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Current Trend in Drug Resistance Pattern Among Extrapulmonary Tuberculosis Cases at Reference Laboratory of Western India

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Research Article

Keywords:

Posted Date: June 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9771204/v1

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Additional Declarations: No competing interests reported.

Abstract

Background: Extrapulmonary tuberculosis (EPTB) remains a major public health challenge because of its atypical presentation, paucibacillary nature, and diagnostic difficulties. The emergence of drug-resistant EPTB (DR-EPTB) further complicates management and threatens tuberculosis control programs. Limited data are available from Western India regarding molecular epidemiology and resistance patterns of EPTB. This study evaluated the distribution, yield, and resistance patterns with molecular and culture-based methods.

Methods A cross-sectional study was conducted at an Reference Laboratory in Western India from January 2024 to December 2025. A total of 6,068 suspected EPTB samples were analyzed using CBNAAT, liquid culture (MGIT), solid culture (LJ), and line probe assays (LPA). Drug susceptibility testing was performed using FL-LPA, SL-LPA, and MGIT 960 where indicated. Statistical analysis was performed using Jamovi software with $p < 0.05$ considered significant.

Results: Of 6,068 samples, 997 (16.43%) were CBNAAT positive. Highest positivity was observed in pus aspirates (35.3%) followed by lymph node samples (20.36%). Overall culture positivity was 3.80%, while positivity among CBNAAT-positive samples was 23.16%. Males constituted 56.36% of cases, and the most affected age group was 21–40 years. Drug resistance was detected in 6.11% cases, with RR-TB being most common (65.57%), followed by isoniazid mono-resistance (16.39%) and MDR-TB (6.55%). Common mutations included rpoB D516V, katG S315T1, inhA C-15T, and gyrA A90V. CBNAAT semi-quantitative grading correlated with culture positivity ($p < 0.001$).

Conclusions: EPTB in Western India shows a substantial burden of drug resistance. molecular diagnostics are crucial for early detection and resistance profiling, highlighting the need for universal DST and surveillance systems.

Introduction

The white plague- Tabes or tuberculosis (TB); though identified in ancient times, still in a quarter year of 21st century (2025), it is world's leading cause of death among top 10 causes [1,2]. According to WHO global TB report -2025, 10.7 million people were incident cases & 1.23 million were died in 2024. Global Tb incidence was 131 cases per 1 lakh population by end of 2024 [2]. India alone contributes 25% of the global TB burden, with EPTB accounting for 24% of total TB cases in the country [2,3].

Pulmonary Tuberculosis (PTB) accounts for 80% of TB cases, while Extrapulmonary Tuberculosis (EPTB) constitutes 20%, commonly affecting lymph nodes, pleura, genitourinary tract, bone, meninges, peritoneum, and pericardium. EPTB occurs mainly through hematogenous spread of bacilli and less commonly via local routes, accounting for 15–20% of all TB cases and up to 50% among HIV-positive patients [1].

Clinicians suspecting EPTB itself consider an uncommon or "out of box diagnosis" as affect any anatomical sites, unusual manifestations, difficulty in sampling, similarity with fungal diseases, and malignancy conditions. EPTB affected patients are non-infectious also [4].

Microbiological confirmation of EPTB is even more challenging due to paucibacillary in nature, less uniform distribution of organism, smear – negativity, precious samples like biopsy, cerebrospinal fluid (CSF) and limited resources. Due to above mentioned reasons, EPTB is diagnosed late or in advanced stage of diseases or missed also.

Microscopy for acid-fast bacilli (AFB) detection in EPTB is simple, rapid, and cost-effective, but low bacillary load and technical difficulty limit its routine use in large populations. Culture of MTB; though considered gold standard for diagnosis of TB, often takes more time of 25±9 days on a Lowenstein-Jensen (LJ) medium and 18±14 days by Mycobacteria growth indicator tube (MGIT) liquid medium [5].

Critical step in EPTB management is faster and accurate testing in laboratory. Considering above factors and to reduce turnaround time (TAT), newer rapid molecular methods are available for diagnosis of EPTB. Since 2011, rapid molecular tests (RMT) have taken over the lab diagnosis part which was previously done by conventional microscopy and culture [1]. This RMT simultaneously detects MTB and drug resistance (DR) which is an age-old phenomenon; a burning problem.

Drug resistant EPTB (DR-EPTB) is still an iceberg phenomenon and has been neglected many times. DR-EPTB can be primary or secondary depending on either patients receive drug resistant strain or prior exposure to anti-TB drugs.

WHO's five categories includes: (1) Isoniazid(Inh)- resistant TB (H- mono), (2) Rifampicin(Rif) resistant TB (RR-TB), (3) Multidrug resistant TB (MDR-TB) which is resistant to both Inh & Rif, (4) Pre-extensively drug Resistant (Pre-XDR) TB having resistance to Rif and any fluroquinolones(FQs), (5) XDR – TB having resistance to Rif, any FQs and either Bedaquiline (Bdq) or Linezolid (Lz) [2].

16–20% of EPTB cases harbour drug-resistant TB (DR-TB), comprising 8–14% mono-H, 2.4–3.9% rifampicin-mono resistant (mono-R), and 2–10% MDR-TB strains [3]. Universal drug susceptibility testing (UDST) for Rif enhances management of EPTB cases by modifying treatment regimen.

While PTB is treated with an all-oral regimen, DR-EPTB requires prolonged treatment with modification of drugs according to the site of involvement. Second-line injectables (SLI) such as Amikacin (Am) and Streptomycin (Sm) can be used in central nervous system (CNS) TB [1].

Without treatment, death rate of TB is close to 50% but with proper treatment as per WHO criteria, approximately 90% of people with TB can be cured [2].

A low complexity CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) system GeneXpert MTB/Rif assay (Cepheid's), used under national tuberculosis elimination program [NTEP] uses molecular beacon to detect DNA sequences amplified in a hemi-nested real time polymerase chain reaction [PCR] with

lowest TAT of < 2 hours. By targeting 81bp core region of *rpoB* gene called RRDR [rifampicin resistant determining region], it simultaneously detects MTB complex [MTBC] DNA with Rif resistance (RR/Rif-R). More than 95% of RR strains possesses mutation within RRDR. Although Inh resistant is not determined [1].

If CBNAAT is not available then the EP specimen should be sent for liquid culture (LC) at culture and DST(C&DST) lab. If LC is positive then it is called microbiologically confirm EPTB case [6].

Automated LC medium [BD BACTEC MGIT 960] works on principle of monitoring oxygen decrease rate consume by MTB as measure by increasing fluorescence [1]. It is also used for phenotypic DST(PDST). Though this LC based PDST is gold standard but having disadvantages like time consuming (4-13 days), laborious, not detecting genetic mutation among first- and second-line drugs.

On the other hand, high complexity NAAT-line probe assay (LPA) targets 23srRNA gene fragment for confirmation of MTBC [1]. It is genotypic DST (GDST) method employed for both 1st and 2nd- line drugs using Genotype MTBDRplus version-2 and MTBDRsl version -2 [Hain life science, Gmbh, Nehren, Germany] kit respectively [7]. WHO has recommended it over PDST for resistance detection among Rif, Inh, FQs, Am because it can reduce TAT compare to PDST [8]. LPA is based on PCR followed by a DNA strip reverse hybridization assay, having pooled sensitivity of 95.8% for Rif, 94.5% for Inh, 86.2% for FQ and 87% for SLI [8,9].

Genotype MTBDRplus targets mutation in RRDR of *rpoB* gene (codon 505 to 533), *inhA* promotor (-16 to -8 nucleotide upstream) for low level resistance and *katG* (codon 315) genomic region for high level resistance whereas MTBDRsl includes quinolones resistance determining region (QRDR) of *gyrA* (codon 85 to 96), *gyrB* (codon 536-541) for FQ and *rrs* (nucleic acid position 1401,1402 and 1484), e*is* promotor region (-37 to -2 nucleotide upstream) for Am resistance [8].

This study focuses on EPTB cases to evaluate their distribution, age-gender prevalence, and drug resistance patterns at the genomic level using rapid molecular tests (RMTs). Given scarce data on DR-EPTB in western India, it aims to guide diagnostic stewardship for EPTB in alignment with the WHO End TB Strategy 2023–2030.

Materials & Methods

A retrospective study was conducted at the IRL (Intermediate Reference Laboratory) under NTEP situated in Gujarat over 6,068 suspected EPTB samples from January 2024 to December 2025, a two-year period, with the aim to detect drug resistance; samples from suspected EPTB patients were transported to the reference laboratory in proper cold chain and packaging [10].

Specimens received for EPTB diagnosis: lymph node biopsy/aspirate for lymphadenitis; cerebrospinal fluid (CSF) for meningitis; pleural, pericardial, ascitic and synovial fluids for body-fluid infections; urine for genitourinary disease; pus, swabs, aspirates, biopsies and FNAC for localized swellings/abscesses or discharges; gastric lavage and stool for gastrointestinal EPTB; bronchoalveolar lavage and bronchial wash for miscellaneous sampling. Inclusion criteria comprised samples from all age groups and both genders, accompanied by a proper request form. Exclusion criteria included sputum samples, leaking specimens, specimens without a Nikshay test request ID, unlabelled/mislabelled samples or mismatch with Nikshay details, overfilled containers, and specimens not collected in appropriate containers [10]. Demographic details of patient such as age, gender were also recorded. No treatment or follow up data was recorded.

Initially all samples received were divided into 2 parts one part is directed for CBNAAT and other is reserved for culture.

- **CBNAAT- GeneXpert MTB/Rif-**

All EPTB samples were offered CBNAAT as a part of initial diagnostic test. Before loading into cartridge, all samples were pretreated and processed as per protocol [11]. 2ml of final sample is loaded in cartridge for further hemi-nested real time PCR. Samples with no MTB detected by CBNAAT were confirmed by conventional Löwenstein–Jensen (LJ) culture. CBNAAT-positive samples were inoculated into liquid culture (for shorter turnaround time) and onto LJ medium (to increase yield). Samples with GeneXpert cycle threshold (Ct) values <16 (high) or 16–22 (medium) were submitted directly for LPA (Ver.-2) to permit faster drug-resistance detection, per laboratory protocol.

- **Culture techniques-**

The second part of the specimen was processed by digestion and decontamination using 4% NaOH (except urine and sterile fluids). For LJ culture, specimens were inoculated on LJ medium and incubated at 35–37°C for 8 weeks, where rough, tough, buff-coloured colonies were identified as MTBC by ZN staining and confirmed by MPT-64 antigen detection [1]. For LC, 0.5 ml sample was inoculated into MGIT tubes supplemented with PANTA and OADC and incubated up to 6 weeks with daily fluorescence monitoring; positive tubes were confirmed by ZN staining or MPT64 antigen detection.

- **LPA-Genotypic DST(GDST)-**

All culture positive isolates were subjected to FLLPA (1st line LPA) for GDST using Genotype MTBDRplus Ver-2.0. LPA is based on 3 steps: (1) DNA extraction (2) PCR amplification (3) reverse hybridization. DR profile is determined by band patterns that represent lines of immobilized probes that are bound/hybridized to MTBC amplicons (DNA amplification products). It detects most common mutation among 1st and 2nd line agents and specific MTBC wild-type (WT) DNA sequences [8]. Mutations are confirmed if amplicons bind to probes that target most common mutations (MUT probes) or by lack of

amplicon binding (i.e., lack of hybridization) to corresponding WT probes known as “inferred resistance” [2]. Strain with any 1st line drug resistance checked for 2nd line LPA (SLLPA) for SLI and FQs.

- **Phenotypic DST (MGIT 960)-**

If resistance was found in SLLPA (pre-XDR or XDR isolates), the isolates were subjected to PDST to assess susceptibility to high-level moxifloxacin (hMfx, using a critical concentration of 1.0 as per WHO), Lz, and Clofazimine (Cfz) [1]. If growth unit (GU) of a drug containing tube is >100 after growth control tube gains GU of>400, it is considered as resistant.

Statistical analysis-

Descriptive statistics were applied to summarize the data in terms of frequencies and percentages. The Chi-square test of independence was used to evaluate associations between categorical variables, including specimen type with CBNAAT positivity and gender distribution. The Chi-square test of goodness of fit was employed to assess the distribution of cases across predefined age groups (0–20, 21–40, 41–60, 61–80, and 81–100 years). Fisher’s exact test was applied where appropriate, particularly for small sample sizes, to examine associations between CBNAAT semi-quantitative levels and culture results. A p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using Jamovi software (version 2.3).

Results

A cross-sectional study was conducted over a total of 2 years on 6,068 samples of suspected EPTB received at the present reference laboratory, including 3,408 samples from the year 2025 and 2,660 samples from 2024.

Specimen-wise distribution of total EP samples (n = 6068) is showing that the highest number of samples received were pleural fluid (1,395 out of 6,068; 22.98%). The second most common was pus aspirate (18.09%), followed by LN (14.96%), gastric aspirate (12.52%), and tissue biopsy samples (11.45%). Other samples received were CSF (5.89%), fluid (4.02%), ascitic fluid (3.7%), BAL (2.9%), and bronchial wash (1.87%). The least common samples were stool (0.03%), blood (0.18%), ET aspirate (0.47%), and urine (0.87%).

Although the most common sample received was pleural fluid, the CBNAAT positivity rate (MTB detection rate) was highest (35.3%) in pus aspirate, followed by LN (20.36%). Pleural fluid showed a CBNAAT positivity of 14.14%. Moderate positivity was found in gastric lavage/aspirate samples (9.62%) and tissue biopsy (9.42%), followed by BAL (4.51%), fluids (2.9%), and bronchial wash (2.0%). The least detection rate was found among blood, urine, ET aspirate, ascitic fluid, and CSF, with percentages of 0.1%, 0.2%, 0.4%, 0.5%, and 0.5%, respectively. No stool sample was found positive by CBNAAT. A Chi-square test of independence showed a statistically significant association between specimen type and CBNAAT positivity ($\chi^2 = 386$, df = 12, $p < 0.001$), indicating that positivity rates varied significantly across different EP samples. The MTB detection rate (NAAT positivity rate) among all samples was 16.43% (997/6,068), while 5,071 samples were negative by CBNAAT (83.56%). The culture positivity rate among CBNAAT-detected samples (997) was 23.16% (231/997), while 759 were culture-negative (76.12%) and 7 were contaminated cultures (0.70%).

To assess the DR profile, positive samples (997) divided into two categories: (1) DS-EPTB and (2) DR-EPTB. DS-EPTB accounted for 93.88% (936/997), while the DR-EPTB burden was 6.11% (61/997) among positive samples. The DR profile was determined using three methods employed for DST: (1) CBNAAT, (2) FL-LPA and SL-LPA, and (3) PDST (for hMfx, Cfz, Lz).

All EPTB-positive cases (997) of DS- and DR-EPTB were analysed for age-wise distribution. The majority of cases were observed in the 21–40 years age group, comprising 49.89% of DS-EPTB and 50.81% of DR-EPTB cases. This was followed by the 0–20 years group (25.21% DS vs 18.03% DR) and the 41–60 years group (18.91% DS vs 21.31% DR). Older age groups (≥ 60 years) contributed relatively fewer cases. Statistical analysis showed no significant association between age group and drug resistance ($\chi^2 = 3.63$, df = 4, $p = 0.459$), indicating a comparable distribution of DS and DR cases across all age groups.

H-mono resistance was found in 10 isolates out of a total of 61 DR-EPTB isolates during the study period (16.39%). H-mono with Lfx resistance was seen in 2 isolates, with one showing high-level and the other showing lMfx resistance. One isolate showed inferred resistance to Lfx & lMfx. RR-TB was found in 40 isolates and was the most common DR-EPTB category (65.57%). The MDR-TB rate was 6.55% (4/61), while the pre-XDR prevalence was 4.9%. Only one isolate was found to be XDR (1.63%) [Figure 1].

Maximum burden among DR-EPTB isolates was observed in pus aspirates (24.48%), followed by pleural fluid and LN (18.36% each). Tissue biopsy contributed 12.24%, followed by gastric aspirate (8.16%), BAL (6.12%), and B. wash (4.08%). The least burden was noted in fluids, CSF, ET aspirate, and blood samples (2.04% each). Out of 61 samples, 32 were from males, while 29 were from females (47.54%). All EP NAAT-positive samples were more commonly from male patients, with the exception of LN and tissue biopsy samples, which were more commonly from females than males.

All DR-EPTB samples were positive for MTBC by NAAT and showed a culture positivity rate of 27.86% (17/61), while 44 (72.13%) were culture-negative. No isolate showed culture contamination. CBNAAT showed Rif sensitivity in 21.31% (13/61), Rif resistance in 47/61 (77.04%), and indeterminate Rif resistance in 1/61 (1.63%).

Tables 1 and 2 show the semi-quantitative analysis of MTBC detection by NAAT into very low, low, medium, and high categories in relation to culture results among H-mono (Rif sensitivity status), and DR-TB (Rif resistance status). This classification is based on cycle threshold (Ct) values corresponding to bacillary load. In DR-EPTB, rifampicin resistance detection was higher at very low bacillary load, followed by low. In contrast, H-mono isolates were detected maximally at Ct values of 16–22 (medium bacillary load).

Table-1: **Correlation of CBNAAT Semi-quantitative Results for Rif-resistance with Culture in Rif-Resistant DR-EPTB Samples**

Culture Status	Very Low (Ct > 28)	Low (Ct 22–28)	Medium (Ct 16–22)	High (Ct < 16)	Total	Statistical Analysis
Negative	25	11	6	0	42	p = 0.088
Positive	1	2	2	0	5	
Total	26	13	8	0	47	
• Fisher's exact test showed no statistically significant association between CBNAAT semi-quantitative levels and culture results among RR EPTB cases (p = 0.088). • Although higher CBNAAT levels showed a trend toward increased culture positivity, the association was not statistically significant, possibly due to the small sample size."						

Table-2: **Correlation of CBNAAT Semi-quantitative Results for Rif-sensitivity with Culture in Rifampicin-sensitive DR-EPTB Samples(H-mono)**

Culture Status	Very Low (Ct > 28)	Low (Ct 22–28)	Medium (Ct 16–22)	High (Ct < 16)	Total	Statistical Analysis
Negative	0	0	1	0	1	p = 1.000
Positive	3	4	5	0	12	
Total	3	4	6	0	13	
• Fisher's exact test showed no statistically significant association between CBNAAT semi-quantitative levels and culture results in H-mono EPTB cases (p = 1.000) • The lack of association may be attributed to the small sample size, limiting the statistical power to detect meaningful differences.						

As per laboratory working protocol, those DR isolates either positive by culture or detected at high/medium bacillary load (in NAAT), irrespective of their culture status, were selected for LPA (GDST). A total of 21 isolates were subjected to LPA. Rif -resistance was detected in 8 (38.09%) isolates, including one with an inferred pattern, while isoniazid resistance was found in 20 (95.23%) isolates. FL-LPA patterns showed IR, RR, and SR (H-mono) patterns in 1, 7, and 13 cases, respectively, where the first character denotes rifampicin pattern and the second denotes isoniazid pattern (S = sensitive, I = inferred, R = resistant). Among the 20 isoniazid-resistant isolates, katG mutation associated with high-level resistance was observed in 14 cases, inhA mutation associated with low-level resistance in 4 cases, and both gene mutations in 2 cases.

Table 3
Interpretation of Mutation Patterns in Drug Resistance Genes of EPTB Isolates Using MTBDRplus and MTBDRsl Assays

Serial no.	Target resistance region (WT band)	Resistance mediating mutations present (MUT probes)	No. of isolates	Possible mutations/locus/region detected by MUT probe	codon analysed by WT band in corresponding gene	Nucleotide change recognized by MUT probes	Interpretations
1	*rpoB* all WT band present	*rpoB* MUT3 present	2	S531L	530–533	TCG > TTG	Resistance to Rif detected
2	*rpoB* WT3 absent	MUT1 present	2	D516V	513–519	GAC > GTC	
3	*rpoB* WT3,4 absent	MUT1 present	2				
4	*rpoB* WT3,4,5,6 absent	MUT1 present	1	D516V(predominant), D516Y,del515,S522L,S522Q	513–519 (PREDOMINANT),518–525	GAC > GTC	
5	*rpoB* WT6 absent	*rpoB* all MUT band absent	1	mutations other than S522L, S522Q	518–525	--	Resistance to Rif inferred
6	*katG* WT band present	*katG*MUT1 present	2	S315T1	315	AGC > ACC	Mutations associated with High-level Isoniazid resistance detected
7	*katG* WT absent	*katG*MUT1 present	11	S315T1		AGC > ACC	
8	*katG* WT absent	*katG*MUT1,2 present	2	S315T1, S315T2		AGC > ACC, AGC > ACA	
9	*katG* WT absent	*katG* all MUT band absent	1	mutations other than S315T1,S315T2		--	Mutations associated with High-level Isoniazid resistance inferred
10	*inhA* WT1 absent	inh A MUT1 present	5	C-15T	-15 mucleic acid position analysed	--	Mutations associated with low-level Isoniazid resistance detected, Resistance to Eto& Pto detected.
11	*inhA* WT2 absent	*inhA* MUT3A present	1	T-8C	-8 mucleic acid position analysed	--	
12	*gyrA* WT3 absent	*gyrA* MUT3B present	2	D94N/D94Y	92–97	GAC > AAC	Resistance to Levofloxacin detected, Mutations associated with high level Moxifloxacin detected.
13	*gyrA* WT2 absent	*gyrA* MUT1 present	2	A90V	89–93	GCG > GTG	Resistance to levofloxacin detected, Mutations associated with low level moxifloxacin detected.
14	*gyrA* WT2 absent	*gyrA* MUT1 present,3A present	1	A90V, D94A		GCG > GTG, GAC > GCC	
15	*gyrA* WT2 absent	*gyrA* MUT2 present	1	S91P		TCG > CCG	
16	*gyrA* WT3 absent	*gyrA* all MUT band absent	1	Mutations in codon 92–96	92–97	--	Resistance to levofloxacin inferred, Mutations associated with low level moxifloxacin inferred.
17	rrs WT2 absent	rrs MUT1,2 present	1	G1484T, A1401G	1484 > 1401 nucleic acid position analysed	--	Resistance to Amikacin, Kanamycin, Capreomycin, Viomycin detected.

As per Table 3, among the *rpoB* gene sequence, the most common mutation was found at codon 513–519, corresponding to D516V (MUT1). The next common mutation was S531L (MUT3) at codon 530–533. One isolate showed inferred resistance. The commonest pattern conferring rifampicin resistance was *rpoB* WT3 absent with MUT1 present.

In the *katG* gene, the most common mutation noted at codon 315 was S315T1 (MUT1), followed by S315T2, both associated with high-level resistance. The commonest pattern was *katG* WT absent with MUT1 present. One isolate showed a mutation other than these two at codon 315, denoting inferred resistance.

Among *inhA* mutations, the most common mutation (C-15T) pattern was *inhA* WT absent with MUT1 present at the analysed nucleic acid position (– 15), followed by T-8C (MUT3A) at the – 8 nucleic acid position. These low-level resistance isolates were also interpreted as resistant to ethionamide (Eto) and prothionamide (Pto).

All 21 isolates were tested by SLLPA, among which 4 isolates with invalid results were excluded. The remaining 17 isolates were further screened, and the SSSSSS pattern was observed in 9 cases, ISSSSS in 1 case, RRRRRR in 1 case, RSRSSS in 4 cases, and RRRSSS in 2 cases. The characters denote patterns for Lfx, hMfx, lMfx, Am/Cap/Kan, Kan, and Am, respectively (S = sensitive, I = inferred, R = resistant).

FQ sensitivity was observed in 9/17 isolates, while resistance was detected in 8/17 isolates. Among the FQ-resistant isolates, no mutations were found in the *gyrB* gene; all mutations were detected in the *gyrA* gene. One isolate showed an inferred pattern of resistance. The most common mutation was A90V(MUT1) at codon 89–93, followed by D94A (MUT3A) and S91P (MUT2), all associated with Lfx and at least low-level moxifloxacin (lMfx) resistance. Two isolates with WT3 absent and MUT3B present showed D94N/Y mutations at codon 92–97, conferring hMfx resistance along with Lfx resistance. SLI resistance was observed in one isolate (XDR), showing *rrs* gene mutations G1484T and A1401G (MUT1,2+) at nucleic acid position 1484, conferring resistance to Am, Kan, Cap, and viomycin.

Seven SLLPA-resistant isolates were further subjected to PDST using MGIT 960 to assess resistance to Mfx (1.0 mg/L), Cfz, and Lz. One isolate was identified as XDR, while the remaining isolates were sensitive.

Discussion

This cross-sectional study demonstrated a year-on-year increase in the annual sample load of EPTB, indicating improved clinical awareness and a higher index of suspicion for EPTB. These findings highlight the importance of simultaneously sending an additional specimen to a reference laboratory during initial histopathological sampling, minimizing the need for repeated invasive or needle-based procedures. A similar rising trend in sample burden has been reported in a study from South India conducted between 2019 and 2023, with a decline observed during the COVID-19 pandemic period [12].

DRTB-

With the rising prevalence of EPTB, it is essential to determine the drug resistance also. Therefore, detailed evaluation of each DR-EPTB case is warranted to ensure appropriate management, improved outcomes as improperly treated cases facilitate transmission of DR strains in community as well.

Table - 4: Comparative Analysis of Rifampicin Resistance, Drug-Resistant TB Patterns, and Demographic Distribution in Extrapulmonary Tuberculosis Across Studies

Serial No.	Study (year of study)	Place	Rifampicin status Rif-S(%)/Rif-R(%)	DSTB(%)/DRTB(%)	DRTB pattern (H-mono/R-mono/RR/MDR/Pre-XDR/XDR among DR-TB)	DRTB sample distribution	Commonest age-group affected by positive DR-EPTB samples (by CBNAAT)	Gender affected in DR-EPTB
1	Present study (Jan-2024 to dec-25)	IRL, Jamnagar (Gujarat)	95.28/4.71 (by CBNAAT)	93.88/6.11 (by CBNAAT & LPA)	16.39/0/65.57/6.55/4.91/1.63 Other resistance pattern not defined =4.91%	Pus (24.48%), Pleural fluid & Lymph node (18.36% each)	21-40 Years	Male
2	Dr. Neha Rajauriya et al. [13] (Nov-2023 to Oct-2024)	TB C&DST lab, Gwalior (central India)	83.87/16.13 (by CBNAAT)	83.87/16.13 (by CBNAAT) Not evaluated other drugs in the study	--	CSF (25%), Pus (16.67%)	11-20 Years	Male
3	Desikan P et al [14] (Jan-2015 to Dec-2023)	NRL, Bhopal (central India)	58.20/41.79 (By LPA)	86.7/13.3 (By LPA)	56.7/3.7/41.79/31.3/--/-- Has inferred resistance in discussion	--	--	--
4	Gong S et al [15] (2016 to 2023)	Wuhan pulmonary hospital, China	92.27/7.73 (By Sensititre)	72.13/27.86 (By Sensititre)	26.89/--/5.04/10.92/11.76/-- Other resistance pattern not defined = 45.38%	TB Meningitis (39.60%), TB Pericarditis (30.77%),	40-59 Years	Male
5	Kumar A et al [16] (Aug-2023 to Nov-2024)	IRL, Uttar Pradesh	92.04/7.96 (by CBNAAT & LPA)	79.95/20.05 (by CBNAAT & LPA)	--/--/39.70/4.13/--/--	--	--	Male
6	Diriba G et al [9] (Oct-2019 to April-2020)	Ethiopia	90.72/9.27 (by MGIT), 90.06/9.93 (by LPA)	80.79/19.20 (by MGIT), 86.1/13.90 (by LPA)	--/--/71.4/66.66/14.28/--	--	--	--
7	Utpat KV et al [4] (2013-2015)	Mumbai	--	--	--/--/--/67.18/29.68/3.12	Lymph node(53.12%), Pleura(28.12%)	18-30 Years	Female
8	Chaubey J et al [17] (April-2016 to March -2017)	IRL, Delhi	87.91/12.08 (by MGIT)	77.85/22.14 (by MGIT)	--/--/54.54/54.54/12.12/2.02 Poly DRTB=45.45 %	--	--	--
9	Cuppuswamy et al [18] (Jan-2016 to Dec-2019)	IRL, Puduchery	95.17/4.82 Rif IND=1.74% (by CBNAAT)	95.17/4.82 (by CBNAAT) Not evaluated other drugs in the study	--	Lymph node (25%), Pleural fluid (19.44%)	--	--
10	Desai U et al [19] (2025)	Mumbai	77.97/19.04 (by CBNAAT)	77.97/19.04 (by CBNAAT) Not evaluated other drugs in the study	--/--/--/71.87/25/3.12	--	--	--
11	Murhar R et al [7] (August-2020 to Dec-2022)	IRL, Nagpur	86.77/13.23 (by CBNAAT) 89.18/10.81 (by LPA)	81.08/18.91 (by LPA)	50/21.42/--/21.42 /7.14/--	Pus (16.66%), B. wash (14.28%)	--	--

The burden of DR-EPTB in the present study was 6.11% among all positive samples. The DR-TB rates reported in various studies, as depicted in the table-4, show considerable variation, ranging from 4.82% in South India to as high as 22.14% in North India, and 27.86% in a study in China [15,17,18]. This variation may be attributed to differences in the diagnostic modalities used for assessing resistance, which significantly impact the reported rates. Studies by Dr. Neha Rajauriya *et al.*, Desai U et al. and VidyaRaj CK et al. utilized only CBNAAT, as per their study design, whereas the study from China employed broth microdilution (BMD) Sensititre for DST [13,15,18,19]. In contrast, the study by Kumar et al. and the present study used a combination of CBNAAT and LPA for the detection of DR-TB, providing a more comprehensive evaluation of resistance patterns [16].

Studies by Chaubey J et al. and Diriba G et al. from Ethiopia evaluated DR profiles using the gold standard PDST (MGIT) [9,17]. The Ethiopian study further compared LPA with PDST and found that LPA has high sensitivity for detecting Rif resistance, but lower sensitivity for Inh resistance when compared to PDST. However, appropriate dose modification for Inh cannot be reliably performed without identifying specific gene mutations, particularly in *inhA* and *katG*, which are commonly detected using FL-LPA. DR-EPTB cases emerge not only from drug defaulters but also from widespread empirical therapy and untreated cases, which amplify DR strains.

In the present study, pus samples constituted the highest proportion of DR-TB cases, a finding similar to studies from Nagpur and Gwalior [7,13]. The next common sample types were pleural fluid and LN. Likewise, several studies have reported LN and PF samples as major contributors to the DR-TB burden [4,18]. In contrast, a study from China reported TB meningitis (CSF samples) as having the highest proportion of resistant cases [15]. The reasons for higher prevalence of DR-TB in pus samples are chronic purulent discharge from various sites, prior invasive procedures or instrumentation, exposure to broad-spectrum antimicrobials (e.g., Lfx), previous anti-tubercular therapy (ATT), incomplete treatment, and delayed presentation to healthcare facilities. In LN TB, nodes are typically discrete in the early stages but may become matted, non-tender over time, eventually undergoing caseation and forming sinus tracts with discharge of caseous material or pus. These factors may contribute to the higher likelihood of detecting DR in both pus and LN samples. Additionally, PF samples may show resistance due to spread of bacilli (from DR-PTB) from infected lung parenchyma as secondary involvement.

The age-wise distribution of DR-TB cases in the present study was not statistically significant, indicating that all age groups were affected to varying extents. However, the highest burden was observed in the 21–40 years age group (productive group), similar to DSTB. In DR-TB cases, the second most common group was 41–60 years (older age group). The higher burden in the older age group may be attributed to the presence of comorbidities, weakened immunity, and prior exposure to anti-tubercular therapy (ATT). Similar findings have been reported in a study from China [15].

Also, this productive age group is commonly affected by DR-TB in India, as noted in studies from Gwalior and Mumbai but the lack of statistical significance suggests that there is no clear association between age group and the occurrence of DR-TB [4,13]. Individuals with above mentioned risk factors may develop DR-TB irrespective of age.

Male preponderance was observed among DR-TB cases in the present study, as well as in studies from Central, North India and China [13,15,16]. However, an earlier study from Mumbai reported a female preponderance [4]. Overall, only a slight difference was noted between male and female cases in DR-EPTB. Nevertheless, since TB in India predominantly affects males, the likelihood of drug-resistant cases being associated with males is correspondingly higher.

Comparison of different modalities with Rif resistance detection-

Among the CBNAAT-detected samples, Rif resistance was observed in 4.71%, while 95.28% were Rif -sensitive. Comparable studies have reported varying proportions of Rif resistance: 4.82% at IRL Pondicherry, 7.96% at IRL Uttar Pradesh, 13.23% at IRL Nagpur, 16.13% at C&DST Gwalior, and 19.04% in Mumbai, indicating regional variation [7,13,16,18,19].

Kumar et al. (IRL–Uttar Pradesh) and Murhar R et al. (IRL–Nagpur) reported that LPA may be superior to CBNAAT for detecting Rif resistance, as few isolates were found to be falsely sensitive by NAAT but resistant on FL-LPA [7,16]. In contrast, a study from Bangladesh reported 100% sensitivity and specificity of LPA for Rif resistance detection compared to NAAT [20].

The possible reasons for this discordance are as follows: (1) Coverage of RRDR by CBNAAT: CBNAAT targets RRDR region, which accounts for more than 95% of RR cases. However, some isolates reported as Rif -sensitive by NAAT may harbour silent or evolving amino acid sequence changes within the 81-bp core region that are not detectable by CBNAAT. When such isolates are tested by LPA, they may show absence of WT bands without corresponding MUT probe binding, resulting in inferred resistance (Rif-R by LPA). (2) Higher resolution of LPA: LPA differentiates multiple WT sequences across different codons within the RRDR, thereby increasing the likelihood of detecting resistance compared to CBNAAT. (3) Mutations outside RRDR: CBNAAT does not detect mutations occurring outside the RRDR, which may contribute to missed detection of Rif resistance.

Diriba et al. from Ethiopia compared LPA with PDST using MGIT for Rif resistance detection and reported one isolate with a silent mutation that led to failure of probe hybridization on the LPA strip [9]. This isolate was interpreted as resistant by LPA but was found to be sensitive by PDST. There is a paucity of data comparing CBNAAT, LPA, and PDST directly. However, based on the available evidence, LPA appears to have an intermediate performance between NAAT (CBNAAT) and PDST. Therefore, all Rif-resistant or indeterminate results detected by NAAT should be confirmed by FLLPA or, if feasible, by PDST, which remains the gold standard. Further studies are required to better evaluate and standardize the comparative performance of these diagnostic modalities. In resource-limited settings, LPA may be relatively cumbersome to perform and has a longer TAT, whereas NAAT, with a rapid TAT of approximately 2 hours, serves as an effective screening tool for *MTB* detection in new or first-time cases. However, all follow-up or previously treated cases should ideally undergo PDST for accurate resistance profiling.

In present study, the prevalence of H-mono was relatively low (16.39%) compared to other studies, where rates as high as 56.7% have been reported from NRL Bhopal [14]. No R-mono isolates were identified in this study, which is a relatively rare occurrence; however, such cases have been reported in other studies [table-4].

RR-TB prevalence in the present study was 65.57%, which is higher than that reported in studies from Bhopal, China, Uttar Pradesh, and Delhi, but slightly lower than that observed in studies from Ethiopia and Mumbai [4,9,14–17]. The proportion of isolates resistant to both Rif and Inh (MDR-TB) was 6.55% in this study. Lower MDR-TB rates have been reported in studies from IRL–Uttar Pradesh and China (4.13% and 10.92%, respectively), whereas higher prevalence has been observed in Mumbai, Bhopal, Ethiopia, Delhi, and Nagpur, as shown in the table-12 [15,16]. The prevalence of pre-XDR isolates in the present study was 4.91%, indicating resistance to second-line drugs such as FQ and/or SLI. The highest prevalence has been reported from Mumbai,

followed by Ethiopia and Delhi [4,19]. The XDR-TB prevalence was 1.63%, which is comparable to findings from studies by Chaubey et al. (2.02%) and from Mumbai [4,17]. Additionally, there was a 4.7% higher culture positivity rate among DR-EPTB cases compared to all NAAT detected cases.

The present study is unique among various studies, as the authors separately analysed Rif-resistant and sensitive isolates using semi-quantitative assessment of MTBC detection in DR-EPTB isolates, along with their corresponding culture results. Higher bacillary load (i.e., lower Ct value) was associated with increased culture positivity. This finding also holds true for H-mono resistant isolates, which interestingly showed the highest culture positivity (12/13) compared to other DR isolates; however, this association was not statistically significant. H-mono isolates were most commonly detected at Ct values of 16–22, indicating a medium bacillary load. This also highlights the effectiveness of NAAT, as it can detect low bacillary load samples that often yield negative culture results, thereby facilitating rapid diagnosis even in paucibacillary cases and also evaluate risk of transmission [21,22].

Rif resistance semi-quantitative analysis of DRTB isolates (47/61) revealed that the majority had a very low bacillary load (55.31%). Similar findings were observed in the IRL-Pondicherry, where most isolates were in the very low category (38.9%), followed by low bacillary load [18]. Although the association was not statistically significant, a trend toward increased culture positivity with lower Ct values was also observed in RR isolates.

Molecular detection of DR genes –

The treatment of DS-EPTB and DS-PTB is generally similar, following the standard regimen [2HRZE → 4HR], with prolonged therapy for CNS and bone-joint TB [1,23]. However, management becomes more complex in DR-EPTB, requiring prolonged all-oral MDR/XDR-TB regimens, adjunctive steroids, MIC-guided dose adjustments, replacement drugs, and mutation analysis. Thus, DR-EPTB requires an individualized, patient-specific treatment approach.

All these factors underscore the importance of LPA as a rapid molecular test for the detection of DR genes, particularly in reference laboratories in developing countries with a high TB burden, such as India, where whole genome sequencing (WGS) cannot be routinely performed for every isolate. RR-TB serves as a surrogate marker for MDR-TB, as more than 90% of RR cases demonstrate coexisting Inh resistance and thus qualify as MDR-TB [24]. LPA serves as a rapid assay for detecting additional resistance (Inh and FQ) among RR-TB cases (pre-MDR stage), thereby facilitating timely modification and appropriate selection of drug regimens.

Table-5: Cross-Study Evaluation of Molecular Mutation Profiles in Drug-Resistant Extrapulmonary Tuberculosis

Serial No.	Study (year of study)	Place	Frequent mutation in *rpoB* GENE	Frequent mutation causing Isoniazid resistance	Frequent mutation in *katG* GENE	Frequent mutation in *inhA* GENE	Frequent mutation in *gyrA* GENE	Frequent mutation in rrs GENE
1	Present study (Jan-2024 to dec-25)	IRL, Jamnagar (Gujarat)	D516V (MUT1) > S531L (MUT3)	*katG>inhA*	S315T1 (MUT1) > S315T2 (MUT2)	C-15T (MUT1)	A90V (MUT1) > D94A (MUT3B)	G1484T, A1401G (MUT1,2)
2	Desikan P et al [14] (Jan-2015 to Dec-2023)	NRL, Bhopal (central India)	S531L(MUT3)> D516V (MUT1)>H526D (MUT2B)	*katG>inhA*	S315T1 (MUT1) > S315T2 (MUT2)	C-15T (MUT1)	--	--
3	Diriba G et al [9] (Oct-2019 to April-2020)	Ethiopia	S531L (MUT3) > H526Y (MUT2A)	*katG>inhA*	S315T1 (MUT1)	C-15T (MUT1)	D94G (MUT3C), D94H (MUT3D)	A1401G (MUT1)
4	Gong S et al [15] (2016 to 2023)	Wuhan pulmonary hospital, China	*RpoB* Ser450Leu (by WGS)	--	*KatG* Ser315Thr (by WGS)	--	--	--

As shown in the table-5, the most frequent mutation in the *rpoB* gene in the present study was D516V at codons 513–519, with the corresponding nucleotide change GAC → GTC. In contrast, studies from Central India and Ethiopia predominantly reported the S531L mutation at codons 530–533 (TCG → TTG) [9,14]. Similarly, in China, *rpoB* Ser450Leu was identified as the most common mutation by WGS [15]. The S531L mutation is known to confer a high level of Rif resistance and poses significant challenges in treatment [14]. The study by Desikan P et al. demonstrated that the absence of WT3 and WT4 bands was associated with inferred Rif resistance, whereas in the present study, absence of the WT6 band was observed, suggesting mutations other than S522L/Q at codons 518–525 leading to inferred resistance [14]. Additionally, the *rpoC* Leu527Val mutation has also been reported in studies from China [15]. Therefore, chronic DSTB cases not responding to standard DSTB drugs may require further evaluation for *rpoC* and other mutations through sequencing methods.

Since Inh and Rif are central components of the treatment regimen, their genomic profiles must be studied in detail. All studies have demonstrated that the *katG* gene is more prone to mutation than the *inhA* gene, resulting in high-level resistance, where high-dose isoniazid is unlikely to be clinically effective [9,14–16].

Mutation in the *katG* gene alters the catalase–peroxidase enzyme, reducing its ability to convert Inh into its active form, thereby leading to resistance. In contrast, mutation in the *inhA* promoter region causes overexpression of enoyl-ACP reductase, which reduces the efficacy of the drug [14].

As shown in the table-5, all studies, including the present study, reported that the most frequent mutation in the *katG* gene was S315T1 (AGC → ACC) at codon 315, followed by S315T2 (AGC → ACA). Mutations at codons other than those specified were interpreted as conferring inferred resistance in the study.

Low-level resistance to Inh, ETO, and PTO (encoded by the *inhA* gene) was primarily associated with the C-15T mutation at the −15 nucleotide position in the promoter region, as observed across all studies, followed by the T-8C mutation.

For DR isolates, it is important to determine resistance to FQ and SLI, for which SL-LPA is performed. In the present study, no *gyrB* mutation was detected, similar to findings reported in a study from Ethiopia [9]. FQ resistance is broadly classified into two types: (1) Lfx resistance and (2) Mfx resistance, which may be of high- or low-level, depending on the specific *gyrA* mutation. Mutations in the *gyrB* gene are generally associated with low-level resistance and are less frequent compared to *gyrA* mutations.

The present study maximally evaluated FQ resistance and its genomic pattern amongst scanty studies. There is absence of data from India regarding evaluation of SLLPA pattern among EPTB cases. Ethiopian study evaluated FQ resistance and noted mutation in codon 92–96 (MUT3C & 3D present, D94G/H) associated with hMfx resistance and Lfx resistance [9]. In such cases, FQs were not effective clinically. In the present study, D94N/Y (GAC → AAC) mutation at codon 92–97 was causing high level Mfx and Lfx resistance, while the overall commonest mutation in *gyrA* was A90V (GCG → GTG) at codon 89–93, coding for low level Mfx and Lfx resistance, followed by D94A and S91P.

gyrA MUT3B/C/D (D94N/Y/G/H) are associated with hMfx resistance. In lMfx resistance, the regimen must be re-evaluated according to PDST results at CB (Mfx 1.0) [8]. With this aim, isolates underwent PDST with three drugs: Mfx 1.0, Cfz, and Lz. Only one isolate was XDR, while the rest showed susceptibility by PDST, and Mfx can be used at higher doses in them. The XDR isolate showed resistance to all SLI drugs, having *rrs* gene mutations G1484T and A1401G (MUT1,2) at nucleotide positions 1484 and 1401 in the present study, while only the A1401G mutation was noted in Ethiopia in XDR isolates [9].

Policy Implications and authors' recommendations for guideline algorithm-

Judicious use of FQs is essential, as widespread and empirical use for UTIs, respiratory and gastrointestinal infections, incomplete treatment courses, efflux pump mechanisms, and over-the-counter availability contribute to resistance. Cross-resistance within the FQ class further reduces susceptibility to other agents once resistance develops to one FQ [17]. Additionally, FQs are frequently used empirically, including in broad-spectrum ATT, before definitive diagnosis.

Guidelines for testing should be regularly updated; for this, the authors suggest:

a. Following initial CBNAAT screening, samples with negative results but high clinical suspicion should undergo LC and LPA for MTB diagnosis. Samples with lower clinical suspicion should preferably be processed by LC or, if unavailable, by LJ culture.

b. NAAT-positive Rif-sensitive samples with medium/high semi-quantitative results should undergo direct FL-LPA using NAAT-extracted DNA, while the remaining sample is inoculated into LC. This reduces DST turnaround time to 4–5 days in samples with higher bacillary load. Samples with low/very low bacillary load should instead be processed on LC. This approach also enables detection of H-mono resistance and inhA/katG mutations, supporting decisions on isoniazid use in treatment regimens.

c. NAAT-detected Rif-resistant (semi-quantification: low/medium/high) isolates should be subjected to simultaneous FL-LPA and SL-LPA (since in RR-TB it is used for presumptive identification of MDR-TB), along with liquid culture (LC). However, discordant results (NAAT-resistant/LPA-sensitive) should be sent for whole genome sequencing (WGS).

d. In all isolates showing *inhA* resistance but no *katG* resistance, sequencing of the *inhA* coding region and *katG* gene is required [8]. All inferred patterns of resistance should also be sent for WGS.

f. In all DR-EPTB cases, SL-LPA must be performed along with PDST for Lz, Cfz, and Mfx (1.0) simultaneously; if not feasible, it should at least be done for MDR-TB cases, as there is a high chance of pre-XDR with FQ resistance.

g. There is no role of the BPaLM regimen in the treatment of CNS TB, spinal TB, miliary TB, and other severe forms of EPTB [1]. Therefore, only limited drugs are available for the management of DR-EPTB in longer oral regimens (Cfz, Lz, Lfx, and Cs). Correlation is required between the frequency of all genetic mutations associated with ATT drugs among EPTB cases with concurrent PTB, to enable better patient management through appropriate modification of treatment regimens.

Limitations of the Study:
Comorbidity status, including HIV and concurrent PTB, was not evaluated. New and follow-up cases, as well as treatment outcomes, were not differentiated. Due to resource-limited settings, it was not feasible to process all samples using LC (gold standard). Additionally, comparison of different AST modalities (PDST/LPA/NAAT) across all samples was not performed as it was not included in the study design. Although agreement among these three AST methods across all ATT drugs is important, detailed evaluation warrants further research.

Strengths of the Study:
A large sample size, recent data, and statistical analysis to evaluate associations, along with comparison to recent studies from different parts of India and globally, strengthen the study. The study further categorized EPTB into DS/DR groups and compared NAAT-positive sample distribution, culture positivity, and age–gender patterns, with special emphasis on NAAT semi-quantitative analysis in DR/H-mono EPTB—likely the first such analysis in India. A key strength is the detailed molecular characterization of each DR-EPTB isolate, including codon-level mutations and nucleotide changes, along with their laboratory reporting and clinical interpretation, representing the first study of its kind from Gujarat and Western India. Additionally, instead of relying solely on culture isolates for DR pattern analysis, the study utilized NAAT-extracted samples for direct FLLPA, reducing turnaround time and enabling detection of DR patterns even in cases with culture contamination or negativity.

Conclusion

From this study, it is inferred that each DR-EPTB case is alarming and warrants thorough investigation and a personalized treatment regimen, with the broader goal of ending the global TB epidemic by 2030. To achieve the global target of 100% coverage of rapid diagnostic testing for TB by 2027, all EPTB cases should be referred to reference laboratories (IRL/C&DST) for comprehensive evaluation.

RR-TB, categorized by WHO as a critical priority pathogen, has shown a rising burden in the post-COVID-19 era and remains insufficiently studied. Therefore, DR-EPTB should be considered a distinct clinical entity and managed with particular caution.

The availability of CBNAAT is a major advantage for TB laboratories, as it is the preferred test in the EPTB diagnostic algorithm. However, a negative result does not always exclude the disease. The present study underscores a layered and targeted testing approach using CBNAAT by integrating semiquantitative results (reflecting bacillary load) with culture findings to better predict risk, minimize missed cases, and improve diagnostic accuracy.

To eliminate TB in the 21st century, informed decision-making is essential through the effective use of line probe assays (LPA). Molecular detection of drug resistance patterns enables estimation of disease burden, optimization of treatment regimens for improved outcomes, and prevention of future resistance. Furthermore, understanding resistance mechanisms at the genetic level, including specific mutations and nucleotide changes, enhances capacity building, supports policy-making, and aids in treatment formulation and genetic research.

An EPTB stewardship program, coupled with robust surveillance, sustained political commitment, and investment in innovation—particularly in RMT—will be crucial steps toward achieving a TB-free world.

Declarations

Competing interests

The authors have declared that no competing interests exist.

Author contributions

G.C. and K.M. were responsible for the conception and design of the study, sample processing, data acquisition, data analysis, and V.S. authored draft of the manuscript. K.M. and P.M. supervised the study design, mentored the laboratory work, and critically revised the manuscript for important intellectual content. G.C., R.S.and T.P. contributed to specimen processing, antimicrobial susceptibility testing and data acquisition. All authors reviewed the data, contributed to manuscript revisions, and read and approved the final version of the manuscript before submission. All authors agree to be accountable for all aspects of the work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval and consent to participate

This was a cross-sectional observational study conducted in IRL (Intermediate Reference Laboratory) under NTEP situated in Western Gujarat, India. The study protocol was reviewed and approved by the Institutional Ethics Committee of MP shah Government medical college, Jamnagar (Reference No: [IEC/215/03/2023], dated 22-07-2024 before commencement. As the study used different samples submitted to the laboratory for clinical diagnosis, with anonymisation of all patient identifiers before analysis, the requirement for individual written informed consent was waived by the Institutional Ethics Committee. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments.

Acknowledgements

The authors would like to thank Almighty God, patients, doctors, and all the staff members of the IRL, Western Gujarat, India.

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Figures

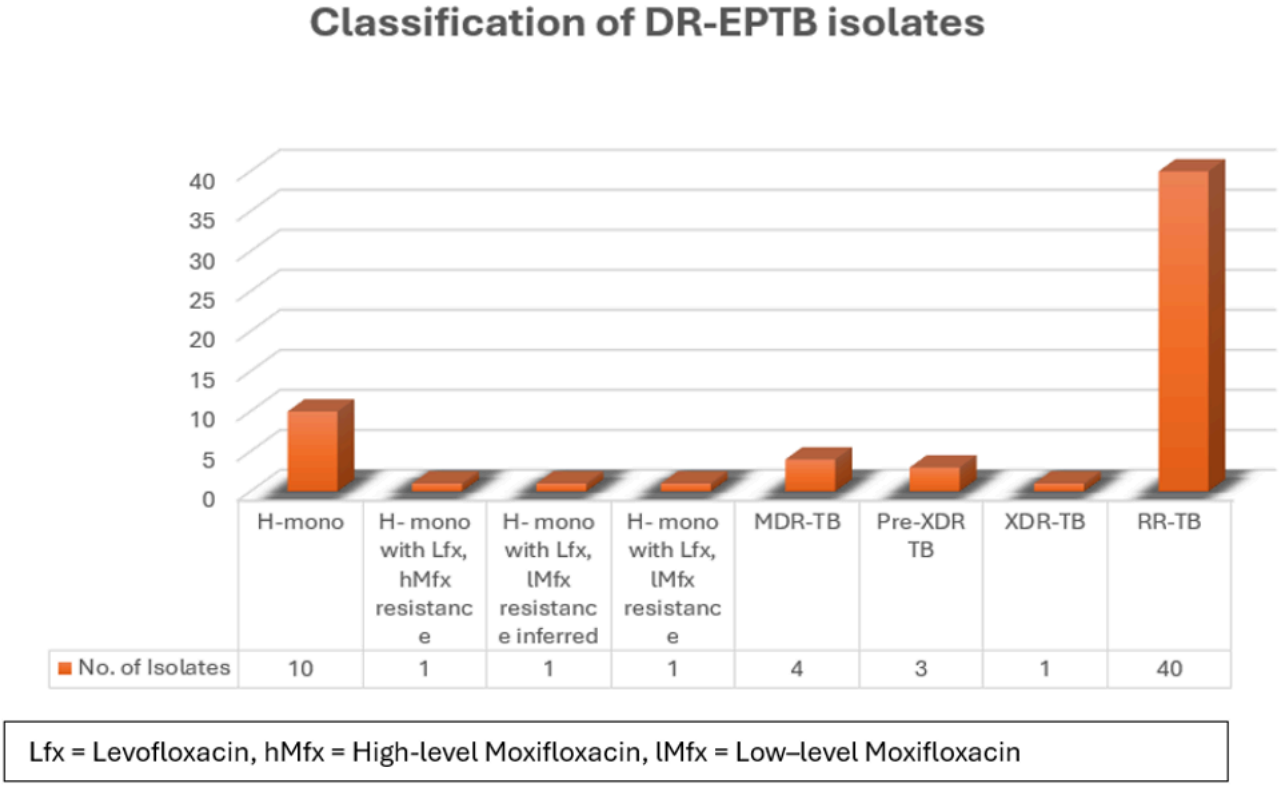


Figure 1

Classification and Distribution of Drug-Resistant Extra-Pulmonary Tuberculosis (DR-EPTB) Isolates