + FLQ resistance) and 4.4% XDR (MDR + FLQ + SLID resistance). This finding is well within the range of our results, which show 56.33% FLQ resistance among Beijing isolates. Our findings of XDR percentage are comparatively higher but consistent with regional escalation trends [32]. A New Delhi hospital reported 21.5% (50/232) isolates as XDR-TB. Among XDR, ~42% belonged to the Beijing genotype. This aligns with our observation of 20% XDR among 55 Beijing isolates [33]. Although many studies focus on pulmonary strains, evidence suggests that Beijing strains often exhibit high-level resistance, particularly in cases involving the lungs. This supports our finding that 78.84% of pulmonary and 100% of extra-pulmonary Beijing isolates were resistant [34]. The findings also imply that the frequency of acquiring drug resistance is greater in the Beijing genotype as compared to the non-Beijing genotype of MTB.

Table 3/Figure 2 elaborates on the drug resistance pattern of 2nd-line LPA-positive 285 non-Beijing genotypes. These non-Beijing strains have shown overall drug resistance of 30.87%, overall drug sensitivity of 69.12%, and pre-XDR resistance to any one of the second-line anti-TB drugs was observed in 24.56%. XDR cases were observed at a rate of 6.31%. Drug resistance was higher in the Beijing genotypes as compared to the non-Beijing genotypes. In a study comparing 217 Beijing/W isolates and 162 non-Beijing isolates, we uncovered findings that have significant implications. We found that 26.7% of Beijing strains were resistant to at least one anti-TB medication, including second-line drugs like ofloxacin, compared to 24.7% of the non-Beijing strains. These results are consistent with the values we reported for the Beijing group of isolates (60%) and the non-Beijing group (24.56%). This study's findings could potentially impact the development of new treatment strategies and the understanding of TB strain resistance. Our study's findings of 24.56% for pre-XDR are supported by the prevalence of Pre-XDR/XDR in non-Beijing groups, which range from approximately 27.9% in Mumbai CAS strains to similar mutation patterns observed worldwide [35]. The non-Beijing resistant strains must be included in thorough surveillance and quick testing protocols due to its similar fitness and spread. The second-line anti-TB drug resistance is primarily caused by non-Beijing strains in India and around the world. They have the same patterns of genetic mutation and contribute significantly to pre-XDR/XDR instances. The non-Beijing lineages must be similarly targeted by surveillance efforts to control the growing drug-

resistant TB issue. The scarcity of local data from Maharashtra highlights the importance of conducting region-specific research on drug resistance and lineage in India [36, 37].

CONCLUSION

This study's findings show that the Beijing genotype of *M. tuberculosis* is not only associated with drug resistance but also occurs at a significantly higher rate than non-Beijing MTB. Our research is the first to report the prevalence of Beijing and non-Beijing genotype isolates from the Marathwada region of Maharashtra, along with their association with drug resistance.

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