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Effectiveness of the Line Probe Assay Compared with Conventional Culture for Detecting Second-Line Anti-Tuberculosis Drug Resistance

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ABSTRACT

Drug-resistant tuberculosis (MDR-TB and rifampicin-resistant TB) is a major challenge in TB control in Indonesia. Rapid detection of second-line anti-TB drug resistance is crucial for timely initiation of therapy. Line Probe Assay (LPA) is a molecular method recommended by WHO as an alternative to conventional culture tests such as Mycobacterium Growth Indicator Tube (MGIT) and Lowenstein Jensen, which have longer detection times. This research aimed to determine the effectiveness of the time and diagnostic efficiency of the Line Probe Assay method compared to conventional cultures (MGIT and Lowenstein Jensen) for identifying second-line anti-TB drug resistance in MDR-TB and rifampicin-resistant TB patients. This study employed an observational analytical design with a cross-sectional approach that incorporated a post-test-only control group framework to compare the performance of the Line Probe Assay (LPA) and conventional culture methods in detecting second-line anti-tuberculosis drug resistanceData were collected from two groups of patients: one group with multidrug-resistant tuberculosis (MDR-TB) and other with rifampicin-resistant TB. All samples were examined using the Line Probe Assay (LPA), Mycobacterium Growth Indicator Tube (MGIT), and Lowenstein-Jensen (LJ) culture methods. A total of 30 collected sputum specimens were analyzed based on the time of the results, ease of interpretation, and readiness for therapy. Findings indicated that the Line Probe Assay (LPA) provided results within 1–2 days, compared to Mycobacterium Growth Indicator Tube (MGIT) (average 17 days) and Lowenstein-Jensen (LJ) culture (average 28–42 days). LPA demonstrated higher efficiency in both laboratory workflow and clinical readiness for early therapeutic decision-making.For the MDR-TB group, LPA successfully detected resistance to fluoroquinolones (gyrA/gyrB mutations) and second-line injectable drugs such as amikacin and kanamycin (rrs/eis mutations), showing full concordance with culture results. Similarly, in the rifampicin-resistant TB group, LPA identified additional resistance patterns consistent with culture findings, confirming its broad applicability for detecting second-line drug resistance. Although the diagnostic accuracy of all three methods was equally high (100% sensitivity and specificity), LPA was clearly superior in terms of turnaround time and overall laboratory efficiency. This study concludes that the Line Probe Assay is more effective and efficient than conventional culture methods for identifying second-line anti-TB resistance in MDR-TB and rifampicin-resistant TB patients, and is strongly suggested for initial screening to accelerate clinical management.

Keywords : Line Probe Assay, Conventional Culture, MDR-TB, Rifampicin-Resistant, Diagnostic Efficiency

INTRODUCTION

Tuberculosis (TB) remains a major global health concern. According to the 2021 WHO report, Indonesia ranks third worldwide in TB burden, following India and China [1]. One of the primary challenges in TB control is the rise of drug-resistant TB, particularly multidrug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB). MDR-TB is defined as infection caused by *Mycobacterium tuberculosis* strains resistant to at least two first-line anti-TB drugs isoniazid and rifampicin making treatment more complex, requiring prolonged therapy, and resulting in lower treatment success rates compared to drug-sensitive TB [2,3]

Previous studies have mainly focused on evaluating the diagnostic accuracy of molecular methods such as the *Line Probe Assay* (LPA) in detecting drug resistance. However, comparative analyses addressing turnaround time, laboratory workflow efficiency, and clinical readiness for treatment initiation particularly within the context of Indonesian healthcare laboratories remain limited. These parameters are crucial in settings with high TB burden, where early detection and timely initiation of appropriate therapy can significantly impact patient outcomes and public health control efforts. Early and accurate detection of drug resistance is crucial to ensure patients receive timely treatment and to prevent further spread of resistant TB strains [4,5]. Conventional diagnostic methods, such as culture using Lowenstein–Jensen media or *the Mycobacterium Growth Indicator Tube* (MGIT) liquid system, have a major limitation: prolonged test times ranging from 5 to 42 days [6,5]. These delays can postpone initiation of therapy and increase the risk of community transmission [7]

To overcome this limitation, WHO recommends molecular diagnostic tools such as the *Line Probe Assay* (LPA), a DNA-based test that can detect genetic mutations associated with resistance to fluoroquinolones and second-line injectable drugs such as amikacin and kanamycin [8,9]. One of the main advantages of LPA is the shorter turnaround time of 24–48 hours while maintaining high sensitivity and specificity [10,8]. Several studies in Indonesia also demonstrated that LPA is reliable for rapid detection of resistance in MDR-TB patients [11,12]

Several studies in Indonesia have compared LPA with conventional culture methods. A study by Gardee [13] showed that the LPA Genotype MTBDRsI ver 2.0 had 100% sensitivity and specificity compared to MGIT for identifying fluoroquinolone resistance in MDR-TB patients. Another study by Yuliana [12] showed similar results in a comparison between LPA and Lowenstein–Jensen culture in rifampicin-resistant TB patients, with 100%

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sensitivity, specificity, positive and negative predictive values, respectively. Both studies demonstrated that LPA can be used as a rapid and accurate diagnostic tool.

However, most previous studies have focused primarily on diagnostic accuracy, with fewer addressing time-effectiveness and laboratory efficiency [14,8]. These aspects are nevertheless crucial for healthcare practice, especially in resource-limited settings. In situations where laboratory capacity is limited and caseloads are high, rapid and efficient methods are essential to expedite clinical management and reduce transmission rates [15,16]

Therefore, this study aims to evaluate the performance of the *Line Probe Assay* (LPA) compared with two conventional culture methods *Mycobacterium Growth Indicator Tube* (MGIT) and *Lowenstein Jensen* (LJ) in detecting second-line anti-tuberculosis drug resistance among patients diagnosed with multidrug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB). Specifically, the research focuses on comparing the turnaround time required by each method to obtain diagnostic results, as well as determining the diagnostic accuracy of the LPA in terms of sensitivity and specificity, using MGIT as the gold standard. In addition, the study assesses the practical efficiency and clinical readiness of the LPA method in supporting timely initiation of appropriate therapy, particularly in settings with limited laboratory resources. Time-effectiveness is assessed based on the speed of the method in producing resistance test results, while diagnostic efficiency is assessed by ease of implementation in the laboratory, the readiness of results for treatment, and the potential for resource savings [16,17,18,12]. It is hoped that the results of this study can strengthen the scientific basis for the use of LPA in initial screening for drug-resistant TB and help optimize TB services in health facilities.

MATERIALS AND METHODS

This study employed a cross-sectional design for diagnostic test evaluation to compare the performance of the Line Probe Assay (LPA) with two conventional culture methods *Mycobacterium* Growth Indicator Tube (MGIT) and Lowenstein Jensen (LJ) in detecting second-line anti-tuberculosis drug resistance. The study was conducted at the Palembang Health Laboratory Center (BBLK) over a six-month period, from January to June 2025. Samples were obtained using a consecutive sampling method from all patients diagnosed with multidrug-resistant TB (MDR-TB) or rifampicin-resistant TB (RR-TB) who met the inclusion criteria. A total of 30 sputum samples were collected, consisting of 15 from MDR-TB patients and 15 from RR-TB patients. Each specimen met the inclusion criteria of having a minimum volume of 1 mL, being AFB-positive, and accompanied by a TB request form (TB-04 or TB-05). Exclusion criteria included non-sterile, broken, leaking, or damaged containers, and non-sputum specimens. For laboratory testing, the LPA was performed using Genotype MTBDRsI ver. 2.0 and Genotype MTBDRsl ver. 2.0 (Hain Lifescience, Germany), while the culture-based methods employed MGIT for MDR-TB specimens and Lowenstein Jensen medium for RR TB specimens. The use of two different culture systems was based on resource availability and laboratory workflow considerations, which is acknowledged as a limitation of the study that may influence the comparability of turnaround times. The study focused on two objective outcome parameters: diagnostic turnaround time and diagnostic accuracy (sensitivity and specificity). Variables such as “ease of interpretation” and “readiness for therapy” were excluded because they were not measured quantitatively or validated

RESULTS

Of the 30 collected sputum specimens from MDR-TB patients tested for fluoroquinolone resistance, 29 (96.7%) showed sensitivity, while 1 (3.3%) was detected as resistant. These results were fully consistent between the Line Probe Assay (LPA) and the Mycobacterium Growth Indicator Tube (MGIT) and Lowenstein–Jensen culture methods. No false-positive or false-negative cases were found.

Table 1. Results of LPA and MGIT Examination of Fluoroquinolone Resistance in MDR-TB Patients

No	Sample Code	LPAResults (MTBDRsI v2.0)	MGIT Results	Information
1	TB001	Sensitive	Sensitive	Suitable
2	TB002	Sensitive	Sensitive	Suitable
3	TB003	Sensitve	Sensitive	Suitable
4	TB004	Sensitive	Sensitive	Suitable
5	TB005	Sensitive	Sensitive	Suitable
6	TB006	Sensitive	Sensitive	Suitable
7	TB007	Sensitive	Sensitive	Suitable

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No	Sample Code	LPAResults (MTBDRsI v2.0)	MGIT Results	Information
8	TB008	Sensitive	Sensitive	Suitable
9	TB009	Sensitive	Sensitive	Suitable
10	TB010	Sensitive	Sensitive	Suitable
11	TB011	Sensitive	Sensitive	Suitable
12	TB012	Sensitive	Sensitive	Suitable
13	TB013	Sensitive	Sensitive	Suitable
14	TB014	Sensitive	Sensitive	Suitable
15	TB015	Sensitive	Sensitive	Suitable
16	TB016	Sensitive	Sensitive	Suitable
17	TB017	Sensitive	Sensitive	Suitable
18	TB018	Sensitive	Sensitive	Suitable
19	TB019	Sensitive	Sensitive	Suitable
20	TB020	Sensitive	Sensitive	Suitable
21	TB021	Sensitive	Sensitive	Suitable
22	TB022	Sensitive	Sensitive	Suitable
23	TB023	Sensitive	Sensitive	Suitable
24	TB024	Sensitive	Sensitive	Suitable
25	TB025	Sensitive	Sensitive	Suitable
26	TB026	Sensitive	Sensitive	Suitable
27	TB027	Sensitive	Sensitive	Suitable
28	TB028	Sensitive	Sensitive	Suitable
29	TB029	Sensitive	Sensitive	Suitable
30	TB030	Resistant	Resistant	Suitable

The diagnostic accuracy calculation showed 100% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 100% negative predictive value (NPV). The results of the sensitivity and specificity calculations are shown in Table 2 below.

Table 2. LPA Diagnostic Test for MGIT (Gold Standard)

Diagnostic Test	MGIT Resistant	MGIT Sensitive	Total
LPA Resistant	1	0	1
Sensitive LPA	0	29	29
Total	1	29	30

From the 30 MDR-TB patient samples tested for fluoroquinolone resistance, 29 (96.7%) showed sensitivity using both LPA and MGIT. Only one sample (3.3%) was detected as resistant, and these results were consistent between the two methods. No false-positive or false-negative cases were found.

Based on the diagnostic comparison between the Line Probe Assay (LPA) and the Mycobacterium Growth Indicator Tube (MGIT) method as the reference standard, the following results were obtained:

1. Sensitivity = 1 / (1 + 0) × 100% = 100%
2. Specificity = 29 / (29 + 0) × 100% = 100%
3. Positive Predictive Value (PPV) = 1 / (1 + 0) × 100% = 100%
4. Negative Predictive Value (NPV) = 29 / (29 + 0) × 100% = 100%
5. Overall Accuracy = (1 + 29) / 30 × 100% = 100%

To account for uncertainty due to the small sample size, 95% Confidence Intervals (CIs) were calculated using the Clopper–Pearson (exact) method:

1. Sensitivity (95% CI): 2.5% – 100%
2. Specificity (95% CI): 88.1% – 100%
3. PPV (95% CI): 2.5% – 100%

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4. NPV (95% CI): 88.1% – 100%

These wide confidence intervals, particularly for sensitivity and PPV, indicate a high level of uncertainty in the 100% estimates, mainly due to the limited number of resistant samples (n = 1). Therefore, while the point estimates suggest perfect diagnostic performance, these results should be interpreted with caution. Future studies involving a larger and more diverse sample population are needed to provide more stable and generalizable estimates of test accuracy%

DISCUSSION

The results of this study indicate that the Line Probe Assay (LPA) method has very high effectiveness in detecting resistance to second-line anti-TB drugs, particularly fluoroquinolones, in MDR-TB patients. Of the 30 sputum samples analyzed, only one (3.3%) showed resistance, and these results were consistent between the LPA and MGIT methods. The diagnostic performance of LPA achieved 100% sensitivity and specificity, as well as perfect predictive values, which is consistent with previous reports [19,10]

These consistent results corroborate findings from various previous studies that suggest LPA is a reliable molecular method for detecting second-line TB drug resistance [20,21]. Research by Jain et al [22] demonstrated that the LPA Genotype MTBDRsI ver 2.0 can detect mutations in the gyrA, gyrB, rrs, and eis genes with high accuracy, mutations that are well known to be associated with resistance to fluoroquinolones and second-line injectable drugs such as amikacin and kanamycin [23,24]

Besides accuracy, the most prominent advantage of LPA is its time efficiency. In this study, LPA test results could be obtained within 24 to 48 hours, significantly faster than the MGIT method, which takes an average of 5 to 19 days depending on bacterial growth in liquid medium. This speed offers major clinical advantages, particularly in making initial therapeutic decisions, as MDR-TB treatment depends heavily on rapid drug susceptibility testing [25]

This improved diagnostic turnaround time aligns with the study by Aricha et al.[25] which found that LPA drastically reduces the time needed to diagnose MDR-TB compared with conventional culture. Delays in obtaining resistance results often cause delays in treatment initiation, ultimately increasing the risk of community transmission and reducing therapeutic success rates [26]

In addition to time efficiency, LPA also demonstrates efficiency in terms of laboratory resources. This molecular method does not require lengthy incubation, does not rely on bacterial growth, and can be performed on decontaminated sputum specimens without waiting for culture isolates. The PCR- and DNA hybridization–based LPA procedure allows for specific tracking of resistance mutations, providing deeper insight into resistance mechanisms [10]

The finding of only **one resistant case (3.3%)** in this study is epidemiologically noteworthy. It may reflect a genuinely low prevalence of second-line drug resistance in Palembang, consistent with national surveillance data showing that most MDR-TB cases in Indonesia remain resistant only to first-line drugs, particularly rifampicin and isoniazid [26]. However, this finding could also be influenced by selection bias, as the samples were obtained from patients who had already received partial therapy or were under routine follow-up rather than from newly diagnosed MDR-TB cases. The small sample size further limits the ability to generalize this prevalence estimate. Nevertheless, these data may suggest that while MDR-TB remains a public health concern, extensive drug resistance (XDR-TB) and second-line resistance are still relatively uncommon in this region, highlighting the importance of early molecular detection and continuous local surveillance.

However, there are several limitations that need to be considered. First, the sample size was relatively small, with only one resistant isolate, resulting in wide confidence intervals and uncertain estimates of diagnostic accuracy. Second, although this study included two culture-based comparison methods (MGIT and Lowenstein–Jensen), the distribution was limited by laboratory resources and sample availability. Third, the analysis focused solely on fluoroquinolone resistance, without extensive evaluation of resistance to second-line injectable drugs. Lastly, as the data were collected from a single laboratory center in Palembang, the findings may not fully represent the regional or national epidemiology of MDR-TB.

Despite these limitations, this study supports the use of LPA as a rapid and reliable diagnostic tool for the detection of second-line drug resistance in MDR-TB and rifampicin-resistant TB cases. Broader, multicenter studies with larger sample sizes are recommended to validate these findings and better estimate the true prevalence of second-line resistance in Indonesia

CONCLUSIONS AND SUGGESTIONS

The Line Probe Assay (LPA) demonstrated excellent diagnostic performance in detecting *fluoroquinolone* resistance among MDR-TB patients, with 100% sensitivity and specificity and a significantly shorter turnaround time of 1–2 days compared to the MGIT liquid culture method, which required an average of 17 days. These findings confirm that LPA is an effective and rapid diagnostic tool specifically for identifying *fluoroquinolone resistance* as part of second-line drug susceptibility testing.

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However, since this study detected resistance only to fluoroquinolones and involved a limited number of samples, the conclusions cannot be generalized to all second-line anti-TB drugs. Further multicenter studies with larger sample sizes are needed to evaluate LPA performance in detecting resistance to other second-line agents, such as amikacin, kanamycin, and capreomycin, and to establish regional data on the prevalence of second-line drug resistance in Indonesia

AUTHOR CONTRIBUTION STATEMENT

Aristoteles conceived and designed the study, supervised the research implementation, performed data interpretation, and critically revised the manuscript for intellectual content. Nurhidayanti conducted laboratory analyses, collected clinical and laboratory data, and contributed to the drafting and editing of the manuscript. Both authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper. All research activities were conducted independently, and no financial, personal, or professional relationships influenced the results or interpretations presented in this study

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