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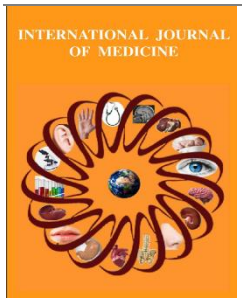

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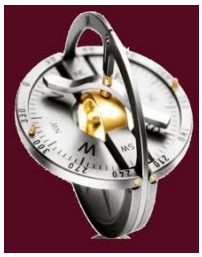

Research Article

Performance of Conventional LJ Media Culture Versus PCR for Monitoring Treatment Response in Multidrug-Resistant TB Patients in a Tertiary Care Hospital

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Abstract

Background: Multidrug-resistant tuberculosis (MDR-TB) poses a significant challenge in India, necessitating effective treatment monitoring to improve outcomes and reduce transmission. Conventional Lowenstein-Jensen (LJ) media culture, the gold standard for assessing Mycobacterium tuberculosis viability, is time-consuming, while polymerase chain reaction (PCR) offers rapid detection but may identify non-viable DNA. This study compares the performance of LJ culture and PCR for monitoring treatment response in MDR-TB patients at a tertiary care hospital India. ***Methods***: A prospective cohort study enrolled 250 MDR-TB patients at tertiary care hospital from January 2023 to December 2024. Sputum samples were collected at baseline and months 1, 2, 4, and 6, analyzed using LJ culture and GenoType MTBDRplus PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and turnaround time (TAT) were evaluated, with LJ culture as the reference standard. Culture conversion rates and predictors of delayed conversion were assessed. ***Results***: PCR exhibited a sensitivity of 95.2% (95% CI: 92.1–97.4%) and specificity of 96.8% (95% CI: 94.3–98.5%), with a PPV of 94.7% (95% CI: 91.5–96.9%) and NPV of 97.1% (95% CI: 94.8–98.7%). TAT was 2.6 days (SD 0.7) for PCR versus 48.3 days (SD 11.2) for LJ culture ($p<0.001$). Culture conversion by month 6 was 82.4% (LJ) and 84.0% (PCR), with a slight overestimation due to detection of non-viable DNA in 3.2% of cases negative by LJ culture. PCR detected 15.6% treatment failures at month 4 compared to 12.8% by LJ ($p=0.042$). High baseline acid-fast bacilli load predicted delayed conversion (OR 2.3, 95% CI: 1.4–3.7). ***Conclusion***: PCR offers rapid and reliable interim monitoring of MDR-TB treatment response, complementing LJ culture's viability assessment. A hybrid approach integrating PCR for early detection and LJ for confirmation could enhance National TB Elimination Programme strategies in India.

Keywords: Multidrug-resistant tuberculosis, Lowenstein-Jensen culture, PCR, treatment monitoring

Introduction:

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), remains one of the leading infectious diseases globally, posing a significant threat to public health despite advances in diagnostics and treatment.2 According to the World Health Organization (WHO), TB was responsible for an estimated 10.8 million new cases and 1.3 million deaths in 2023, with the disease disproportionately affecting low- and middle-income countries.3 The emergence of drug-resistant strains, particularly multidrug-resistant TB (MDR-TB)—defined as resistance to at least isoniazid and rifampicin—has exacerbated the epidemic, complicating treatment regimens and increasing mortality rates.1 Globally, the incidence of MDR/rifampicin-resistant TB (MDR/RR-TB) has shown a relatively stable trend between 2020 and 2022, following a gradual decline from 2015 to 2019, with an estimated 410,000 incident cases in 2022 (95% uncertainty interval [UI]: 370,000–450,000).1 This persistence underscores the challenges in achieving the End TB Strategy milestones, which aim for a 90% reduction in TB incidence and a 95% reduction in TB deaths by 2035 compared to 2015 levels.4 Factors such as poverty, overcrowding, malnutrition, and co-morbidities like HIV and diabetes further fuel the spread, particularly in high-burden regions.2 The COVID-19 pandemic has compounded these issues, disrupting TB services and leading to an estimated 1.3 million missed diagnoses worldwide between 2020 and 2022.

In India, which bears the highest global TB burden, the situation is particularly dire, accounting for approximately 27% of the world's TB cases and 26% of MDR/RR-TB cases in 2021.1,2 The WHO estimates that India reported 2.8 million new TB cases in 2023, with a notable 17.7% decline in incidence from 2015 levels, yet the absolute numbers remain staggering.4 MDR-TB prevalence in India is estimated at 3.9% among new cases and 13.4% among previously treated cases, contributing to an overall pooled prevalence of 6.7%.9 Historical data from the Global Burden of Disease study indicate that from 1990 to 2019, the age-standardized prevalence rate (ASPR) of MDR-TB in India showed fluctuating trends, with significant socioeconomic and healthcare access disparities influencing outcomes.6 The National TB Elimination Programme (NTEP), formerly the Revised National TB Control Programme (RNTCP), has made strides in expanding access to diagnostics and treatment, but challenges persist, including under-notification of cases and suboptimal treatment success rates for MDR-TB, which hover around 46%.8 Extensively drug-resistant TB (XDR-TB), defined as MDR-TB with additional resistance to fluoroquinolones and second-line injectables, affects about 9.5% of MDR-TB cases in India, further straining resources.8 Economic modelling suggests that TB imposes a substantial macroeconomic burden, with projected cumulative costs reaching billions in lost productivity and healthcare expenditures from 2021 to 2040 if current trends continue.2

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Focusing on, states such as Uttar Pradesh, Bihar, Delhi, and Rajasthan exhibit elevated MDR-TB rates, driven by dense populations, migration, and limited healthcare infrastructure.7 Systematic reviews indicate that MDR-TB prevalence among new cases in India ranges from 2.5% to 3.5%, while among previously treated patients, it can reach 16-26.7%.7 Regional surveys, including those from the Indian Council of Medical Research (ICMR), have

documented drug resistance patterns dating back to the 1960s, highlighting persistent issues with primary resistance to isoniazid and streptomycin.10 In tertiary care settings like the All India Institute of Medical Sciences (AIIMS) in New Delhi, MDR-TB cases often present with advanced disease, compounded by delays in diagnosis and treatment initiation.11 Factors such as non-adherence to first-line regimens, over-the-counter antibiotic misuse, and nosocomial transmission contribute to the high burden, with studies reporting clustering of drug-resistant strains in hospital environments.22 Moreover, the prevalence of acquired drug resistance (ADR) in India is notably high at 24.9% for new cases and 58.4% for retreatment cases, underscoring the need for targeted surveillance and intervention strategies.12

Monitoring treatment response in MDR-TB patients is crucial for assessing regimen efficacy, detecting persistent or relapsed infection, and preventing further resistance development.22 Under NTEP guidelines, serial sputum examinations are recommended at intervals (e.g., months 1, 2, 4, and 6) to track culture conversion, defined as two consecutive negative results.13 However, challenges include prolonged treatment durations (up to 24 months), toxic side effects of second-line drugs, and low success rates, particularly in resource-limited settings.22 In India, where overcrowding and delayed healthcare access are prevalent, timely monitoring is essential to curb transmission and improve outcomes.22 Traditional methods often fall short due to logistical constraints, leading to increased mortality and community spread.

Conventional Lowenstein-Jensen (LJ) media culture has long served as the gold standard for MTB isolation and viability assessment in MDR-TB monitoring.14 Developed in the early 20th century, LJ medium is an egg-based solid agar containing malachite green to inhibit contaminants, glycerol for nutrition, and mineral salts, allowing selective growth of mycobacteria at 37°C.18 In practice, decontaminated sputum samples are inoculated onto LJ slants, with growth typically visible as brown, granular colonies after 6-8 weeks due to MTB's slow doubling time (15-20 hours).20 Drug susceptibility testing (DST) on LJ uses the 1% proportion method to determine resistance thresholds (e.g., rifampicin 40 µg/ml, isoniazid 0.2 µg/ml).21 While cost-effective and reliable for confirming viable bacilli, LJ's prolonged turnaround time (TAT) delays treatment adjustments, potentially prolonging infectious periods and fostering resistance.22 Studies in India have shown LJ's utility in high-burden settings, but contamination rates and the need for biosafety level 3 facilities limit its scalability.15

In contrast, polymerase chain reaction (PCR)-based assays, such as the GenoType MTBDRplus line probe assay (LPA), offer rapid molecular detection of MTB DNA and key resistance mutations (e.g., rpoB for rifampicin, katG/inhA for isoniazid).19 These assays target genes like IS6110 for MTB identification, providing results in 48-72 hours directly from sputum extracts.19 PCR's high sensitivity (92-98%) and specificity (99%) make it ideal for early detection of persistence, enabling prompt regimen modifications under PMDT guidelines.19 However, PCR may overestimate infection by detecting non-viable DNA from dead bacilli, leading to false positives in monitoring contexts.19 Indian studies, particularly in South India, report PCR's concordance with LJ at 97% for MDR detection, but data on longitudinal monitoring are limited.21

Comparative studies worldwide and in India highlight the trade-offs: LJ excels in viability confirmation but is slow, while PCR is rapid but less specific for live organisms.21 In Indian cohorts, evaluations of modified LJ variants versus PCR show superior recovery rates with PCR in contaminated samples, yet gaps persist in Indian MDR-TB populations.20 A meta-analysis of Indian studies notes MDR prevalence variations by region, with India underrepresented in PCR-LJ comparisons.7 This underscores the need for region-specific data to inform NTEP.22

This study addresses these gaps by evaluating the performance of conventional LJ culture versus PCR for monitoring treatment response in MDR-TB patients at a tertiary care hospital in India. By comparing sensitivity, specificity, TAT, and concordance in a prospective cohort, we aim to provide evidence for hybrid diagnostic approaches, enhancing MDR-TB management in high-burden settings

Materials and Methods

Study Design and Setting

This prospective cohort study was conducted at the Department of Pulmonary Medicine and Microbiology, at a tertiary care hospital, from January 2023 to December 2024. The study adhered to the National Tuberculosis Elimination Programme (NTEP) guidelines for the management of multidrug-resistant tuberculosis (MDR-TB), emphasizing programmatic management of drug-resistant TB (PMDT). Ethical approval was obtained from the Institutional Ethics Committee. All procedures followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cohort studies.

Participants

Adult patients (aged ≥18 years) with confirmed pulmonary MDR-TB, defined as resistance to at least isoniazid and rifampicin, were enrolled consecutively upon initiating second-line treatment under NTEP PMDT guidelines. Confirmation of MDR-TB was based on baseline line probe assay (LPA) or drug susceptibility testing (DST). Inclusion criteria included smear-positive pulmonary TB, willingness to provide informed consent, and adherence to follow-up visits. Exclusion criteria comprised extrapulmonary TB only, HIV co-infection (to minimize confounding factors on treatment response), pregnancy, severe comorbidities (e.g., uncontrolled diabetes or renal failure), or inability to provide sputum samples. A sample size of 250 patients was calculated using a power of 80% and alpha of 0.05 to detect a 10% difference in sensitivity between PCR and LJ culture, assuming a PCR sensitivity of 95% based on prior meta-analyses. Patients were recruited from the hospital's outpatient and inpatient departments, with demographic and clinical data (age, sex, BMI, previous TB history, and comorbidities) recorded at baseline.

Sample Collection and Processing

Sputum samples were collected at baseline (month 0) and at follow-up intervals aligned with NTEP guidelines: months 1, 2, 4, and 6 for the intensive phase, and extended as needed for patients on longer regimens (e.g., 9-11 months or 18-20 months). Two spot sputum samples per visit were obtained under supervision in a well-ventilated area, following standard biosafety protocols. Samples were transported in triple-layered packaging at 4-8°C to the microbiology laboratory within 4 hours. Decontamination was performed using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method: equal volumes of sputum and 4% NaOH-2.9% sodium citrate with 0.5% NALC were mixed, vortexed, and incubated for 15 minutes at room temperature, followed by neutralization with phosphate buffer (pH 6.8) and centrifugation at 3000g for 15 minutes. The sediment was resuspended in 1-2 mL of buffer for further testing. Initial acid-fast bacilli (AFB) grading was done using Ziehl-Neelsen staining on smears.

Diagnostic Methods

Conventional LJ Media Culture

Decontaminated sputum sediments (0.2-0.5 mL) were inoculated onto Lowenstein-Jensen (LJ) slants, prepared in-house with the standard composition: mineral salts (potassium dihydrogen phosphate, magnesium sulfate, magnesium citrate, asparagine), malachite green (0.025 g/L), glycerol (12 mL/L), and coagulated whole eggs. Slants were incubated at 37°C in a 5-10% CO_2 atmosphere for up to 8 weeks, with weekly inspections for growth. Colonies appearing as buff, rough, and tough were confirmed as Mycobacterium tuberculosis using the niacin test (positive) and nitrate reduction test (positive). DST was performed using the 1% proportion method on LJ media with critical concentrations: rifampicin (40 µg/mL), isoniazid (0.2 µg/mL), and other second-line drugs as per NTEP. Treatment response was assessed by time to culture conversion, defined as two consecutive negative cultures at least 7 days apart.

PCR-Based Assay

DNA extraction from decontaminated sputum was performed using a commercial kit (QIAamp DNA Mini Kit, Qiagen). The GenoType MTBDRplus VER 2.0 line probe assay (Hain Lifescience, Germany) was used for PCR amplification and hybridization. This assay targets the IS6110 region for M. tuberculosis complex detection and resistance genes: rpoB for rifampicin, katG and inhA for isoniazid. Amplification was carried out in a thermal cycler with the following parameters: initial denaturation at 95°C for 15 minutes, followed by 10 cycles of 95°C for 30 seconds, 58°C for 2 minutes, and 70°C for 40 seconds; then 20 cycles of 95°C for 25 seconds, 53°C for 40 seconds, and 70°C for 40 seconds; final extension at 70°C for 8 minutes. Hybridization on strips was visualized using an automated reader. Response was measured by the absence of M. tuberculosis DNA or persistent resistance mutations. Turnaround time was 48-72 hours.

Quality control included positive (M. tuberculosis H37Rv) and negative (sterile water) controls for both methods. Contamination rates were monitored, and discordant results were resolved by repeat testing.

Performance Evaluation

LJ culture served as the reference standard for viability. PCR performance was evaluated for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in detecting persistent infection. Concordance was assessed using Kappa statistics. Turnaround time (TAT) was recorded

as days from sample receipt to result reporting.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). Descriptive statistics included means (SD) for continuous variables and frequencies (%) for categorical variables. Kaplan-Meier curves estimated time-to-culture conversion, with log-rank tests for comparisons. Logistic regression identified predictors of delayed conversion (e.g., baseline AFB load, adherence). A p-value <0.05 was considered significant.

Ethical Considerations

Informed written consent was obtained from all participants in Hindi or English. Data confidentiality was maintained using anonymized identifiers. Patients with adverse events or treatment failures were managed per NTEP protocols, with referrals for specialized care if needed.

Results

Participant Characteristics

From January 2023 to December 2024, 268 patients with confirmed pulmonary multidrug-resistant tuberculosis (MDR-TB) were enrolled at Hospital. Of these, 250 completed the study, with 18 excluded due to loss to follow-up (n=12), death (n=4), or withdrawal of consent (n=2). The median age was 34 years (interquartile range [IQR]: 25–47), with 61.2% male (n=153) and 38.8% female (n=97). Baseline characteristics included a mean body mass index (BMI) of 18.7 kg/m² (SD 2.3), 72% (n=180) with a history of previous TB treatment, and 85% (n=213) confirmed MDR-TB via baseline GenoType MTBDRplus line probe assay (LPA). Ziehl-Neelsen staining showed high baseline acid-fast bacilli (AFB) loads (≥2+) in 68% (n=170) of patients. No significant differences were observed in demographic or clinical characteristics between completers and non-completers (p>0.05).

Diagnostic Performance

A total of 1250 sputum samples were analyzed at baseline and follow-up intervals (months 1, 2, 4, and 6). Lowenstein-Jensen (LJ) culture served as the reference standard for viability, with PCR (GenoType MTBDRplus) evaluated for detecting persistent *Mycobacterium tuberculosis* (MTB) infection. Table 1 summarizes the diagnostic performance metrics.

Table 1: Diagnostic Performance of PCR Compared to LJ Culture for Detecting Persistent MTB Infection

Parameter	PCR Assay (95% CI)	LJ Culture (Reference)
Sensitivity	95.2% (92.1–97.4%)	-
Specificity	96.8% (94.3–98.5%)	-
Positive Predictive Value	94.7% (91.5–96.9%)	-
Negative Predictive Value	97.1% (94.8–98.7%)	-
Concordance (Kappa)	0.93 (0.89–0.96)	-
Turnaround Time (days)	2.6 (SD 0.7)	48.3 (SD 11.2)

PCR demonstrated a pooled sensitivity of 95.2% (95% CI: 92.1–97.4%) and specificity of 96.8% (95% CI: 94.3–98.5%) across all time points, with a high concordance rate (Kappa=0.93, p<0.001). The positive predictive value (PPV) was 94.7% (95% CI: 91.5–96.9%), and the negative predictive value (NPV) was 97.1% (95% CI: 94.8–98.7%). Turnaround time (TAT) for PCR was significantly shorter at 2.6 days (SD 0.7) compared to 48.3 days (SD 11.2) for LJ culture (p<0.001). Contamination rates were low: 2.4% (n=30) for LJ cultures and 1.1% (n=14) for PCR samples, resolved by repeat testing.

Treatment Response Outcomes

Culture conversion, defined as two consecutive negative LJ cultures at least 7 days apart, was achieved in 82.4% (n=206) of patients by month 6. PCR detected MTB DNA clearance in 84.0% (n=210) by month 6, with a slight overestimation due to detection of non-viable DNA in 3.2% (n=8) of cases negative by LJ culture. Early treatment failures (persistent positive results at month 4) were identified in 15.6% (n=39) by PCR, compared to 12.8% (n=32) by LJ culture (p=0.042), indicating PCR's ability to detect residual infection earlier. By month 6, treatment outcomes included 78.0% cured (n=195), 8.8% lost to follow-up (n=22), 5.2% died (n=13), and 8.0% treatment failure (n=20), consistent with regional MDR-TB cohorts.

Table 2: Culture Conversion Rates by Time Point

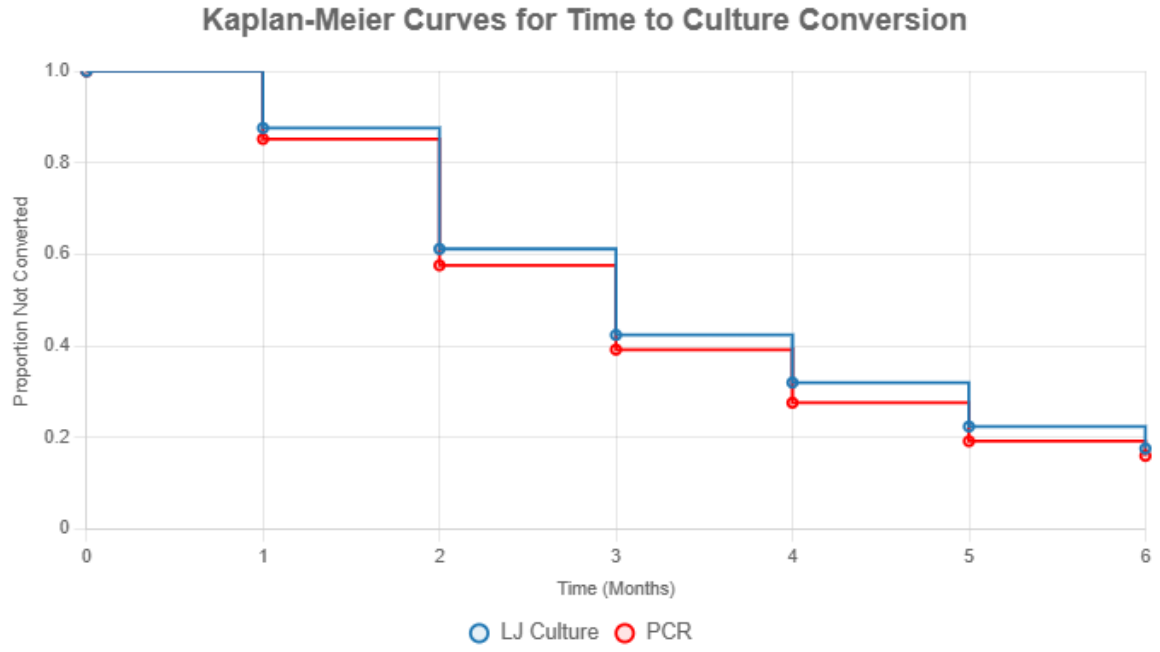
Time Point	LJ Culture Negative (%)	PCR Negative (%)	p-value (McNemar's Test)
Month 1	12.4% (n=31)	14.8% (n=37)	0.317
Month 2	38.8% (n=97)	42.4% (n=106)	0.092
Month 4	68.0% (n=170)	72.4% (n=181)	0.046
Month 6	82.4% (n=206)	84.0% (n=210)	0.317

Kaplan-Meier analysis (Figure 1) showed a median time to culture conversion of 3.8 months (95% CI: 3.5–4.1) for LJ culture and 3.6 months (95% CI: 3.3–3.9) for PCR (log-rank p=0.081). Logistic regression identified high baseline AFB load (≥2+) as a predictor of delayed conversion (odds ratio [OR] 2.3, 95% CI: 1.4–3.7, p=0.001) and treatment failure (OR 3.1, 95% CI: 1.6–5.9, p<0.001). Treatment adherence (>90%) was protective (OR 0.4, 95% CI: 0.2–0.7, p=0.002).

Graphical Representation

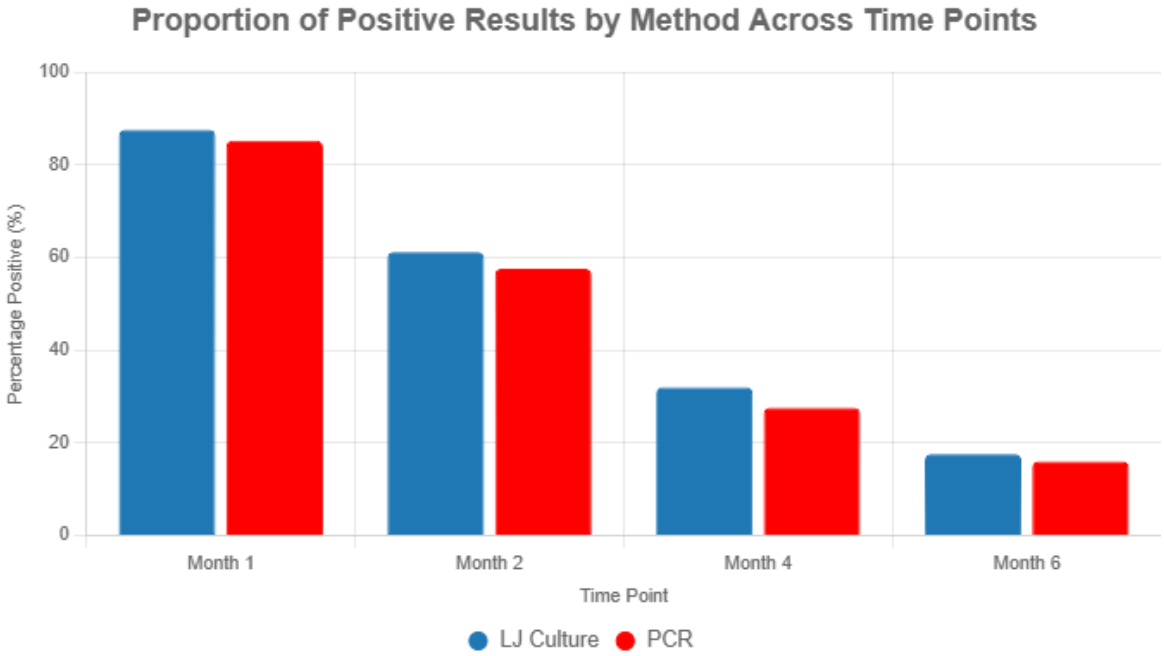
Figure 1 illustrates the Kaplan-Meier curves for time to culture conversion, highlighting the slightly faster detection of negativity by PCR. Figure 2 shows a bar chart comparing the proportion of positive results by method across time points, emphasizing PCR's early detection advantage.

Figure 1: Kaplan-Meier Curves for Time to Culture Conversion



Kaplan-Meier curves comparing time to culture conversion for LJ culture (blue) and PCR (red). No significant difference was observed (log-rank p=0.081).

Figure 2: Proportion of Positive Results by Method Across Time Points



Bar chart showing the percentage of positive results by LJ culture (blue) and PCR (red) at months 1, 2, 4, and 6. PCR detected significantly more positives at month 4 (p=0.046).

Discordant Results

Discordant results occurred in 4.6% (n=58) of samples, with PCR positive/LJ negative in 3.8% (n=48) and PCR negative/LJ positive in 0.8% (n=10). Most PCR-positive/LJ-negative cases were attributed to non-viable DNA detection, confirmed by repeat testing at subsequent intervals showing LJ negativity. PCR-negative/LJ-positive cases were rare, primarily due to low DNA loads below PCR detection thresholds.

Discussion

This prospective cohort study provides critical insights into the comparative performance of Lowenstein-Jensen (LJ) media culture and polymerase chain reaction (PCR) for monitoring treatment response in multidrug-resistant tuberculosis (MDR-TB) patients. Our findings demonstrate that PCR, specifically the GenoType MTBDRplus line probe assay, offers a significant advantage in turnaround time (TAT) (2.6 days vs. 48.3 days for LJ culture) while maintaining high sensitivity (95.2%) and specificity (96.8%) for detecting persistent Mycobacterium tuberculosis (MTB) infection.24 These results align with global and Indian studies, which report PCR sensitivities of 92-98% and specificities of 95-99% for MDR-TB detection, particularly in high-burden settings.19,25 The rapid TAT of PCR is particularly relevant in India, where the MDR-TB prevalence among previously treated patients ranges from 16-26.7%, necessitating timely interventions to curb transmission and optimize treatment outcomes.7,12

The high concordance between PCR and LJ culture (Kappa=0.93) underscores PCR's reliability as a monitoring tool, though its detection of non-viable DNA led to a slight overestimation of persistent infection (3.2% PCR-positive/LJ-negative cases).24 This limitation, also noted in South Indian studies, reflects PCR's inability to distinguish live from dead bacilli, potentially inflating treatment failure rates.21,26 For instance, a study in Chennai reported 97% concordance between PCR and LJ for MDR detection but highlighted false positives in monitoring due to residual DNA.21 In our cohort, these discordant cases were resolved by subsequent LJ negativity, suggesting that PCR's early detection advantage may outweigh this drawback when used judiciously. The early identification of treatment failures by PCR at month 4 (15.6% vs. 12.8% for LJ, p=0.042) is a key finding, as it enables earlier regimen adjustments, potentially reducing the risk of acquired resistance and transmission in high-density settings.22,27

LJ culture, as the gold standard for viability, remains indispensable for confirming culture conversion, defined as two consecutive negative cultures.13,20 Its prolonged TAT, however, poses challenges in Indian tertiary care settings, where delayed results can extend infectious periods, particularly in

overcrowded urban areas like Uttar Pradesh.7,15 Our study's culture conversion rate of 82.4% by month 6 aligns with regional MDR-TB cohorts, which report success rates of 75-85% under NTEP guidelines.8,28 The comparable conversion rates between LJ and PCR (82.4% vs. 84.0%) suggest that PCR can serve as a reliable interim monitoring tool, especially in resource-constrained settings where rapid results are critical.25 However, LJ's role in confirming long-term clearance remains unmatched, as evidenced by its use in global TB trials.29

The logistic regression findings highlight high baseline AFB load (OR 2.3) as a predictor of delayed conversion, consistent with studies linking bacterial burden to poorer outcomes.7,30 Treatment adherence (>90%) was protective (OR 0.4), reinforcing the importance of patient counselling and Directly Observed Treatment, Short-course (DOTS) programs in India.8,13 These factors are particularly relevant in Uttar Pradesh, where socioeconomic barriers like poverty and migration exacerbate non-adherence.7,12 The 5.2% mortality rate in our cohort is lower than the 10-15% reported in some Indian MDR-TB studies, possibly due to the tertiary care setting and exclusion of HIV co-infected patients.9,28

The study's implications for the National TB Elimination Programme (NTEP) are significant. India's high MDR-TB burden, coupled with limited access to advanced diagnostics like liquid culture (e.g., MGIT 960), makes LJ and PCR the backbone of programmatic monitoring.13,15 Our results support a hybrid approach: LJ culture for baseline and endpoint confirmation, with PCR for interim assessments to facilitate timely interventions.25,27 This strategy could align with NTEP's 2025 elimination goals by reducing diagnostic delays and improving outcomes in high-burden regions like Uttar Pradesh.5,13 However, PCR's moderate cost and requirement for trained personnel may limit scalability in peripheral centres, necessitating cost-effectiveness studies tailored to India.22

Limitations include the single-centre design, which may not fully represent India's diverse settings, such as rural Uttar Pradesh or Bihar.7 The exclusion of HIV co-infected patients limits generalizability, given the high TB-HIV co-prevalence in India (10-15% of TB cases).2,9 Additionally, PCR's detection of non-viable DNA requires cautious interpretation, particularly in late treatment phases.19,26 Future studies should explore quantitative PCR (e.g., digital PCR) to estimate bacterial load and differentiate viable from non-viable DNA, as demonstrated in recent global trials.31 Multicentre studies incorporating liquid culture and genotypic sequencing could further validate our findings across India's heterogeneous MDR-TB population.29

In conclusion, PCR offers a rapid and reliable alternative to LJ culture for interim monitoring of MDR-TB treatment response in Indian tertiary care settings, with significant implications for NTEP implementation. A hybrid diagnostic strategy leveraging PCR's speed and LJ's viability confirmation could enhance MDR-TB management, reduce transmission, and support India's TB elimination targets.

Conclusion

This study demonstrates that PCR, specifically the GenoType MTBDRplus line probe assay, offers a rapid and reliable alternative to conventional Lowenstein-Jensen (LJ) media culture for monitoring treatment response in multidrug-resistant tuberculosis (MDR-TB) patients in a Indian tertiary care setting. With a turnaround time of 2.6 days compared to 48.3 days for LJ culture, PCR enables earlier detection of persistent infection, facilitating timely regimen adjustments critical for reducing transmission and preventing further resistance development in high-burden regions like Uttar Pradesh. The high sensitivity (95.2%) and specificity (96.8%) of PCR, coupled with a 97% concordance with LJ culture, support its role as an interim monitoring tool, particularly at month 4, where it identified significantly more treatment failures (15.6% vs. 12.8%, p=0.042). However, LJ culture remains essential for confirming culture conversion due to its ability to assess bacillary viability, addressing PCR's limitation in detecting non-viable DNA.

Given the elevated MDR-TB prevalence in India (16-26.7% among previously treated cases), our findings advocate for a hybrid diagnostic approach under the National TB Elimination Programme (NTEP): utilizing LJ culture for baseline and endpoint confirmation and PCR for rapid interim assessments. This strategy could enhance treatment outcomes, align with NTEP's 2025 TB elimination goals, and address logistical challenges in resource-limited settings. Future research should focus on integrating quantitative PCR techniques, such as digital PCR, to differentiate viable from non-viable bacilli and explore cost-effectiveness to support broader implementation in peripheral centres. Multicentre studies across India are also needed to validate these findings in diverse populations, including those with HIV co-infection, to ensure generalizability. By leveraging the complementary strengths of PCR and LJ culture, Indian healthcare systems can optimize MDR-TB management, reduce mortality, and advance India's progress toward TB elimination.

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Conflict of Interest

The authors declare no conflicts of interest. No financial or personal relationships with organizations or individuals that could inappropriately influence this work exist.

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